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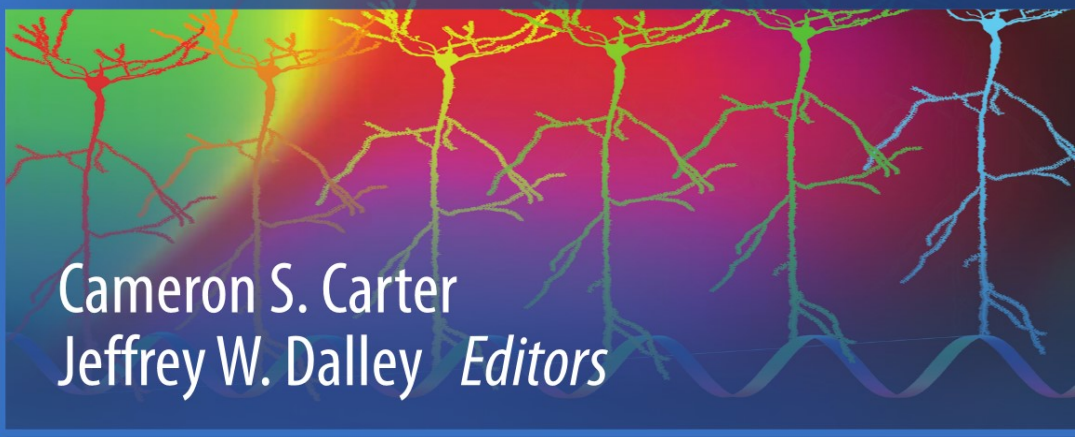

Cameron S. Carter
Jeffrey W. Dalley *Editors*

Brain Imaging in Behavioral Neuroscience

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Cameron S. Carter · Jeffrey W. Dalley
Editors

Brain Imaging in Behavioral Neuroscience

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Preface

No technological advance has had greater impact on our understanding of human brain function or our approach to understanding the underlying mechanisms of brain disease than the emergence of methods for the non-invasive imaging of the human brain. Over a period of three short decades we have moved from an almost complete dependence on animal models and post mortem analysis to an era in which the structure, function, and molecular composition of the brain may be reliably measured with increasing precision and resolution. This has led to a revolutionary approach to modern neuroscience whereby neuroimaging studies enable us to bridge from a cellular, molecular and systems level understanding derived from animal model systems all the way to human cognition, emotion and behavior. The chapters in this volume describe the major technical advances in non-invasive neuroimaging along with important clinical and translational applications in a range of human developmental states and diseases. The first several chapters focus on molecular imaging approaches using Positron Emission Tomography (PET), the second set of chapters focuses on using Magnetic Resonance Imaging (MRI) to reveal the structure, function, and molecular makeup of the brain.

Historically, PET research has focused on the brain dopaminergic systems, not least given the rich bounty of PET tracers targeting dopamine synthesis, plasma membrane transporters, and receptor binding sites. The first chapter by Volkow—a pioneering scientist in the use of PET to investigate dopamine transmission in addiction—gives a compelling insight into the brain mechanisms of this chronically relapsing brain affliction and the strong parallels of this disorder with obesity. This research has paved the way for neuroscientists interested in the causal attributes of dopamine dysfunction in psychopathology, specifically by aiding the development of translational models that capture key elements of clinical syndromes, including the addiction cycle. This has been no better exploited than the work of Gould and colleagues using a non-human primate model of stimulant addiction. Their review on neural vulnerability markers underlying addiction is thematic and timely to the concept of shared predispositions and clinical trajectories of several, often inter-related disorders such as addiction and attention

deficit hyperactivity disorder (ADHD), a point emphasized by Del Campo and colleagues where they discuss the neuroimaging correlates of ADHD and prospects for treatment.

While PET offers the ability to non-invasively assess alterations in receptor function, it does routinely require restraint of the animal in order to keep it stationary during the period of data acquisition. In pre-clinical research this is usually achieved by general anaesthesia and other supplementary devices to fix, for example, the head in one position. This approach places a serious impediment to correlating PET signals with behavior. A quantitative solution to this problem is discussed by Aarons and co-authors, which utilises the gold standard tracer [18F]-fluorodeoxyglucose. This is a must-read chapter for neuroscientists considering using this approach for their own experiments.

Other chapters highlight the importance of PET in the domain of neurological disorders, which by virtue of their progressive underlying molecular neuropathology are well-matched to powerful longitudinal studies to facilitate diagnosis and the development of new therapies. Huguchi and colleagues discuss this important and growing area of research by drawing on evidence from animal models of Alzheimer's disease (AD) and the targeting of biomarkers underlying the immune response to neurodegeneration.

Following this article Cumming and Borghammer provide a comprehensive synthesis of molecular imaging studies in Parkinson's disease (PD) that extend beyond dopaminergic biomarkers in the basal ganglia. The chapter highlights the extraordinary heterogeneity of PD in terms of motor and cognitive impairment as well as underlying neurochemical pathology.

Finally in this section, Praschak-Rieder and Willeit discuss the astonishing relationship between rhythmical seasonal variations (e.g. in light intensity) and monoamine markers in the brain. The authors focus on the serotonergic systems, and their own remarkable contribution to this field that have implications for understanding the neurobiology of seasonal affective disorder.

The second section of the book, focusing largely on MRI based measures, begins with an article by Hall, which reviews the exciting application of magnetic resonance spectroscopy (MRS) to profile metabolic abnormalities in neurological disorders such as AD and PD. A comprehensive review then follows by Maddock and Buonocore on the major metabolites and neurotransmitters that can be measured in the human brain using MRS, along with a discussion of the insights and controversies regarding the functional significance of these measures. It then reviews the spectroscopic findings in a broad range of psychiatric diseases including the anxiety disorders, major depression, bipolar disorder and schizophrenia. This exhaustive distillation of a very large and complex literature is a must read for experts and beginners alike pursuing spectroscopy in patients with mental disorder.

Functional MRI has become a very widely applied methodology for understanding the relationship between discrete functional neural circuits, cognitive and emotional processing and symptoms in psychiatric and neurological disease. One area that has been a proving ground for this methodology has been the

investigation of the neural basis of cognitive deficits in schizophrenia. Libby and Ragland review this literature and chart the evolution in thinking about the mechanisms underlying cognitive dysfunction in schizophrenia, which has paralleled our growing understanding of the cognitive and neural basis of higher cognitive functions such as attention and memory in basic cognitive neuroscience. They also emphasize the evolution of thinking from a lesion deficit model to a systems level analysis of cognition related brain activity.

Two chapters address important issues in the use of MRI to understand the ageing brain. Andreescu and Aizenstein review methodological issues related to conducting fMRI studies of cognition in ageing subjects, as well as findings related to functional changes of brain circuitry associated with late life mood disorders. Next, Carmichael and Lockhart describe the use of diffusion tensor imaging (DTI) to study white matter tracts in the human brain along with its application in understanding the important and potentially partly reversible contribution of white matter disease to cognitive ageing.

Salo and Fassbender review non-invasive neuroimaging studies of individuals with methamphetamine addiction, a major public health problem in the Western United States and many other areas of the world. These authors review MRI studies of brain structure and function and chemistry as well as a body of PET neurochemistry studies in this disorder. This work is discussed in the context of the known neurotoxic effect of methamphetamine as well as documenting the improvements in brain structure and function that are seen with abstinence from the drug.

In the final chapter Minzenberg provides a comprehensive review of the conceptual and methodological issues related to the use of pharmacological MRI in studies investigating the mechanisms of action of psychopharmacological treatments. This chapter also highlights the value of neuroimaging as a source of biomarkers, which may enhance clinical diagnosis and provide surrogate measures of treatment effects, an important advance for treatment development for brain disorders, which has lagged behind that for many other medical conditions.

The broad range of topics covered as well as the methodological depth provided throughout the volume will ensure that the reader is left with a strong appreciation for the progress that has been made in the development and application of brain imaging in behavioral neuroscience as well as the tremendous potential that exists for further major advances in the coming years.

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Contents

Food and Drug Reward: Overlapping Circuits in Human Obesity and Addiction

N. D. Volkow, G. J. Wang, J. S. Fowler, D. Tomasi and R. Baler

Abstract Both drug addiction and obesity can be defined as disorders in which the saliency value of one type of reward (drugs and food, respectively) becomes abnormally enhanced relative to, and at the expense of others. This model is consistent with the fact that both drugs and food have powerful reinforcing effects—partly mediated by dopamine increases in the limbic system—that, under certain circumstances or in vulnerable individuals, could overwhelm the brain's homeostatic control mechanisms. Such parallels have generated significant interest in understanding the shared vulnerabilities and trajectories between addiction and obesity. Now, brain imaging discoveries have started to uncover common features between these two conditions and to delineate some of the overlapping brain circuits whose dysfunctions may explain stereotypic and related behavioral deficits in human subjects. These results suggest that both obese and drug-addicted individuals suffer from impairments in dopaminergic pathways that regulate neuronal systems associated not only with reward sensitivity and incentive motivation, but also with conditioning (memory/learning), impulse control (behavioural inhibition), stress reactivity, and interoceptive awareness. Here, we integrate findings predominantly

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derived from positron emission tomography that shed light on the role of dopamine in drug addiction and in obesity, and propose an updated working model to help identify treatment strategies that may benefit both of these conditions.

Keywords Dopamine · Addiction · Obesity · Reward · Inhibitory control · Positron emission tomography

Contents

1 Background

Dopamine (DA) is considered a key to the rewarding effects of natural and drug rewards. However, its role in the loss of control and compulsive behaviours that are associated with addiction and obesity are much less clear. PET studies have played a crucial role in characterizing the role of the brain DA systems in addiction (in addition to its role in drug reward) and in obesity. Indeed, drugs of abuse (including alcohol) are consumed by humans or self-administered by laboratory animals because they are inherently rewarding, an effect that is mediated through their DA-enhancing properties in the mesolimbic system (Wise 2009). However, in the case of addiction, imaging studies have revealed that the disorder affects not only the DA reward circuit but also other DA pathways involved in the modulation of conditioning/habits, motivation, and executive functions (inhibitory control, salience attribution, and decision-making), and that DA deficits may also participate in the enhanced stress reactivity and disruption of interoceptive awareness associated with addiction. Preclinical and clinical studies have also revealed other neurotransmitters (and neuropeptides) that play important roles in drug reward and addiction (i.e., cannabinoids, opioids) and are intimately involved in the neuroplastic changes that follow repeated drug use (i.e., glutamate, opioids, GABA, corticotropin-releasing factor). The glutamatergic system is particularly prominent in this regard because it mediates the disruptions in both long-term potentiation and long-term depression that have been observed in animal models of chronic drug administration (Thomas et al. 2008). Reviews pertaining to these additional systems can be found elsewhere (Kalivas 2009; Koob 1992).

Since drugs activate the same reward systems that underlie food reward, it is not totally unexpected that, in general, brain imaging studies have supported the notion that impairments in DA-modulated circuits be also implicated in pathologic, compulsive eating behaviours. Food cues, like drug cues, increase striatal extracellular DA and drive the motivation to engage in the behaviours that are necessary to procure and eat the food, providing evidence for the involvement of DA not just in food reward but also in the non-hedonic motivational properties of food (i.e., caloric requirements) and the decrease in inhibitory control seen in compulsive overeating (Avena et al. 2008; Volkow et al. 2008a).

Here, we review findings from imaging studies that specifically focus on the overlaps in the brain circuits that are disrupted in obesity and in drug addiction. It is worth remembering, however, that the regulation of food intake behaviours is much more complex than the regulation of drug intake. The latter is predominantly mediated by the rewarding effects of drugs whereas the former is modulated not just by its rewarding effects (hedonic factors) but also by multiple peripheral and central factors that sense nutrient requirements in the body necessary for survival (homeostatic factors). Interestingly, there is growing evidence that homeostatic factors (e.g., insulin, leptin, ghrelin) modulate food intake in part by increasing or decreasing the sensitivity of brain reward circuits to food stimuli (Volkow et al. 2011a).

2 The Role of Dopamine in Acute Reward to Drugs and Food

Whether directly or indirectly, all addictive drugs display an ability to increase DA in nucleus accumbens (NAc) via specific interactions with different molecular targets (Nestler 2004) (Fig. 1). The mesolimbic DA pathway [DA cells in ventral tegmental area (VTA) that project into the NAc] seems to be crucial for drug reward (Wise 2009). However, as described below, other DA pathways [mesostriatal (DA cells in substantia nigra projecting into dorsal striatum) and mesocortical (DA cells in VTA projecting into frontal cortex)] also contribute to drug reward and addiction (Wise 2009). Overall, it appears that the rewarding and conditioning effects of drugs are predominantly driven by phasic DA cell firing, which leads to large and transient DA increases. In contrast, the downstream changes in executive function that occur in addiction are linked with changes in tonic DA cell firing and result in lower but more stable DA levels (Grace 2000; Wanat et al. 2009). This, in turn, point to the D1 receptors (D1R), which are low affinity DA receptors that stimulate cyclic AMP signaling, as being involved both in acute drug reward as well as in conditioning, since these are associated with the high DA concentrations necessary to stimulate D1R. In contrast, D2Rs, which inhibit cyclic AMP signaling, are stimulated by both phasic and tonic DA. Note that, due to the lack of specific radiotracers for the PET imaging of DA receptors of the D1, D3, D4, and D5 types, most studies on the effects of drugs of abuse and addiction in the human brain have focused on D2Rs.

In humans, PET studies have shown that several drugs [stimulants (Drevets et al. 2001; Volkow et al. 1999b), nicotine (Brody et al. 2009), alcohol (Boileau et al. 2003),

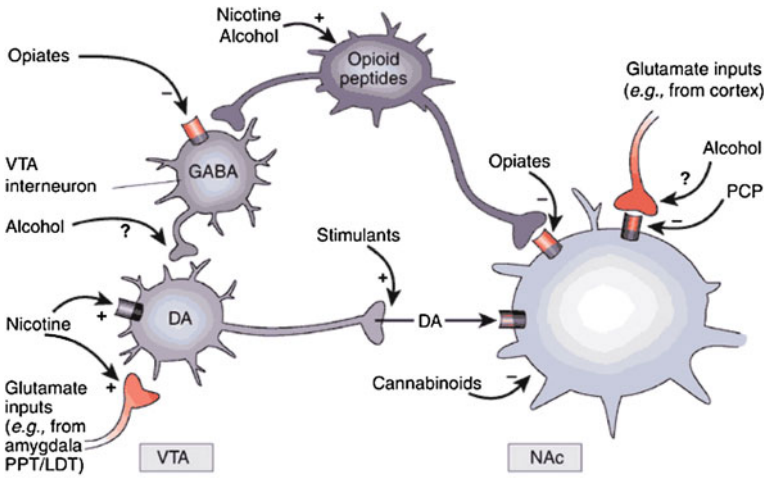

Fig. 1 Drugs of abuse act on the reward and ancillary circuits through different mechanisms, however, they all lead to similar dopaminergic effects in the VTA and NAc. Thus, stimulants boost acumbal DA directly, while opiates do this by lowering the inhibitory tone of GABAergic interneurons on DA signaling both either in the VTA or in then NAc. While the mechanisms of other drugs of abuse is less clear, there is evidence suggesting that nicotine may activate VTA DA *directly* through nicotinic acetylcholine receptor (nAChR) on those neurons and *indirectly* via stimulation of its receptors on glutamatergic nerve terminals that innervate the DA cells. Alcohol appears to inhibit GABAergic terminals in VTA, leading to DA neurons disinhibition in the VTA. Cannabinoids act, among others, through the activation of CB1 receptors on glutamatergic and GABAergic nerve terminals in the NAc, and on NAc neurons themselves. Phencyclidine (PCP) may act by inhibiting postsynaptic NMDA glutamate receptors in the NAc. In addition, there is some evidence suggesting that nicotine and alcohol may also interact with endogenous opioid and cannabinoid pathways (not shown). PPT/LDT, peduncular pontine tegmentum/lateral dorsal tegmentum. Reprinted with permission Nestler (2005)

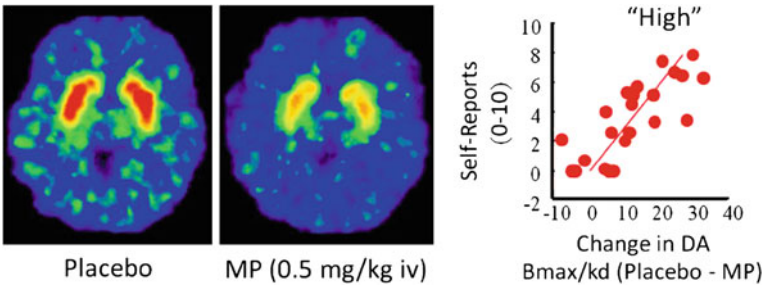

Fig. 2 Effects of intravenous methylphenidate (MP) in raclopride binding and relationship between striatal DA increases induced by MP in the striatum and the self-reports of "high". Modified from Volkow et al. (1999b)

and marijuana (Bossong et al. 2009)] increase DA in dorsal and ventral striatum (where the NAc is located). These studies take advantage of several radiotracers, such as [^{11}C]raclopride, that bind to D2R but only when these are not binding endogenous DA

(unoccupied), which under baseline conditions corresponds to 85–90% of the striatal D2R (Abi-Dargham et al. 1998). Thus, a comparison of [^{11}C]raclopride binding after placebo and after drug administration can help us estimate the decreases in D2R availability induced by the drug (or other stimuli that can increase DA). These decreases in [^{11}C]raclopride binding are proportional to the DA increases (Breier et al. 1997). These studies have shown that drug-induced DA increases in striatum are proportional to the intensity of the subjective experience of euphoria or "high" [see review (Volkow et al. 2009a)] (Fig. 2).

PET studies have also revealed a clear, direct relationship between a drug's pharmacokinetic profile (i.e., the speed with which it enters and leaves the brain) and its reinforcing effects. Specifically, the faster a drug reaches peak levels in the brain the more intense the "high" (Volkow et al. 2009a)**.** For example, for an equivalent level of cocaine reaching the brain (assessed through PET), when cocaine entered the brain rapidly (smoked or i.v. administration), it elicited a more intense "high" than when it entered at a slower rate (snorted) (Volkow et al. 2000). This is consistent with preclinical studies showing a similar correlation between a drug's pharmacokinetic profile and its reinforcing properties (Balster and Schuster 1973). It is reasonable to hypothesize that such abrupt and large DA increases as triggered by drugs of abuse may mimic the fast and large DA increases that result from phasic DA firing that have been associated, in the brain, with the processing of information about reward and saliency (Schultz 2010). Such drug-induced DA increases in the NAc may be necessary for addiction, but the fact that they occur also in non-addicted individuals indicates that they are insufficient to explain the impulsive and compulsive drug use characteristic of addiction.

There is now evidence that comparable dopaminergic responses are linked with food reward and that these mechanisms are also likely to play a role in excessive food consumption and obesity. It is well known that certain foods, particularly those rich in sugars and fat, are potently rewarding (Lenoir et al. 2007). High-calorie foods can promote over-eating (eating that is uncoupled from energetic needs) and trigger learned associations between the stimulus and the reward (conditioning). In evolutionary terms, this property of palatable foods *used* to be advantageous in environments where food sources were scarce and/or unreliable, because it ensured that food was eaten when available, enabling energy to be stored in the body (as fat) for future use. Unfortunately, in societies like ours, where food is plentiful and constantly available, this adaptation has become a liability.

Several neurotransmitters, including DA, cannabinoids, opioids, and serotonin, as well as hormones and neuropeptides involved in homeostatic regulation of food intake, such as insulin, orexin, leptin, and ghrelin, have been implicated in the rewarding effects of food (Atkinson 2008; Cason et al. 2010; Cota et al. 2006). Of these, DA has been the most thoroughly investigated and is the best characterized. Experiments in rodents have shown that, upon first exposure to a food reward, the firing of DA neurons in the VTA increases with a resulting increase in DA release in NAc (Norgren et al. 2006). Similarly, in healthy, normal-weight human subjects, the ingestion of palatable food has been shown to release DA in the dorsal striatum in proportion to the ratings of meal pleasantness (Small et al. 2003) (Fig. 3). However,

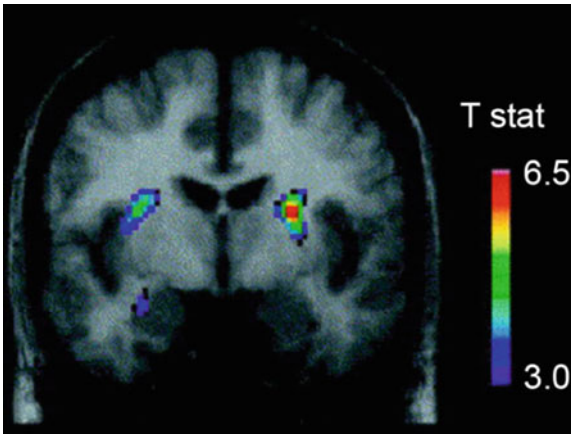

Fig. 3 Dopamine release induced by feeding. Coronal section from the T-map of statistically significant reductions in [^{11}C]raclopride's binding potential (BP) following feeding. The colour bar represents the *t* statistic values. (Reprinted with permission Small et al. 2003)

and as seen in studies with drug abusers, food-induced increases in striatal DA alone cannot explain the difference between normal food intake and excessive compulsive food consumption since these also occur in healthy individuals who do not eat excessively. Thus, as is the case for addiction, downstream adaptations are likely to be involved in the loss of control over food intake.

3 Imaging DA in Response to Drugs and to Conditioned Cues in Addiction

DA's role in reinforcement is more complex than just coding for reward per se (hedonic pleasure); for example, stimuli that induce fast and large DA increases also trigger conditioned responses and elicit incentive motivation to procure them (Owesson-White et al. 2009). This is important because, through the process of conditioning, neutral stimuli that are linked to the reinforcer (whether a natural or a drug reinforcer) acquire the ability *by themselves* to increase DA in striatum (including NAc) in anticipation of the reward, thus engendering a strong motivation to seek the drug (Owesson-White et al. 2009). However, uncoupling reward and conditioning mechanisms in the process of drug addiction is more challenging than for food consumption because drugs of abuse, through their pharmacological effects, directly activate DA neurons (i.e., nicotine) or increase DA release (i.e., amphetamine).

Brain imaging studies that compared the DA increases induced by the stimulant drug methylphenidate (MP) or amphetamine (AMPH) among cocaine addicted subjects vs. controls showed a marked attenuation of MP or AMPH-induced DA increases in striatum (50% lower in detoxified abusers and 80% in active abusers) and lower self-reports of the drug's rewarding effects relative to non-drug-abusing controls (Martinez et al. 2007; Volkow et al. 1997) (Fig. 4). This was surprising since MP and AMPH are pharmacologically similar to cocaine and methamphetamine, respectively, and drug abusers cannot distinguish between them when

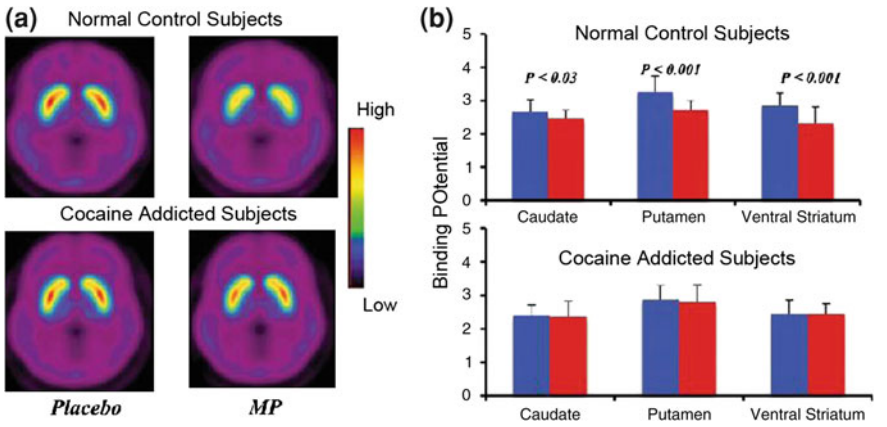

Fig. 4 DA changes induced by i.v. MP in controls and in active cocaine-addicted subjects. **a** Average nondisplaceable biding potential (BPND) images of [^{11}C]raclopride in active cocaine-addicted subjects ($n = 19$) and in controls ($n = 24$) tested after placebo and after i.v. MP. **b** D2R availability (BPND) in caudate, putamen, and ventral striatum after placebo (*blue*) and after MP (*red*) in controls and in cocaine-addicted subjects. MP reduced D2R in controls but not in cocaine-addicted subjects. Note that cocaine abusers show both decreases in baseline striatal D2R availability (placebo measure) and decreases in DA release when given i.v. MP (measured as decreases in D2R availability from baseline). Although one could question the extent to which the low striatal D2R availability in cocaine-addicted subject limits the ability to detect further decreases from MP, the fact that cocaine-addicted subjects show reductions in D2R availability when exposed to cocaine cues indicates that the attenuated effects of MP on [^{11}C]raclopride binding reflect decreased DA release. Reprinted with permission (Volkow et al. 1997; Wang et al. 2010)

they are administered intravenously. Since the marked reductions in the drug-induced DA increases were observed whether the cocaine abusers had been detoxified or not, this indicates that the state of withdrawal is not a confounding factor (Volkow et al. 2011b). These and related results (Volkow et al. 2009a) are consistent with the hypothesis that the hedonic response becomes deficient in drug-addicted individuals, and further strengthen the notion that the acute pharmacological DA-enhancing effects of the drug in NAc cannot explain by themselves the increased motivation to consume them.

The response of VTA DA neurons to rewarding stimuli changes with repeated exposure. While DA cells fire upon the first exposure to a novel reward, repeated exposure to DA causes the neurons to stop firing upon reward consumption and fire instead when they are exposed to stimuli that are predictive of the reward (Schultz et al. 1997). This is likely to underlie DA's role in learning and conditioning. Indeed, drug-induced phasic DA signaling can eventually trigger neuroadaptations in ancillary circuits that are related to habit formation and behavioural conditioning. These changes are predominantly induced by D1R signaling and synaptic changes in glutamate-modulated NMDA and AMPA receptors (Luscher and Malenka 2011; Zweifel et al. 2009). Recruitment of these circuits is significant in disease progression

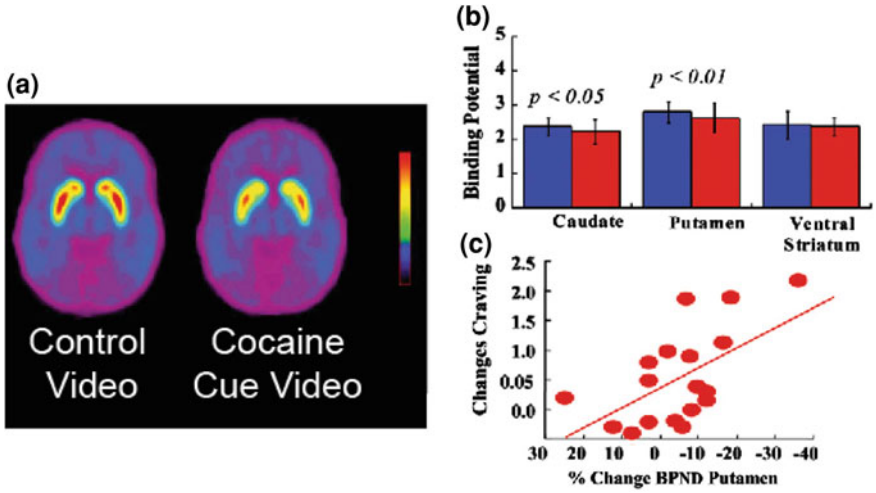

Fig. 5 DA changes induced by conditioned cues in active cocaine-addicted subjects. **a** Average nondisplaceable binding potential (BPND) images of [11C]raclopride in cocaine-addicted subjects ($n = 17$) tested while viewing a neutral video (nature scenes) and while viewing a cocaine-cues video (subjects administering cocaine). **b** D2R availability (BPND) in caudate, putamen, and ventral striatum for the neutral video (*blue*) and the cocaine-cues video (*red*). The cocaine cues decreased D2R in caudate and putamen. **c** Correlations between changes in D2R (reflecting DA increases) and self-reports of cocaine craving induced by the cocaine-cues video. Modified from ref. (Volkow et al. 2006b)

because the ensuing conditioned responses help explain the intense desire for the drug (craving) and the compulsive use that occurs when addicted subjects are exposed to drug-associated cues. This hypothesis is consistent with independent observations (Volkow et al. 2006b; Wong et al. 2006) that show the power of cocaine-associated cue exposure to raise DA levels in the dorsal striatum and trigger a concomitant increase in the subjective experience of craving in detoxified cocaine abusers (Fig. 5). Since the dorsal striatum plays a role in habit learning (Belin et al. 2009; Yin et al. 2004), the association is likely to reflect the strengthening of habits as chronicity of addiction progresses. This suggests that a basic disruption in addiction might relate to the DA-triggered conditioned responses that result in habits leading to intense craving and compulsive drug consumption. Interestingly, in actively using cocaine-addicted subjects, the DA increases triggered by conditioned cues appear to be *even larger* than those produced by the stimulant drug itself as assessed in two separate group of subjects (Volkow et al. 2011b, 2006b), suggesting that conditioned responses may drive the DA signaling that maintains the motivation to take the drug even when its pharmacological effects appear to be reduced. Thus, although drugs may initially induce feelings of immediate reward through DA release in the ventral striatum, with repeated use, and as habit develops, there appears to be a shift from the drug to the conditioned stimulus. According to studies in laboratory animals, glutamatergic projections from prefrontal cortex and from amygdala into VTA/SN

and NAc mediate these conditioned responses (Kalivas 2009). In this manner, the mere prediction of a reward may eventually become the reward that motivates the behaviour necessary for drug (or food) consumption.

Interestingly, this type of functional "switch" has also been reported for *natural reinforcers*, which are likely to induce an equivalent and gradual shift in DA increases, from ventral to more dorsal regions of the striatum during the transition from a novel stimulus that is inherently rewarding to that of the associated cues that predict it. This transition is conveyed through DA signaling, which appears to code for a "reward prediction error" (Schultz 2010). The extensive glutamatergic afferents to DA neurons from regions involved in the processing of sensory (insula or primary gustatory cortex), homeostatic (hypothalamus), reward (NAc), emotional (amygdala and hippocampus), and multimodal (orbitofrontal cortex for salience attribution) information, modulate their activity in response to rewards and to conditioned cues (Geisler and Wise 2008). More specifically, projections from the amygdala and the orbitofrontal cortex (OFC) to DA neurons and to NAc are involved in conditioned responses to food (Petrovich 2010). Indeed, imaging studies showed that when non-obese male subjects were asked to inhibit their craving for food -while being exposed to food cues-, they exhibited decreased metabolic activity in amygdala and OFC (as well as in hippocampus), insula and striatum, and that the decreases in OFC were associated with reductions in food craving (Wang et al. 2009). A similar inhibition of the metabolic activity in the OFC (and also in NAc) has been observed in cocaine abusers when they were asked to inhibit their drug craving upon exposure to cocaine-cues (Volkow et al. 2009b).

Still, the emergence of such powerfully cue-conditioned cravings, which for food also occur in healthy individuals who do not overeat, would not be as devastating were they not coupled with growing deficits in the brain's ability to inhibit maladaptive behaviours.

4 The Impact of Dysfunction in Inhibitory Control

The capacity to inhibit prepotent responses is bound to contribute to an individual's ability to avoid engaging in inappropriate behaviours, such as taking drugs or eating past the point of satiety, and thus increasing his/her vulnerability to addiction (or obesity) (Volkow and Fowler 2000; Volkow et al. 2008a).

PET studies have uncovered significant reductions in D2R availability in the striatum of addicted subjects that persist for months after protracted detoxification [reviewed in (Volkow et al. 2009a)]. Similarly, preclinical studies in rodent and non-human primates have shown that repeated drug exposures are associated with reductions in striatal D2R levels (Nader et al. 2006; Thanos et al. 2007; Volkow et al. 2001). In the striatum, D2Rs mediate signaling in the striatal indirect pathway that modulates prefrontal regions; and its downregulation has been shown to enhance sensitization to the effects of drugs in animal models

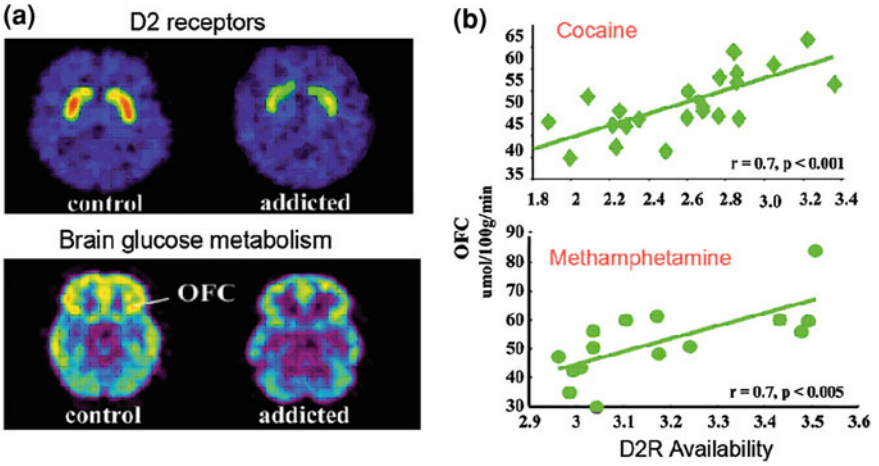

Fig. 6 Correlations between striatal D2R availability and metabolism in prefrontal brain regions. **a** Axial brain images for a control and for a cocaine-addicted subject for baseline images of D2R availability in striatum (obtained with [^{11}C]raclopride) and of brain glucose metabolism in OFC (obtained with [^{18}FDG). **b** Correlations between striatal D2R and metabolism in OFC in cocaine-addicted and methamphetamine-addicted subjects. Reprinted from Volkow et al. (2009a) Copyright (2009), with permission from Elsevier

(Ferguson et al. 2011). In humans addicted to drugs, the reduction in striatal D2R is associated with decreased activity of prefrontal regions as evidenced by decreases in baseline glucose metabolism (a marker of brain function) in OFC, anterior cingulate gyrus (ACC), and dorsolateral prefrontal cortex (DLPFC) (Volkow et al. 2001, 1993, 2007) (Fig. 6). Inasmuch as OFC, ACC, and DLPFC are involved with salience attribution, inhibitory control/emotion regulation, and decision-making, respectively, it has been postulated that their improper regulation by D2R-mediated DA signaling in addicted subjects could underlie the enhanced motivational value of drugs in their behaviour and the loss of control over drug intake (Volkow and Fowler 2000). In addition, because impairments in OFC and ACC are associated with compulsive behaviours and impulsivity (Fineberg et al. 2009), DA's impaired modulation of these regions is likely to contribute to the compulsive and impulsive drug intake seen in addiction (Goldstein and Volkow 2002). Indeed, in methamphetamine abusers, low striatal D2R was associated with impulsivity (Lee et al. 2009), and it also predicted compulsive cocaine administration in rodents (Everitt et al. 2008). A reverse scenario, in which an initial vulnerability for drug use preexists in prefrontal regions, and whereby repeated drug use triggers further decreases in striatal D2R, is also possible. Indeed, a study done in subjects who, despite having a high risk for alcoholism (positive family history of alcoholism) were not alcoholics, revealed a higher than normal striatal D2R availability that was associated with normal metabolism in OFC, ACC, and DLPFC (Volkow et al. 2006a). This suggests that, in these subjects at risk for

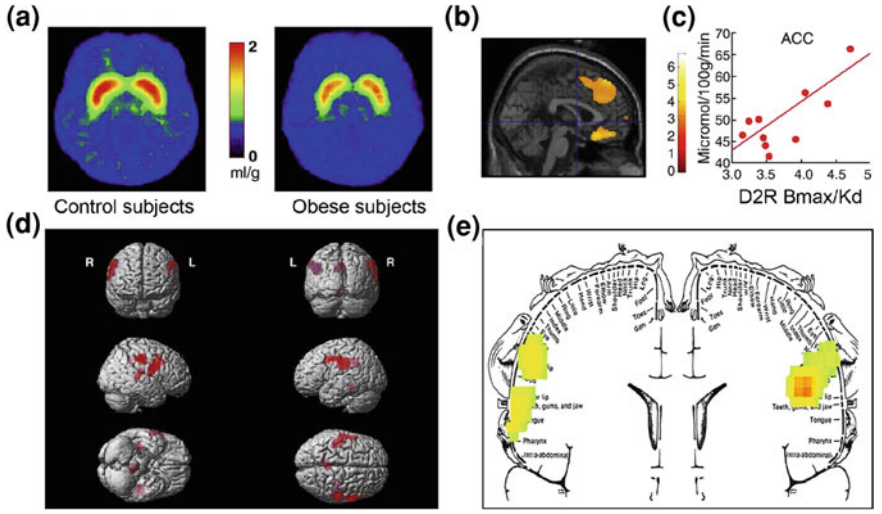

Fig. 7 Hyperphagia could result from a drive to compensate for a weakened reward circuit (processed through dopamine regulated corticostriatal circuits) combined with a heightened sensitivity to palatability (hedonic properties of food processed in part through the somatosensory cortex). **a** Averaged images for DA D2 receptor (D2R) availability in controls ($n = 10$) and in morbidly obese subjects ($n = 10$). **b** Results from (Statistical Parametric Mapping) SPM identifying the areas in the brain where D2R was associated with glucose metabolism, these included the medial OFC, ACC, and the dorsolateral PFC (region not shown). **c** Regression slope between striatal D2R and metabolic activity in ACC in obese subjects. **d** Three-dimensionally rendered SPM images showing the areas with higher metabolism in obese than in lean subjects ($P < 0.003$, uncorrected). **e** Colour coded SPM results displayed in a coronal plane with a superimposed diagram of the somatosensory homunculus. The results (z value) are presented using the rainbow scale where red > yellow > green. When compared with lean subjects, obese subjects had higher baseline metabolism in the somatosensory areas where the mouth, lips, and tongue are represented and which are involved with processing food palatability. Modified, with permission, from Volkow et al. (2008a) (**a**–**c**) and Wang et al. (2002) (**d**, **e**)

alcoholism, the normal prefrontal function was linked to enhanced striatal D2R signaling, which in turn may have protected them from alcohol abuse.

Predictably, evidence of dysregulation in control circuits has also been found among obese individuals. Both preclinical and clinical studies have provided evidence of decreased striatal D2R signaling, which, as mentioned above, is linked with reward (NAc) but also with the establishment of habits and routines (dorsal striatum) in obesity (Geiger et al. 2009; Wang et al. 2001). Importantly, decreased striatal D2R availability has been linked to compulsive food intake in obese rodents (Johnson and Kenny 2010) and with decreased metabolic activity in OFC and ACC in obese humans (Volkow et al. 2008b) (Fig. 7a–c). Given that dysfunction in OFC and ACC results in compulsivity [see review (Fineberg et al. 2009)], this might be part of the mechanism by which low striatal D2R signaling facilitates hyperphagia (Davis et al. 2009). In addition, since decreased D2R-related signaling is also likely to reduce the sensitivity to other natural rewards,

this deficit in obese individuals might also contribute to compensatory overeating (Geiger et al. 2008).

This hypothesis is consistent with preclinical evidence showing that decreased DA activity in VTA results in a dramatic increase in the consumption of high-fat foods (Stoeckel et al. 2008). Similarly, compared with normal-weight individuals, obese individuals who were presented with pictures of high-calorie food (stimuli to which they are conditioned) showed increased neural activation in regions that are part of reward and motivation circuits (NAc, dorsal striatum, OFC, ACC, amygdala, hippocampus, and insula) (Killgore and Yurgelun-Todd 2005). By contrast, in normal-weight controls, the activation of the ACC and OFC (regions involved in salience attribution that project into the NAc) during presentation of high-calorie food was found to be negatively correlated with their body mass index (BMI) (Stice et al. 2008b). This suggests a dynamic interaction between the amount of food eaten (reflected in part in the BMI) and the reactivity of reward regions to high-calorie food (reflected in the activation of OFC and ACC) in normal-weight individuals, which is lost in obesity.

Surprisingly, obese individuals exhibited less activation of reward circuits from actual food consumption (referred to as *consummatory* food reward) than lean individuals, whereas they showed greater activation of somatosensory cortical regions that process palatability when they anticipated consumption (Stice et al. 2008b). The latter observation corresponded to regions where a previous study had revealed enhanced activity in obese subject tested at baseline (non stimulation) (Wang et al. 2002) (Fig. 7d, e). An enhanced activity of regions that process palatability could make obese subjects favour food over other natural reinforcers, whereas decreased activation of dopaminergic targets by the actual food consumption might lead to overconsumption as a means to compensate for weak D2R-mediated signaling (Stice et al. 2008a). This reduced response of the reward circuitry to food consumption in obese subjects is reminiscent of the reduced DA increases triggered by drug consumption in addicted individuals when compared to non-addicted subjects.

The prefrontal cortex (PFC) plays a crucial role in executive function, including inhibitory control (Miller and Cohen 2001). These processes are modulated by D1R and D2R (presumably also D4R) and thus, the decreased activity in PFC, both in addiction and in obesity, is likely to contribute to poor control and high compulsivity. The lower-than-normal availability of D2R in the striatum of obese individuals, which has been associated with reduced activity in PFC and ACC (Volkow et al. 2008b) is therefore likely to contribute to their deficient control over food intake. Indeed, the negative correlation between BMI and striatal D2R reported in obese (Wang et al. 2001) and in overweight (Haltia et al. 2007a) individuals supports this. A better understanding of the mechanisms that lead to impaired PFC function in obesity (or addiction) could facilitate the development of strategies to ameliorate, or perhaps even reverse, specific impairments in crucial cognitive domains. For example, delay discounting, which is the tendency to devalue a reward as a function of the temporal delay of its delivery, is one of the most extensively investigated cognitive operations in relation to disorders associated with impulsivity and

compulsivity. Delay discounting has been most exhaustively investigated in drug abusers who exhibit an exaggerated preference for small-but-immediate over large-but-delayed rewards (Bickel et al. 2007). However, the few studies performed with obese individuals have also uncovered evidence of a preference for high, immediate rewards, despite an increased chance of suffering higher future losses (Brogan et al. 2010; Weller et al. 2008). And more recently, another study found a positive correlation between BMI and *hyperbolic* discounting, whereby future *negative* payoffs are discounted less than future positive payoffs (Ikeda et al. 2010). Interestingly, delay discounting seems to depend on the function of ventral striatum (Gregorios-Pippas et al. 2009) and of the PFC, including lateral OFC (Bjork et al. 2009), and is sensitive to DA manipulations (Pine et al. 2010). Specifically, enhancing DA signaling (with L DOPA treatment) increased impulsivity and temporal discounting.

5 Involvement of Motivation Circuits

Dopaminergic signaling also modulates motivation. Behavioural traits such as vigor, persistence, and investing a continued effort towards achieving a goal, are all subject to modulation by DA acting through several target regions, including NAc, ACC, OFC, DLPFC, amygdala, dorsal striatum, and ventral pallidum (Salamone et al. 2007). Dysregulated DA signaling is associated with enhanced motivation to procure drugs, a hallmark of addiction, which is why drug-addicted individuals often engage in extreme behaviours to obtain drugs, even when they entail known severe and adverse consequences (Volkow and Li 2005). Because drug taking becomes the main motivational drive in drug addiction (Volkow et al. 2003), addicted subjects are aroused and motivated by the process of obtaining the drug but tend to become withdrawn and apathetic when exposed to non-drug-related activities. This shift has been studied by comparing the brain activation patterns occurring with exposure to conditioned cues with those occurring in the absence of such cues. In contrast to the decreases in prefrontal activity reported in detoxified cocaine abusers when not stimulated with drug or drug cues [see review (Volkow et al. 2009a)], these prefrontal regions become activated when cocaine abusers are exposed to craving-inducing stimuli (either drugs or cues) (Grant et al. 1996; Volkow et al. 1999a; Wang et al. 1999). This result is reminiscent of the observation that cocaine abusers, studied shortly after an episode of cocaine binging, showed an increase in metabolic activity in OFC and ACC (also dorsal striatum) that was associated with craving (Volkow et al. 1991).

Moreover, when the responses to i.v. MP are compared between cocaine-addicted and non-addicted individuals, the former responded with increased metabolism in ventral ACC and medial OFC (an effect associated with craving), while the latter showed the opposite response, namely decreased metabolism in these regions (Volkow et al. 2005). This suggests that the activation of these prefrontal regions with drug exposure may be specific to addiction and associated with the enhanced desire for the drug. In addition, a study that prompted cocaine-addicted

subjects to purposefully inhibit craving when exposed to drug cues showed that those subjects who were successful at inhibiting craving displayed decreased metabolism in medial OFC (which processes motivational value of a reinforcer) and NAc (which predicts reward) (Volkow et al. 2009b). These findings further corroborate the involvement of OFC, ACC, and striatum in the enhanced motivation to procure the drug seen in addiction.

Predictably, the OFC has also been implicated in attributing salience value to food (Grabenhorst et al. 2008; Rolls and McCabe 2007), helping to assess its expected pleasantness and palatability as a function of its context. PET studies with FDG to measure brain glucose metabolism in normal weight individuals reported that exposure to food-cues increased metabolic activity in OFC, which was an effect associated with the perception of hunger and the desire for food (Wang et al. 2004). The enhanced OFC activation by the food stimulation is likely to reflect downstream dopaminergic effects and participate in DA's involvement in the drive for food consumption. The OFC plays a role in learning stimulus-reinforcement associations and conditioning (Cox et al. 2005; Gallagher et al. 1999), supports conditioned-cue elicited feeding (Weingarten 1983), and probably contributes to overeating irrespective of hunger signals (Ogden and Wardle 1990). Indeed, dysfunction of the OFC has been linked to overeating (Machado and Bachevalier 2007).

In spite of some inconsistencies among studies, brain imaging data also support the notion that structural and functional changes in brain regions implicated in executive function (including inhibitory control) may be associated with high BMI in otherwise healthy individuals. For example, an MRI study done in elderly women, using voxel-based morphometry, found a negative correlation between BMI and gray matter volumes (including frontal regions), which, in the OFC, was associated with impaired executive function (Walther et al. 2010). Using PET to measure brain glucose metabolism in healthy controls, we reported a negative correlation between BMI and metabolic activity in DLPFC, OFC, and ACC. In this study, the metabolic activity in prefrontal regions predicted the subjects' performance in tests of executive function (Volkow et al. 2009c). Similarly, a nuclear magnetic resonance (NMR) spectroscopic study in healthy middle age and elderly controls showed that BMI was negatively associated with the levels of N-acetyl-aspartate (a marker of neuronal integrity) in frontal cortex and ACC (Gazdzinski et al. 2008; Volkow et al. 2009c).

Brain imaging studies comparing obese and lean individuals have also reported lower gray matter density in frontal regions (frontal operculum and middle frontal gyrus) and in post-central gyrus and putamen (Pannacciulli et al. 2006). Another study, found no differences in gray matter volumes between obese and lean subjects, however, it did record a positive correlation between white matter volume in basal brain structures and waist to hip ratios, a trend that was partially reversed by dieting (Haltia et al. 2007b). Interestingly, cortical areas, like the DPFC and OFC that are involved in inhibitory control, have also been found to become activated in successful dieters in response to meal consumption (DelParigi et al. 2007), suggesting a potential target for behavioural retraining in the treatment of obesity (and also in addiction).

6 Involvement of Interoceptive Circuitry

Neuroimaging studies have revealed that the middle insula plays a critical role in cravings for food, cocaine, and cigarettes (Bonson et al. 2002; Pelchat et al. 2004; Wang et al. 2007). The importance of the insula has been highlighted by a study that reported that smokers with damage to this region (but not control smokers who had suffered extra-insular lesions) were able to stop smoking easily and without experiencing either cravings or relapse (Naqvi et al. 2007). The insula, particularly its more anterior regions, is reciprocally connected to several limbic regions (e.g., ventromedial prefrontal cortex, amygdala, and ventral striatum) and appears to have an interoceptive function, integrating the autonomic and visceral information with emotion and motivation, thus providing conscious awareness of these urges (Naqvi and Bechara 2009). Indeed, brain lesion studies suggest that the ventromedial PFC and insula are necessary components of the distributed circuits that support emotional decision-making (Clark et al. 2008). Consistent with this hypothesis, imaging studies consistently show differential activation of the insula during craving (Brody et al. 2009; Goudriaan et al. 2010; Naqvi and Bechara 2009; Wang et al. 1999). Accordingly, the reactivity of this brain region has been suggested to serve as a biomarker to help predict relapse (Janes et al. 2010).

The insula is also a primary gustatory area, which participates in many aspects of eating behaviours, such as taste. In addition, the rostral insula (connected to primary taste cortex) provides information to the OFC that influences its multimodal representation of the pleasantness or reward value of incoming food (Rolls 2008). Because of the involvement of the insula in the interoceptive sense of the body, in emotional awareness (Craig 2003) and in motivation and emotion (Rolls 2008), a contribution of insular impairment in obesity could be expected. Indeed, gastric distention results in activation of the posterior insula, which is likely to reflect its role in the awareness of body states (in this case of fullness) (Wang et al. 2008). Moreover, in lean, but not in obese subjects, gastric distention resulted in activation of the amygdala and deactivation of the anterior insula (Tomasi et al. 2009). The lack of amygdala response in obese subjects could reflect a blunted interoceptive awareness of bodily states linked with satiety (full stomach). Even though the modulation of insular activity by DA has been poorly investigated, it is recognised that DA is involved in the responses to tasting of palatable foods that are mediated through the insula (Hajnal and Norgren 2005). Human imaging studies have shown that tasting palatable foods activated the insula and midbrain areas (DelParigi et al. 2005; Frank et al. 2008). However, the DA signaling may also be necessary for sensing the calorie content of food. For example, when normal weight women tasted a sweetener with calories (sucrose), both the insula and dopaminergic midbrain areas became activated, whereas tasting a calorie-free sweetener (sucralose) only activated the insula (Frank et al. 2008). Obese subjects exhibit greater insular activation than normal controls when tasting a liquid meal that consists of sugar and fat (DelParigi et al. 2005). In contrast, subjects who have recovered from anorexia nervosa show less activation in the insula when tasting

sucrose and no association of feelings of pleasantness with insular activation as observed in the normal controls (Wagner et al. 2008). When combined, these results make it likely that dysregulation of the insula in response to taste stimuli might be involved in the impaired control of various appetitive behaviours.

7 The Circuitry of Aversion

As mentioned before, training (conditioning) on a cue that predicts reward leads to dopaminergic cells firing in response to the prediction of reward, and not to the reward itself. On the other hand, and consistent with this logic, it has been observed that dopaminergic cells will fire *less than normal* if the expected reward fails to materialise (Schultz et al. 1997). Cumulative evidence (Christoph et al. 1986; Lisoprawski et al. 1980; Matsumoto and Hikosaka 2007; Nishikawa et al. 1986) points to the habenula as one of the regions that controls the decreases in firing of dopaminergic cells in VTA that may follow the failure to receive an expected reward (Kimura et al. 2007). Thus, an enhanced sensitivity of the habenula, as a result of chronic drug exposures, could underlie a greater reactivity to drug cues. Indeed, activation of the habenula, in cocaine-addicted subjects, has been associated with behavioural relapse to drug taking upon cue exposure (Brown et al. 2011; Zhang et al. 2005). In the case of nicotine, the $\alpha 5$ nicotinic receptors in the habenula appear to modulate the aversive responses to large doses of nicotine (Fowler et al. 2011); and the $\alpha 5$ and $\alpha 2$ receptors in the habenula are implicated in nicotine withdrawal (Salas et al. 2009). Because of the habenula's opposite response to that of DA neurons to reward (deactivation) and its activation upon exposure to aversive stimuli, we refer here to the habenula signaling as one conveying an "antireward" input.

The habenula appears to play a similar role with regards to food reward. A highly palatable food diet can induce obesity in rats, with the weight increases correlating with increases in μ-opioid peptide binding in the basolateral and basomedial amygdala. Interestingly, the medial habenula showed significantly higher μ-opioid peptide binding (by approximately 40%) after exposure to the palatable food in the rats that gained weight (those that consumed more food) but not in those that did not (Smith et al. 2002). This suggests that the habenula may be involved in overeating under conditions of availability of palatable food. Moreover, neurons in the rostromedial tegmental nucleus, which receive a major input from the lateral habenula, project to VTA DA neurons and are activated after food deprivation (Jhou et al. 2009). These findings are consistent with a role for the habenula in mediating responses to aversive stimuli or states such as those that occur during dieting or drug withdrawal.

The involvement of the habenula as an antireward hub within emotional networks is consistent with prior theoretical models of addiction that postulated sensitized anti-reward responses (mediated through enhanced sensitivity of the amygdala and increased signaling though the corticotropin releasing factor) as

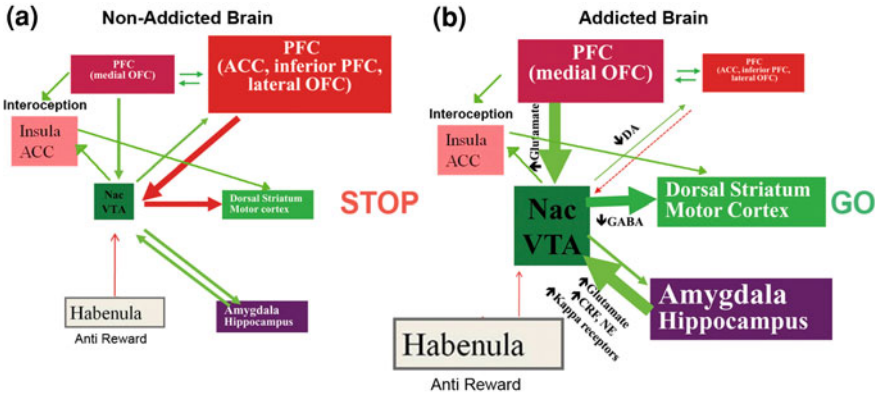

Fig. 8 Model proposing a network of interacting circuits, disruptions which contribute to the complex set of stereotypic behaviours underlying drug addiction and chronic overeating: reward (nucleus accumbens, VTA, and ventral pallidum), conditioning/memory (amygdala, medial OFC for attribution of saliency, hippocampus, and dorsal striatum for habits), executive control (DLPFC, ACC, inferior frontal cortex, and lateral OFC), motivation/drive (medial OFC for attribution of saliency, ventral ACC, VTA, SN, dorsal striatum, and motor cortex). Nac, nucleus accumbens, interoception (Insula and ACC), and aversion/avoidance (Habenula). **a** When these circuits are balanced, this results in proper inhibitory control and decision making. **b** During addiction, when the enhanced expectation value of the drug in the reward, motivation, and memory circuits overcomes the control circuit, favouring a positive-feedback loop initiated by the consumption of the drug and perpetuated by the enhanced activation of the motivation/drive and memory circuits. These circuits also interact with circuits involved in mood regulation, including stress reactivity (which involves the amygdala, hypothalamus, habenula) and interoception (which involves the insula and ACC and contributes to awareness of craving). Several neurotransmitters are implicated in these neuroadaptations, including glutamate, GABA, norepinephrine, corticotropin-releasing factor, and opioid receptors. CRF, corticotropin-releasing factor; NE, norepinephrine. Modified with permission from Volkow et al. (2011b)

driving drug intake in addiction (Koob and Le Moal 2008). Similar antireward responses may also contribute to excessive food consumption in obesity.

8 Pathological Drug and Food Reward: An Updated Working Model

The ability to resist the urge to use a drug or eat past the point of satiety requires the proper functioning of neuronal circuits involved in top-down control to oppose the conditioned responses that predict reward from ingesting the food/drug and the desire to ingest the food/drug. Here, we highlighted six of these circuits: reward/saliency, conditioning/habits, inhibitory control/executive function, motivation/drive, interoception, and aversion avoidance/stress reactivity (Fig. 8). Based on the imaging data presented here, we postulate that it is the

discrepancy between the expectation for the drug/food effects (conditioned responses) and the blunted neurophysiological effects that maintains the taking of drugs or the overconsumption of foods in an attempt to attain the expected reward. Also, whether tested during early or protracted periods of abstinence/dieting, addicted/obese subjects show lower D2R in striatum (including NAc), which are associated with decreases in baseline activity in frontal brain regions implicated in salience attribution (orbitofrontal cortex) and inhibitory control (ACC and DLPFC), whose disruption results in compulsivity and impulsivity. Finally, evidence has also been emerging on the role of interoceptive and aversive circuitry in the systemic imbalances that result in the compulsive consumption of either drugs or food.

As a consequence of the sequential disruption in these circuits, individuals may experience 1) an enhanced motivational value of the drug/food (secondary to learned associations through conditioning and habits) at the expense of other reinforcers (secondary to decreased sensitivity of the reward circuit), 2) an impaired ability to inhibit the intentional (goal-directed) actions triggered by the strong desire to take the drug/food (secondary to impaired executive function) that result in compulsive drug/food taking, and 3) enhanced stress reactivity and aversive avoidance that results in impulsive drug taking to escape the aversive state.

This model suggests a multipronged therapeutic approach to addiction designed to decrease the reinforcing properties of drug/food, reestablish/enhance the rewarding properties of natural reinforcers, inhibit conditioned learned associations, enhance motivation for non-drug/food-related activities, decrease stress reactivity, improve mood, and strengthen general-purpose inhibitory control.

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Nonhuman Primate Models of Addiction and PET Imaging: Dopamine System Dysregulation

Robert W. Gould, Linda J. Porrino and Michael A. Nader

Abstract This chapter highlights the use of nonhuman primate models of cocaine addiction and the use of positron emission tomography (PET) imaging to study the role of individual differences in vulnerability and how environmental and pharmacological variables can impact cocaine abuse. The chapter will describe studies related to the dopamine (DA) neurotransmitter system, and focus primarily on the D2-like DA receptor, the DA transporter and the use of fluorodeoxyglucose to better understand the neuropharmacology of cocaine abuse. The use of nonhuman primates allows for within-subject, longitudinal studies that have provided insight into the human condition and serve as an ideal model of translational research. The combination of nonhuman primate behavior, pharmacology and state-of-the-art brain imaging using PET will provide the foundation for future studies aimed at developing behavioral and pharmacological treatments for drug addiction in humans.

Keywords Animal models · PET imaging · Dopamine · D2-like receptors · Fluorodeoxyglucose (FDG) · Nonhuman primates

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1 Introduction

Drug dependence remains a consistent societal problem resulting in deleterious consequences on an individual's health, work, and family that resonates throughout communities worldwide and bears with it an overwhelming financial burden (WHO 2004). In the United States alone, over 20 million people over the age of 12 met the DSM-IV criteria for drug abuse or dependence in 2008 (translating into nearly 1 in every 15 people; SAMHSA 2009) including 1.6 million cocaine users (SAMHSA 2010). Within the European Union, 56% of all countries reporting on cocaine trends documented increases (WHO 2004). Although numerous advances have been made to improve our understanding of addiction including effectively demonstrating addiction is a brain disease, treatment for numerous addictions, including psychostimulant abuse have remained elusive. Development of successful treatment relies on a strong understanding of the neurobiological etiology of addiction. The goal of the current review is to describe the influence of environmental, physiological, and pharmacological factors contributing to changes in the dopamine (DA) neurotransmitter system across various correlates of the addiction cycle including vulnerability, maintenance, abstinence, and relapse to cocaine addiction, as assessed via positron emission tomography (PET) imaging in nonhuman primate (NHP) models.

1.1 Positron Emission Tomography and Dopamine Neurotransmission

PET is an imaging technique used to visualize and quantify the interaction of a radiolabelled molecule of known structure within an organism in a noninvasive manner. The present focus will be the utility of PET neuroimaging to characterize

the DA neurotransmitter system and its malleability following physiological, environmental, or pharmacological manipulations in NHPs. Briefly, the DA system is comprised of four neuronal pathways originating from midbrain nuclei with projections to various brain structures (see Beaulieu and Gainetdinov 2011 for review). The nigrostriatal pathway innervates the dorsal striatum (caudate-putamen) and is involved in motor control. The mesolimbic pathway projects to the ventral striatum (nucleus accumbens) and other limbic structures including the amygdala, hippocampus, and cingulate gyrus and mediates actions related to reward, reinforcement, emotion, and motivation. The mesocortical pathway innervates frontal cortical regions and is implicated in learning and memory. Lastly, the tuberoinfundibular pathway projects to the hypothalamus and influences anterior pituitary gland function. Dysregulation of the mesencephalic DA system through neurodegeneration or pharmacological insult can contribute to a number of disease states in addition to Parkinson's Disease, including depression, attention-deficit/hyperactivity disorder (ADHD), schizophrenia, and addiction (for reviews see Vallone et al. 2000; Beaulieu and Gainetdinov 2011).

There are two superfamilies of DA receptors, the D1-like and D2-like G-protein coupled-receptors, originally distinguished by their ability to stimulate and inhibit adenylyl cyclase activity, respectively. D1-like receptors are primarily located postsynaptically whereas D2-like receptors are located pre- and post-synaptically functioning as autoreceptors as well as post-synaptic effectors. There are currently no D1-like PET studies in NHP models of cocaine abuse. The D2-like superfamily consists of D_2, D_3, and D_4 receptor subtypes, which have been investigated in PET studies with [^{18}F]N-methylspiperone (NMSP), [^{11}C]raclopride and [^{18}F]fluoroclebopride (FCP), among other tracers. Much of our research utilizes [^{18}F]FCP, which binds with high affinity at D2-like receptors (Mach et al. 1996) to allow examination DA-rich region such as the basal ganglia (Fig. 1a). As it relates to cocaine addiction, the D_3 receptor subtype has received recent attention because its expression is limited to limbic regions. However, D_3 receptor-selective radioligands have only recently become available for PET studies and will not be described in this review. Also located on presynaptic DA nerve terminals are DA transporters (DAT) that function to transport synaptic DA intracellularly, where it can be repackaged in vesicles through the action of the vesicular monoamine transporters (VMAT) or degraded by catechol-O-methyltransferase (COMT; for reviews see Vallone et al. 2000; Beaulieu and Gainetdinov 2011), although the main pathway for intracellular DA catabolism is mediated by monoamine oxidase (MAO; Kopin 1985). In this review, we will describe studies using [^{18}F](+)-N-(4-fluorobenzyl)-2β-propanoyl-3β-(4-chlorophenyl)tropane (FCT) and [^{18}F]-[8-(2-fluoroethyl)-2β-carbomethoyx-3β-(4-chlorophenyl)nortropane] (FECNT) to label the DAT. PET studies examining VMAT and COMT will not be reviewed.

Another utility for PET imaging is the in vivo investigation of the consequences of dopaminergic activity in the central nervous system using tracers that can measure blood flow or energy use, as a means of measuring neural activity. [^{15}O]H_2O for example, is a marker of blood flow and can be used to characterize the acute effects of a pharmacological stimulus. Another tracer is [^{18}F]-fluorodeoxyglucose (FDG)

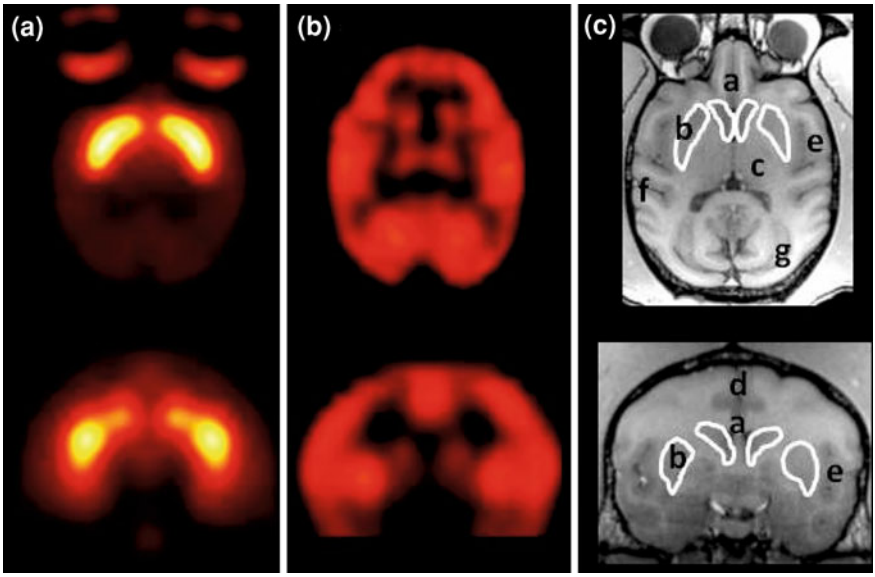

Fig. 1 PET imaging in NHPs**.** FCP binding to D2-like receptors in the basal ganglia of an anesthetized monkey (**a**), FDG uptake occuring over 40 min of responding maintained by 190-g pellet delivery measuring cerebral glucose utilization in cortical and subcortical regions (**b**), and corresponding MR. **c** Each scan occurred in a single rhesus monkey; *a* caudate nucleus; *b* putamen; *c* thalamus; *d* anterior cingulate cortex; *e* insular cortex; *f* temporal cortex; *g* occipital cortex; *top* horizontal; *bottom* coronal slices

which is an analog of glucose, the sole energy substrate in the brain under normal conditions. Increased rates of uptake of FDG reflect increases in local energy use and, in turn, functional activity. These methods are intended for the evaluation of manipulations that occur over relatively short time frames (e.g., drug administration or brief behavioral task) with changes in rates of glucose utilization calculated by comparison to scans obtained during baseline conditions. For example, Fig. 1b highlights FDG uptake in cortical and subcortical regions in a rhesus monkey. FDG was administered prior to the start of a baseline motor task. Following 40 min of behavior during which each response resulted in pellet delivery, the monkey was sedated and FDG uptake was examined via PET. In addition, FDG methods can be used to assess differences in basal brain glucose metabolism associated with disease states or phenotypes. Altered basal rates of glucose utilization generally have longer time courses (hours, days, and weeks), and reflect intrinsic and ongoing changes in brain activity representing the cumulative effects of experience with the internal and external milieu (e.g., Borghammer et al. 2010). For a more comprehensive list of PET radiotracers targeting the DA and serotonin systems and their contributions to the field of addiction studies in NHPs see Howell and Murnane (2011).

Cocaine is a psychomotor stimulant that binds with near equal affinity to the DAT, serotonin and norepinephrine transporter (SERT and NET, respectively) but

its reinforcing effects are thought to be mediated mainly through DAT blockade, and subsequent elevations of extracellular DA, as first evidenced by in vivo microdialysis studies in rodents (Di Chiara and Imperato 1988). Radiolabelled cocaine ([^{11}C]-cocaine) was tested in baboons in the late 1980s, showing substantially higher binding in DAT-rich striatal regions, thus providing a noninvasive method for visualizing the binding site for cocaine within the CNS (Fowler et al. 1989). Further, [^{11}C]-cocaine receptor availability in striatum decreased following pretreatment with unlabeled cocaine and other DAT inhibitors, but not administration of NET or SERT inhibitors providing evidence of predominately DAT-specific binding (Fowler et al. 1989). Other displacement studies have involved administering compounds known to elevate DA during a PET study with a tracer that competes with DA. In one example using FCP (Mach et al. 1997), several psychomotor stimulants (including cocaine) displaced the tracer in the basal ganglia (caudate nucleus and putamen) in an orderly fashion similar to DA elevations seen in microdialysis studies. In addition, Kimmel et al. (2008) noted an inverse relationship between reinforcing effects of cocaine analogs, quantified as the peak number of injections during self-administration (SA), and the time to peak uptake of the same [^{11}C]-labeled cocaine analogs. Together, these studies demonstrated the binding site of cocaine within the CNS, biodistribution and pharmacokinetic differences between psychostimulants, and correlations of PET with behavioral measures to provide data relevant to understanding abuse liability and pharmacotherapeutic development for potential DAT inhibitors (see Murnane and Howell 2011).

1.2 Nonhuman Primates as Research Subjects

The development of new imaging modalities has made in vivo small animal imaging using rodents an exciting line of research. For this review, however, we will focus only on PET imaging research utilizing NHPs. As it relates to translational research, NHPs are more phylogenetically related to humans and, along with baboons, Old World macaques (rhesus, *Macaca mulatta*, and cynomolgus, *M. fascicularis*) are the closest relatives of humans approved for biomedical research in the United States. Macaques have close homology to humans in terms of developmental and aging processes, neurotransmitter distribution, and complex social and cognitive behavioral repertoires (see Weerts et al. 2007 for review). For example, humans and NHPs share greater than 95% overall gene homology and greater than 98% homology in monoaminergic transporters (Hacia et al. 1998; Miller et al. 2001). Further, documented differences in DA neuron innervation (Berger et al. 1991; Joel and Weiner 2000) and affinity of DA for receptors between monkey and rodent (Weed et al. 1998) may be indicative of other differences in drug biodistribution, pharmacokinetic or pharmacodynamic interactions within the DA system (e.g., Lyons et al. 1996; Roberts et al. 1999; Lile et al. 2003). Using animal models with neurotransmitter distribution and receptor

localization resembling that in humans is critical when using PET imaging to generalize preclinical results to the human condition (Nader and Czoty 2008). An additional advantage of NHP research is the ability for longitudinal study designs within a laboratory setting. Baseline behavioral, neurochemical, and hormonal measures can be correlated with changes following an experimental manipulation (e.g., chronic drug administration) while controlling for such factors as stress and nutrition over many years. As it relates to drug addiction, the longer the history of drug exposure, the stronger the face validity of the animal model.

NHPs have an extensive and complex behavioral repertoire. Macaques form a linear social hierarchy that can be quantified by measuring the number of agonistic interactions between monkeys (e.g., Kaplan et al. 1982). Typically, the most dominant monkey aggresses toward all other animals and has access to the largest allotment of resources whereas the most subordinate monkey does not aggress, submits and avoids more dominant monkeys, spends more time alone, and has less access to resources. We describe this continuum as one in which the living conditions of dominant monkeys can be considered an enriched environment while subordinate monkeys are exposed to an environment of chronic social stress. This continuum allows an examination of how social and environmental variables (i.e., acute or chronic stress and enrichment) affect neuroendocrine function (Czoty et al. 2009a; Riddick et al. 2009) or neurobiology influencing vulnerability to self-administer drugs (Morgan et al. 2002; Czoty et al. 2004; Nader and Czoty 2005). These studies provide direct translational applicability toward understanding how social stressors may influence the propensity to initiate drug use, relapse during abstinence, or conversely, positively influence sustained abstinence.

Another advantage presented by NHP models is their capacity for learning complex cognitive tasks similar to those administered to humans. Human cocaine abusers show signs of cognitive impairments on tasks measuring strategic planning, such as the ability to withhold or modify a behavioral response, working memory and measures of impulsivity (Fillmore and Rush 2002; Bolla et al. 2004; Hester and Garavan 2004; Goldstein et al. 2007, 2010); similar impairments have been shown in monkeys following cocaine SA (Liu et al. 2008, 2009; Porter et al. 2011). Cognitive impairments have been hypothesized to perpetuate a cycle of compulsive drug use and increase the prevalence of relapse (Goldstein and Volkow 2002; Koob and Volkow 2011). Therefore, understanding when (duration or dose) and how (neurobiological alterations) cocaine affects executive function is integral in developing effective treatment strategies. Longitudinal studies examining cognitive performance in monkeys allows for the assessment of environmental (e.g., stressors) or pharmacological agents that can be assessed using PET (e.g., Porrino et al. 2005; Hampson et al. 2009, 2011).

Often overlooked until recently, sex differences are emerging as an important variable in preclinical (e.g., Lynch 2006; Terner and de Wit 2006) and clinical settings (Zilberman et al. 2003; O'Brien and Anthony 2005; Greenfield et al. 2010). Similar to humans, female macaques have a $\sim$28 day estrous cycle marked by similar fluctuations in hormone levels, notably those of estrogen and progesterone (e.g., Dukelow et al. 1979). Influence of hormones on various drug-related

behaviors (e.g., reinforcing or discriminative stimulus effects) and effects of drugs on hormonal regulation are empirical questions beginning to be delineated (see Mello and Mendelson 2009; Evans and Foltin 2010 for reviews). For example, the relationship between menstrual cycle phase and D2-like receptor availability has been examined in women, with three different results. Using [^{18}F]NMSP, Wong et al. (1988) observed a trend toward lower striatal uptake during the follicular versus luteal phase, indicating either lower D2-like receptor densities or higher striatal DA concentrations during the follicular phase. In a second study from this group, [^{11}C]raclopride binding potential was lower in the putamen (but not caudate nucleus or ventral striatum) in women during the luteal versus follicular phase (Munro et al. 2006). In contrast, Nordström et al. (1998) observed no menstrual-cycle dependent variation in [^{11}C]raclopride availability in the putamen of five women. Of course, there are many factors that could influence D2-like measures, so it is not surprising that in different populations of women, the interaction between menstrual cycle phase and D2-like receptor availability is complicated. In experimentally naïve female macaques, though, the relationship was quite straightforward and orderly (Czoty et al. 2009b). In that study using a within-subjects design in which seven female cynomolgus monkeys were scanned in the follicular and luteal phases of the menstrual cycle, D2-like receptor availability using [^{18}F]FCP was significantly lower during the follicular phase compared to the luteal phase. As will be described in greater detail, D2-like receptor availability is intimately related to vulnerability to self-administer drugs of abuse.

1.3 Animal Models

There are several advantages to using animal models, including the ability to perform experiments that are ethically or practically impossible in humans. For example, as it relates to addiction, the use of animal models allows for imaging the brain of individuals prior to any drug exposure, so as to permit the assessment of trait variables (i.e., whether that brain measure represents a pre-existing characteristic that is predictive of an outcome) and state variables (i.e., whether the independent variable was associated with a change in brain function; see Nader and Czoty 2005 for further discussion). Before discussing animal models of cocaine addiction and brain imaging studies, it is worthwhile to first describe the types of animal models (see also Katz and Higgins 2003).

At its simplest level, there are two methods for animals to receive drugs of abuse: non-contingent, i.e., administered by the experimenter, or contingent, i.e., self-administered by the animal. The choice of methods depends on the research question. If the researcher simply wants to know where in the brain a drug is binding, then non-contingent administration will suffice. This has been used with great success as part of ligand-development studies. However, if the researcher wants to understand the role of environment, the organism's phenotype and environment x organism interactions mediating drug abuse, then contingent drug SA studies are

necessary. Differences in response to contingent vs. non-contingent cocaine include the pattern and magnitude of DA efflux measured via microdialysis (Bradberry 2000; Lecca et al. 2007), HPA axis responsivity (Galici et al. 2000), and lethality (Dworkin et al. 1995), supporting the notion that addiction is more complex than just neurobiological changes resulting from drug-receptor interactions. In humans, drug abuse is a chronic condition, such that long-term SA studies in NHPs provide a homologous model. Equally important, SA paradigms allow animals to self-administer at rates and intakes that provide an index of individual differences, in contrast to fixed experimenter-administered regimens that may affect neurobiology differently.

The distinction between contingent and non-contingent drug administration can also be considered in terms of models of "formal equivalence" (Carlton 1983). As described by Katz and Higgins (2003), these are models developed to simulate a specific symptom of the human disease. In short, these types of models have established "face validity"—the endpoint in animals resembles some aspect of the human condition. Chronic drug administration impacts on receptors in the brain, resulting in (perhaps) long-term changes in brain function that can be modeled in animals and imaged using the same instruments as those used for humans. In terms of face validity, as will be described below, chronic drug administration is certainly more relevant to human addiction than, for example, acute drug treatments, but SA has higher face validity than non-contingent drug administration.

2 Models of Cocaine Addiction and DA Receptor Function

Drug SA models have been used for decades to examine the reinforcing effects of compounds (e.g., Woolverton and Nader 1990; Mello and Negus 1996), including examination of potential pharmacotherapies. Animal drug SA resembles human drug use (Griffiths et al. 1980) and produces parallel neurobiological effects (e.g., Beveridge et al. 2008; Everitt et al. 2008). For example, in monkeys, chronic cocaine SA induced long-lasting changes in the mesolimbic DAergic system (Moore et al. 1998; Letchworth et al. 2001; Nader et al. 2002, 2006; Porrino et al. 2007) and altered cerebral blood flow, metabolism, and function (Lyons et al. 1996; Beveridge et al. 2006) in a manner similar to what has been observed in human cocaine users (Strickland et al. 1993; Volkow et al. 1993).

2.1 Models of Vulnerability

As mentioned above, perhaps the best use of animals, including NHPs, in models of addiction involves assessing phenotypic traits that render an individual more or less vulnerable to addiction. Currently, among the best examples of the use of imaging to study trait variables have involved vulnerability to drug abuse in relation to the DA D2-like receptor. In humans, Volkow et al. (1999) reported an

inverse relationship between D2-like receptor availability and subjective effects of intravenous methylphenidate (MP). In that study, 23 non-drug abusing males were scanned with [^{11}C]raclopride in a drug-free condition, and at another time were administered MP and asked to rate its subjective effects. Volkow et al. (1999) found that men with lower D2-like binding potentials found MP "pleasant", while men with higher D2-like binding potentials found the same dose to be "noxious". This suggests that individuals with low D2-like receptor availability would be more vulnerable to stimulant abuse than individuals with high D2-like measures. This same inverse relationship was observed in male rhesus monkeys (Nader et al. 2006). In that study, 12 experimentally naïve monkeys were scanned with [^{18}F]FCP and then trained to respond on a lever maintained by banana-flavored food pellets. When responding was stable, the reinforcer was changed from banana-flavored pellets to 0.2 mg/kg intravenous cocaine. Over the first 10 weeks of exposure, cocaine-maintained responding was higher in monkeys with lower D2-like receptor measures compared to monkeys with higher baseline D2-like receptor availability. Taken together, these two findings support the hypothesis that low D2-like receptor availability makes individuals more vulnerable for stimulant abuse. The use of NHP models in the latter study controlled for many variables that exist in humans, such as past drug use, stress, etc. However, the use of NHPs can also be used to determine if these D2-like receptor measures are malleable to environmental and pharmacological variables.

If D2-like receptor availability influences vulnerability, can these levels be modified (increased or decreased) before exposure to cocaine and thereby alter vulnerability? The answer appears to be "yes". In one study, baseline PET scans were conducted in individually housed male cynomolgus monkeys prior to random placement in social groups of four monkeys/pen (Morgan et al. 2002). As mentioned above, macaques form linear hierarchies, with the most dominant monkeys (#1) winning all fights, being groomed more, having access to the entire pen, etc., while the most subordinate monkeys (#4) being the target of most aggression, they are groomed least and are frequently used as a model of socially derived stress. Morgan et al. (2002) reported no relationship between initial D2-like receptor measures and eventual social rank, indicating that D2-like receptor availability is not a trait marker for social rank. However, D2-like receptor availability changed significantly after stable social hierarchies were formed and the change occurred in the dominant monkeys. On average, D2-like receptor availability increased by 20% after monkeys became dominant. This increase in D2-like receptor availability subsequently protected the monkeys from the initial reinforcing effects of cocaine (Morgan et al. 2002). In fact, not only did subordinate monkeys, who had the lower D2-like receptor measures, self-administer cocaine at higher rates compared to dominant animals, the dominant monkeys, with their elevated D2-like receptors, didn't find cocaine reinforcing. These two monkey studies highlight an important role for D2-like receptors in vulnerability to cocaine abuse and suggest viable targets for pharmacotherapy development.

An important consideration that will require much future research involves the use of female subjects. The human and monkey research described above involved

males. We have recently replicated the findings above using initially individually housed female cynomolgus monkeys who were then randomly assigned to social groups of four monkeys/pen. We found similar results with D2-like receptor availability as seen in males—D2-like receptors at baseline were not trait markers for eventual social rank, but these measures increased significantly in females who became dominant. However, the relationship between D2-like receptor availability and vulnerability to cocaine reinforcement was opposite. Dominant females, with higher D2-like receptor measures, acquired cocaine reinforcement at lower doses compared to subordinate females who had low D2-like receptor availability (Nader et al. under review). These findings suggest that treatment strategies that target the D2-like receptor should be opposite in males and females. For example, drugs that increase D2-like receptor availability would be hypothesized to be effective in males, but would likely fail in females. Clearly, more research involving females is required to validate this conjecture.

2.2 Models of Maintenance

Drug addiction is characterized as a chronic, relapsing condition. Thus, it is imperative for our animal models to incorporate long-term chronic drug administration. The use of NHPs is particularly advantageous in this regard, because monkeys can be used in intravenous drug SA studies for many years, and can self-administer extremely high doses of drug that better model the human condition compared to rodent models. In male monkeys, we found that long-term cocaine SA produced robust decreases in D2-like receptor availability as assessed with [^{18}F]FCP and PET (Nader et al. 2006). The mechanism(s) for this reduction in D2-like receptor availability may be (1) receptor down-regulation, (2) elevations in extracellular DA concentrations or (3) both. Without additional studies, it is impossible to determine which mechanism mediates the effects we are observing. There have been, however, several studies by multiple groups that can provide important information related to the mechanisms involved in the reductions in D2-like availability. Our group has conducted terminal in vitro receptor autoradiography studies confirming that level of protein (i.e., D2-like receptor density) is significantly lower in monkeys with a cocaine SA history compared to control monkeys (Moore et al. 1998; Nader et al. 2002). Concerning the second possible mechanism, acute cocaine elevates extracellular DA (Czoty et al. 2000), and acute methamphetamine reduced the apparent affinity of [^{11}C]-raclopride (Doudet and Holden 2003) consistent with increased interstitial DA levels, but it is unlikely that chronic cocaine SA produces long-lasting elevations since Bradberry (2000) reported within-session tolerance to cocaine-induced elevations in DA. Another method used in conjunction with PET imaging is the co-administration of a "cold" compound that can reduce extracellular DA concentrations, thereby increasing the likelihood that the assessment of receptor availability will occur under conditions of low competition with DA. Using baboons, Dewey et al. (1992) conducted two studies in the same animal on the same day to investigate GABA-mediated

inhibition of DA release. The first PET study of the day was a baseline measure of D2-like receptor availability using [^{11}C]raclopride. The second PET study was preceded by administration of either the benzodiazepine lorazepam or the indirect-acting GABA agonist gamma-vinyl-GABA (GVG); both compounds should decrease extracellular DA concentrations. Consistent with their hypothesis, administration of lorazepam and GVG increased D2-like receptor availability by nearly 50 and 30%, respectively. The PET studies showing reductions in D2-like availability following cocaine SA included the use of lorazepam in order to decrease DA concentrations (Nader et al. 2006).

Up to this point, we could summarize our findings as follows: in males, low D2-like receptor availability appears to increase vulnerability to cocaine abuse and that continued cocaine use results in further decreases in D2-like receptor availability, perhaps developing a spiral cycle toward addiction (Koob and Le Moal 2000). Another question that can be explored with PET involves "drug seeking" vs. "drug taking". Volkow et al. (1993) noted that there was no relationship between D2-like receptor function and her subjects' typical cocaine dose, but there was a significant correlation between D2-like binding potential and duration of cocaine use. To better explore this relationship, we conducted a study in 12 experimentally naïve male rhesus monkeys in which baseline D2-like receptor availability and DAT availability was determined in all monkeys prior to cocaine access (Czoty et al. 2007). Monkeys were then trained to self-administer cocaine under a very lean schedule of reinforcement—a fixed-interval 30 min schedule of 0.03 mg/kg cocaine. The cocaine dose was low and the contingency was such that the first response after 30 min resulted in a cocaine injection; sessions ended after 2 injections. Thus, monkeys were "drug seeking" every day, but were not self-administering very high doses. Monkeys self-administered cocaine under these conditions for approximately 9 weeks and were then rescanned. The mean cocaine intake over that time was approximately 5 mg/kg. There were no effects of cocaine SA on D2-like or DAT availability in the caudate nucleus and putamen—two regions that have been shown to be sensitive to environmental and pharmacological manipulations (e.g., Morgan et al. 2002; Czoty et al. 2005; Nader et al. 2006). Such findings suggest that during acquisition, the pharmacological effect of cocaine, and not drug seeking per se, is the critical determinant of decreases in D2-like receptor availability.

Howell and colleagues have extended the use of PET to examine relationships between receptor occupancy and the ability of drugs to decrease cocaine SA (e.g., Lindsey et al. 2004; Howell et al. 2007). In one study (Lindsey et al. 2004), several drugs that bind to the DAT were substituted for cocaine in rhesus monkeys trained to SA drugs under a second-order schedule of reinforcement. These same drugs were also tested for their ability to decrease cocaine SA. In a final experiment, DAT occupancy was measured by administering behaviorally active doses during a PET study with the DAT ligand [^{18}F]FECNT. These investigators found that selective DAT inhibitors required high DAT occupancy (between 50% and 90%) to reduce cocaine SA and to function as reinforcers; such information will be critical for the development of long-acting compounds that reduce cocaine abuse and that have low abuse liability.

2.3 Models of Abstinence and Relapse

The previous sections have documented a relationship between D2-like receptor availability and vulnerability and changes during chronic cocaine exposure. Of critical relevance is whether these changes in D2-like receptor availability persist during abstinence. Volkow et al. (1993), using [^{18}F]NMSP, reported significantly lower D2-like binding potentials in abstinent cocaine abusers compared to control subjects. These investigators were only able to examine abstinence periods up to 4 months; all subjects had relapsed by then. Although it would be several years later before a potential inverse relationship between D2-like receptor availability and vulnerability would be identified, the authors speculated that it was possible the lower D2-like receptor measures during abstinence were due to a predisposition of individuals with low D2-like receptor availability to use cocaine, rather than a consequence of chronic cocaine exposure. The studies described in the previous two sections indicate that low D2-like receptor availability increases vulnerability to use drugs and that chronic cocaine use further decreases D2-like receptor function (in males). The use of NHPs has allowed for long-term study of SA and permits the study of abstinence beyond the 4 months reported in human cocaine abusers.

Nader et al. (2006) examined recovery of D2-like receptor availability in male rhesus monkeys that had been self-administering cocaine. In one study, short access to cocaine reduced D2-like receptor availability, but recovery occurred in all monkeys within 1–3 weeks. Five animals were studied in abstinence after 1 year of cocaine SA; D2-like receptor availability was reduced by $\sim$20% in all monkeys. For three monkeys, there was complete recovery of [^{18}F]FCP signal within 3 months of abstinence. However, in two monkeys, there was no recovery of D2-like receptor availability after 1 year of abstinence. There were no differences in the amount of cocaine self-administered over the 1 year or in the rate of cocaine SA between monkeys that showed D2-like receptor recovery and those that did not. However, there were subtle behavioral differences that may have predicted a lack of recovery (see Nader et al. 2006). The main point is that the combination of behavioral pharmacology and PET imaging provided novel and important information that may guide future research into environmental and/or pharmacological modifications of D2-like receptor availability.

3 Functional Sequelae of Cocaine Administration

3.1 Blood Flow and Metabolic Responses to Cocaine-Related Stimuli

Physiological and functional changes can be measured via PET in response to environmental (e.g., conditioned stimuli) or pharmacological (e.g., cocaine) challenges that may contribute to repeated drug use, cognitive decline and relapse.

Howell et al. (2001) trained drug-naïve rhesus monkeys to lie still in a head and body restraint system to allow examination of cerebral blood flow (CBF) in the awake, unstressed condition. An acute non-contingent cocaine injection increased cerebral blood flow primarily in the dorsolateral prefrontal cortex (PFC), in a dose-dependent manner, measured with [^{15}O]H_2O-PET (Howell et al. 2002). Further, pretreatment with the SERT inhibitor alaproclate blocked the cocaine-induced elevation in CBF (Howell et al. 2002).

Using a within-subject design and FDG-PET, Henry et al. (2010) examined the effects of a single non-contingent cocaine dose compared to saline, on glucose metabolism prior to and following a history of cocaine SA in rhesus monkeys. In this study, cocaine-induced elevations in glucose metabolism were restricted to the PFC in cocaine-naïve monkeys. Following 60 sessions of limited access (1 h) cocaine SA, the same non-contingent cocaine injection increased glucose metabolism in the PFC and also striatum (primarily dorsal striatum). Following an additional 60 sessions of extended access (4 h) cocaine SA, cocaine increased glucose metabolism throughout the PFC and striatum. This pattern of activation was diminished following 1 month of cocaine abstinence, compared to the response in the cocaine-naïve state. This study showed a progressive expansion of cocaine's effects from cortical to mesolimbic regions with extended access to cocaine in a pattern similar to studies using the [^{14}C]-deoxyglucose autoradiographic technique (2DG method; see Porrino et al. 2007 for review). However, FDG-PET revealed progressive increases in glucose metabolism following non-contingent cocaine exposure, whereas results from 2DG studies showed progressive decreases in these dopaminergic-rich brain regions. The differences in directional effects may be due to differences in reinforcement schedule, dose, or timing of drug administration and data collection between studies.

While the previous studies examined non-contingent administration of cocaine, similar activation patterns were shown when rhesus monkeys were allowed to self-administer cocaine during PET image acquisition (Howell et al. 2010). In addition to the PFC and striatum the anterior cingulate cortex (ACC) was metabolically more active following cocaine SA, a region widely shown to be hypoactive during executive function tasks in recent cocaine users (e.g., Bolla et al. 2004; Hester and Garavan 2004; Kubler et al. 2005; Tomasi et al. 2007a, b). Further, when saline was substituted for cocaine during a subsequent PET scan such that only the conditioned stimuli (e.g., lights) were present, activation of the dorsomedial PFC was still present. Brain activation patterns in these NHP studies utilizing awake PET imaging during cocaine SA or extinction conditions are similar to those induced by acute cocaine injections (Breiter et al. 1997) or cocaine-related visual cues (e.g., Wilcox et al. 2011) in humans measured with functional MRI. Such functional responses to drug-related cues are in part attributed to increased DA release determined via displacement of [^{11}C]-raclopride and PET imaging in humans (Volkow et al. 2006). These studies present a neurobiological mechanism underlying drug stimuli-induced craving that may contribute to compulsive drug use and relapse in humans. PET studies in NHPs may be utilized to assess novel, putative pharmacotherapies to block the CNS response to cocaine-related stimuli during abstinence to aid in relapse prevention.

FDG-PET can also be used to examine the effects of various environmental influences on drug-related behavior. For example, preliminary FDG-PET data from our laboratory have shown that glucose metabolism is differentially altered following a stressful encounter with an unknown monkey based on social rank. Dominant monkeys showed increased glucose metabolism in mesocorticolimbic regions and chose fewer cocaine injections and more food pellets in a food-drug choice procedure following this encounter, whereas their subordinate counterparts showed reduced metabolism in mesolimbic regions and an increased preference for cocaine. This model can be used to assess neurobiological differences influencing subsequent behavior based on different environmental histories.

3.2 Models of Cognitive Decline

One of the hallmark consequences of cocaine addiction is a disruption of cognitive behavior. In human cocaine users, fMRI studies complement PET studies and have provided new opportunities to examine specific brain function underlying cognitive tasks. Recent fMRI studies have shown differences associated with impaired executive function compared to control groups across cognitive domains such as behavioral inhibition, cognitive flexibility, updating or working memory, and measures of impulsivity (Fillmore and Rush 2002; Bolla et al. 2004; Hester and Garavan 2004; Goldstein et al. 2007, 2010; Woicik et al. 2011). Despite strong evidence supporting cocaine-induced cognitive impairments, studies cannot discount cognitive predispositions such as heightened impulsivity or risk-taking behavior that may lead to drug use.

Recent studies have used FDG-PET to identify the substrates underlying cognitive behavior. For example, Porrino et al. (2005) used a delay match-to-sample (DMS) task as a measure of working memory in NHPs. In this task monkeys are shown a 'sample' image followed by a delay of varying length (1–30 s). The monkeys are then shown an array of 2–8 images from which they must 'match' the one from the sample phase of the task for a juice reward. The task ranges in difficulty with short intervals and a small number of images during the match phase (low cognitive load) to long intervals with a high number of images (high cognitive load). Performance was associated with increases in glucose utilization in the hippocampal region, dorsal striatum, and the dorsolateral prefrontal cortex. When cocaine was substituted for juice as the reward, there was a dose-dependent decline in performance particularly in high-load trials that was associated with increased activation in the dorsolateral PFC. The basis for the increased activation was a disruption in the firing patterns of dorsolateral PFC neurons recorded in the same animals under the same conditions (Hampson et al. 2011).

NHPs can be trained to perform tasks probing specific cognitive domains known to be impaired in human cocaine users. Following establishment of a stable cognitive baseline, cocaine SA can be initiated. The effects of acute and chronic drug exposure, or abstinence from cocaine on cognitive performance can

be systematically examined, controlling for both intake and duration. Cocaine SA in monkeys brought about impairments in stimulus discrimination and reversal learning, response inhibition, measures of impulsivity, and working memory (Liu et al. 2008, 2009; Porter et al. 2011). In another paradigm of similar design, sleep deprivation impaired percent accuracy on a DMS task and was accompanied by reductions in glucose metabolism in working memory-related cortical areas (Porrino et al. 2005). Administration of the ampakine, CX717 or orexin-A attenuated the deleterious effects of sleep deprivation on both percent accuracy and glucose metabolism (Porrino et al. 2005; Deadwyler et al. 2007; Hampson et al. 2009). Putative cognitive enhancers aimed to attenuate cocaine-induced hypoactive brain function are also being tested in monkeys and humans. For example, recent cocaine users showed deficits in an attention task and showed lower activation within the ACC compared to controls (Goldstein et al. 2010). Following administration of methylphenidate, ACC activity and percent accuracy improved, demonstrating the attenuation of functional alterations underlying cognitive deficits via a pharmacological treatment. The generalizability of methylphenidate to enhance cocaine-associated neural and cognitive deficits has yet to be examined across other cognitive domains.

4 Summary

This review has attempted to briefly highlight the use of NHP models of cocaine addiction and in vivo brain imaging using PET to better understand the neuropharmacology of drug addiction. While we describe key PET studies using DAT tracers and FDG, the focus on DA D2-like receptors has allowed for an assessment of a potential trait marker, which was also influenced by environmental, social and pharmacological variables. D2-like receptor availability influenced vulnerability to cocaine abuse in a manner that was orderly and quite malleable to environmental or pharmacological manipulations. Certainly future studies must examine how other neurotransmitter and neurohormone systems change in concert with brain DA systems in these NHP models.

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PET Applications in Animal Models of Neurodegenerative and Neuroinflammatory Disorders

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Abstract Studies on hereditary neurological disorders such as familial Alzheimer's disease (AD) have revealed abnormalities of pathogenic proteins causative of neurodegeneration, while molecular initiators of sporadic neuropsychiatric conditions remain unidentified. Such disorders are characterized by collections of molecular abnormalities that may be critically involved in synaptic dysfunctions and other deteriorations in neurons. Diverse classes of radiochemicals designed for positron emission tomographic (PET) imaging facilitate delineation of mechanistic links among key molecules in these processes by tracking their spatiotemporal correlations. This assay technique is of particular utility when applied to rodent and nonhuman primate models given their suitability for invasive genetic and pharmacological interventions. In addition, the detection of neurochemical and neuropathological changes by PET can be examined in laboratory animals when combined with invasive antemortem and postmortem investigations such as in vivo microdialysis, electrophysiological and histopathological techniques. This review primarily covers the use of small animal models of brain disorders using PET to elucidate etiological molecular cascades to facilitate in turn the search for diagnostic and therapeutic agents applicable to AD and related disorders in humans.

Keywords Positron emission tomography (PET) · Alzheimer's disease · Transgenic mouse · Amyloid β peptide · Tau protein · Translocator protein · Imaging · Rodent model · Neurodegenerative disorders · Psychiatric diseases

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Contents

1 Introduction

Researchers have taken advantage of positron emission tomographic (PET) imaging technologies in both nonclinical and clinical in vivo studies primarily because they offer an excellent alignment of results between laboratory animals and humans. This approach also opens an avenue to bidirectional translational research between bench and clinic through a feedback loop of pathological and pharmacological information. The limited availability of biopsy specimens and essential need for assessing penetration of drugs across the blood–brain barrier lend rationale to the application of PET to central nervous system assays in humans, while animal studies benefit from the capability of PET to measure neuronal activities and neurotransmissions and to monitor longitudinally brain pathologies in the same individuals, thereby enhancing statistical power.

In relation to small laboratory animal research, the latest progress in PET imaging instruments offer the precise mapping of radiotracer distribution in rodent brains with an in-plane spatial resolution approximating 1.5 mm (Fig. 1a–d) (Tai et al. 2005). This technology is of particular benefit to the use of genetically engineered mice and rats for proving specific interactions between radioligands and target molecules by overexpressing and/or knocking down such binding components, and for pursuing pathophysiological events downstream from the genetic modification. It is also noteworthy that the throughput of PET scans may be somewhat improved by utilizing mice, as dynamic PET data of four mice can be acquired in a single session (Fig. 1e). Furthermore, estimation of rate constants for transfer of a radiotracer between plasma and brain tissue and association with, and dissociation from, specific and nonspecific binding sites has been successful in PET imaging of mouse brains on the basis of compartment models requiring a metabolite-corrected arterial input function (Seki et al. 2008). Effects of neuroactive drugs possibly influenced by anesthetics are also assessable by small-animal PET devices with the use of conscious rats (Tokunaga et al. 2009) and mice (Mizuma et al. 2010). Congenic and inbred rodents with minimal or no genetic variations tend to produce less interindividual variability in levels of endogenous components and consequently kinetic parameter values for exogenous tracers than do primates, including humans. These technical merits notwithstanding, however,

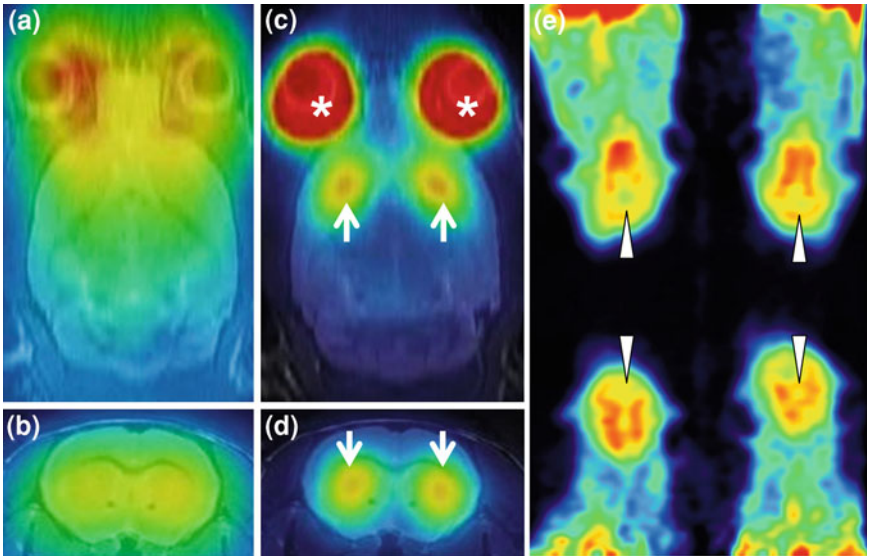

Fig. 1 Horizontal (**a**, **c**) and coronal (**b**, **d**) images of wild-type mouse brains acquired by Hamamatsu SHR7700 (**a**, **b**) and Siemens microPET Focus 220 (**c**, **d**) scanners with in-plane spatial resolutions of 2.6 and 1.3 mm, respectively, full-width at half-maximum at the center of the field of view. Images were generated by averaging dynamic PET data at 0–90 min after intravenous injection of a radioligand for the D2 dopamine receptor, [^{11}C]raclopride, and are superimposed on an MRI template. Despite concentrated radioactivity in the Harderian glands (*asterisks*), intense in vivo radiolabeling of the striatum (*arrows*) is detectable with a Focus 220 system. **e** Simultaneous scanning of four mice with a Focus 220 scanner. Images were constructed as an average of dynamic data collected at 0–30 min after intravenous injection of [^{11}C]PIB. Radiotracer uptake in the brain is indicated by *arrowheads*

rodent PET imaging may not be a full replacement for non-human primate assays prior to clinical applications, in consideration of interspecies heterogeneities of immunity, life span, higher brain functions and several other biological factors. Quantitative determination of radioprobe kinetics could be hampered in small rodents due to 'mass effects', which are primarily attributable to the occupancy of target and off-target binding elements by unlabeled tracer compounds (Hume et al. 1998; Kung and Kung 2005), and are inevitable unless scans with a very small mass dose are enabled by a profound enhancement of either specific radioactivity or an increased sensitivity of PET detectors.

Pharmacokinetic characterization of potential diagnostic and therapeutic compounds with PET is generally performed by injecting radiolabeled variants of these chemicals or measuring competition between test chemicals and established radioprobes. This assay can be conducted in normal animals if target molecules are expressed in a physiological condition. More specifically for neurodegenerative conditions, probes producing positive signals by capturing pathological aggregates of misfolded proteins/peptides and inflammatory glia can be characterized by the

use of model animals with or without genetic modifications. In the interim, these models are also applicable to evaluations of the capability of radiochemicals for negative signs of pathologic injuries such as loss of neurons, neuroreceptors, neurotransmitter transporters and energy metabolism. This approach can be contrasted with models of psychiatric illnesses, of which positive and negative biological signs in the brain remain largely elusive or are relatively subtle. However, PET research on animals together with candidate genetic and other etiological culprits of mental disorders may reveal neurochemical consequences of these abnormalities, and such alterations of neurotransmission components are potentially relevant as endophenotypes of psychiatric diseases (Gottesman and Gould 2003; Martinez et al. 2001).

2 Visualization of Fibrillar Protein Assemblies

A wide variety of neurodegenerative disorders can be neuropathologically characterized by either extracellular or intracellular deposition of proteinaceous fibrils (Forman et al. 2004). Senile plaques and neurofibrillary tangles, composed of amyloid β peptide (Aβ) and tau protein, respectively, are histopathological hallmarks in Alzheimer's disease (AD), while neuronal and glial tau inclusions feature in a subset of non-AD neurodegenerative diseases collectively referred to as tauopathies (Higuchi et al. 2002a). Genetic mutations of a precursor of Aβ, amyloid precursor protein (APP), and catalytic subunits of an enzyme responsible for Aβ production, presenilin-1 (PS1) and presenilin-2, are causative of familial AD (Chartier-Harlin et al. 1991; Rogaev et al. 1995; Sherrington et al. 1995), and tau gene mutations have been discovered in family members of hereditary non-AD tauopathy termed frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Hutton et al. 1998; Poorkaj et al. 1998; Spillantini et al. 1998), indicating pivotal roles of Aβ, tau and other fibrillogenic molecules in the neurodegenerative pathway. The demonstration in autopsied brains that accumulation of senile plaques and neurofibrillary tangles is initiated decades before the symptomatic onset of AD (Price and Morris 1999) supports the significance of these fibrillar lesions as primary targets of early diagnostic and disease-modifying therapeutic approaches. However, it is yet to be clarified whether amounts of total aggregates or certain assembly forms are intimately associated with neurotoxicity.

A notable property of these pathological fibrils is that constituent proteins are homologously oligomerized and polymerized through formation of a β-pleated sheet as a secondary structure (Berriman et al. 2003). Radiolabeled small molecules binding to β-sheets are accordingly applied to the visualization of such aggregates in living brains (Cai et al. 2007). Although the vast majority of β-sheet ligands exhibit high in vitro affinity for diverse self-assemblies of synthetic Aβ peptides and recombinant tau proteins, only a restricted subgroup of these compounds is capable of interacting with tau depositions in the brain (Okamura et al. 2005; Maruyama et al. 2009; Fodero-Tavoletti et al. 2011). In addition, selectivity of β-sheet ligands

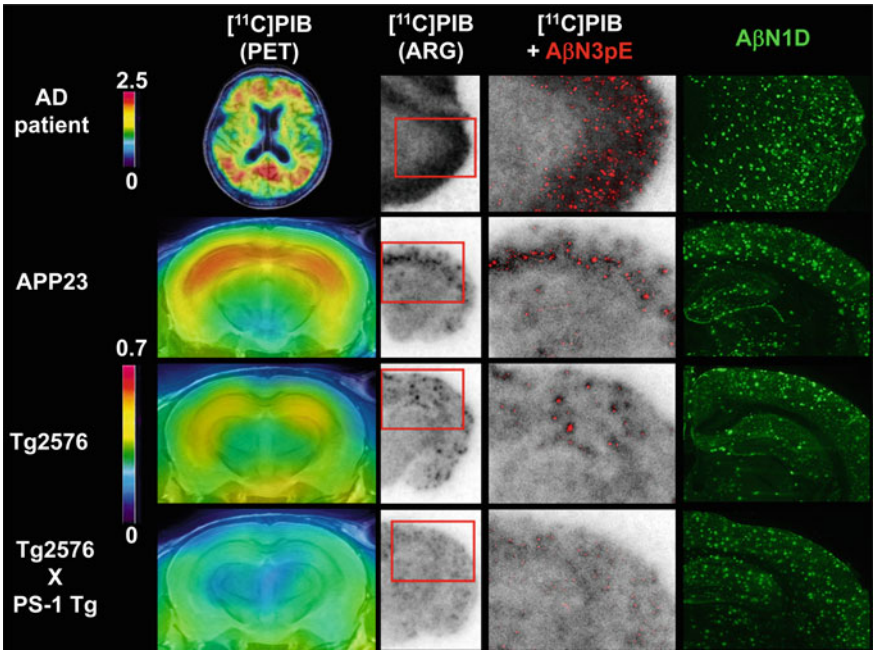

Fig. 2 Preferential binding of [^{11}C]PIB to AβN3pE aggregates characteristic of AD pathologies. Scale bars indicate binding potential for amyloid deposits in an AD patient and transgenic (Tg) mice modeling plaque formation, including APP23 (23 months old), Tg2576 (23 months old) and double PS-1/APP (8 months old) transgenics. The human PET image is superimposed on an individual MR image, and mouse PET images on an MRI template. Frozen sections of human and mouse brains used for in vitro autoradiography with [^{11}C]PIB were subsequently used for immunohistochemical mapping of AβN3pE and N-terminally intact Aβ, AβN1D

for subspecies of Aβ and tau is frequently indicated by comparative studies in diseased patients and animal models. High-contrast imaging of senile plaques in AD patients by PET with ^{11}C-labeled Pittsburgh Compound-B ([^{11}C]PIB) was first reported by Klunk et al. 2004. However, other more recent [^{11}C]PIB-PET studies have failed to detect Aβ deposits with a high contrast in APP transgenic mice (Toyama et al. 2005), which overexpress APP and developed amyloid (Tg2576; Hsiao et al. 1996), as well as mice doubly transgenic for mutant APP and PS-1 (Klunk et al. 2005), which show accelerated Aβ fibrillogenesis (Holcomb et al. 1996). By contrast, we have found detectable plaque lesions in several different mouse models using [^{11}C]PIB-PET, including Aβ deposition in an aged APP transgenic line (Sturchler-Pierrat et al. 1997; Maeda et al. 2007a; Fig. 2). Indeed, a longitudinal PET assay of the same APP23 mice illustrated intensification of plaque signals with aging (Maeda et al. 2007a). By contrast, in vivo binding of [^{11}C]PIB to aggregated Aβ in Tg2576 and APP/PS1 double transgenic mice was only marginally detectable in animals of 24 months of age (Fig. 2).

Binding potential based on specific binding compared to nondisplaceable uptake for [^{11}C]PIB was quantified with dynamic PET scan data, and was approximately 0.2 and 0.6 in the neocortex at 18 and 26 months of age, respectively, being linearly proportional to age during this period. These values much exceeded those in Tg2576 ($\sim$0.3 at 26 months) and APP/PS1 double transgenic (<0.1 at 9 months) mice, but were far below levels in the neocortex of AD patients, which reportedly approximated 1.3–1.5 in the frontal cortex (Price et al. 2005; Lopresti et al. 2005). Moreover, binding of [^{11}C]PIB to the cerebral cortices was shown to reach a plateau at a prodromal stage of AD often referred to as mild cognitive impairment (MCI) (Engler et al. 2006), unlike the continuous elevation of [^{11}C]PIB signals throughout the lifetime of APP23 mice (Maeda et al. 2007a). The distinct characteristics of amyloid ligand binding among different species and transgenic strains were overtly discrepant with the biochemical results demonstrating that amounts of total Aβ and two major C-terminal variants of Aβ ending at Val40 (Aβ40) and Ala42 (Aβ42) in insoluble protein fractions extracted from brains of all examined APP transgenic lines were equally abundant and were greater than those in AD patients (Kawarabayashi et al. 2001; Klunk et al. 2005; Maeda et al. 2007a).

We subsequently sought to identify the main component(s) of binding sites for [^{11}C]PIB, as accumulation of such elements has been hypothesized to be unique to the continuum from pathological aging to AD in humans (Findeis 2007; Gunn et al. 2010). Combined autoradiographic and immunohistochemical analyses were performed to assess the association of N-terminal and C-terminal heterogeneities of Aβ with [^{11}C]PIB binding sites. The levels and distribution of an Aβ subtype lacking two N-terminal amino acid residues and beginning with pyroglutamate (pGlu3), AβN3pE, were found to correlate with those of [^{11}C]PIB signals (Fig. 2) (Maeda et al. 2007a). As expected from biochemical assays of brain homogenates, Aβ40 and Aβ42 were not consistently co-localized with binding sites for [^{11}C]PIB in humans and APP transgenics. The preferential binding of [^{11}C]PIB to AβN3pE was further supported by an in vitro binding assay with synthetic Aβ peptides (Maeda et al. 2007a). Thus, we postulated that this N-terminal variant of Aβ was a primary element of binding components for [^{11}C]PIB.

The significance of AβN3pE as a major component of AD plaques was documented by histopathological studies (Saido et al. 1995), followed by reports by different groups demonstrating fibrillogenic and neurotoxic propensities of this Aβ subtype (He and Barrow 1999; Russo et al. 2002; Schilling et al. 2006), and biochemical assays of AD and transgenic mouse brains (Kawarabayashi et al. 2001). Incorporation of the PS1 mutation into Tg2576 mice induced no signal intensification in [^{11}C]PIB-PET despite an enhanced accumulation of total Aβ and Aβ42, which might amplify disproportionate deposition of N-terminally unprocessed Aβ to AβN3pE. Such PIB-negative Aβ plaques are also characteristic of Tg2576 and APP23 mice at 9–15 months, and correspond to the transition from normal to pathological aging of human brains. Similarly, linear increases of [^{11}C]PIB-PET signals with aging in these mouse models at 15 months or older resembles the progression of pathological aging toward MCI. This notion may be

justified by the fact that no marked neuronal loss is observable in most APP transgenic lines during their lifetime (Van Dam et al. 2005). Accumulation of AβN3pE and emergence of [^{11}C]PIB signals predate overt cognitive impairment in humans (Mintun et al. 2006; Rowe et al. 2010), which contrasts to the antecedence of abnormal electrophysiological and behavioral phenotypes to the formation of AβN3pE- and [^{11}C]PIB-positive plaques in APP transgenics (Huang et al. 2006). In these contexts, APP transgenic models may not be of utility in elucidating roles played by Aβ subtypes deleterious to neuronal functionality and viability in aged human brains.

PET studies in different animal species and transgenic strains have also provided insights into the development of animal models with Aβ pathologies. Aβ is primarily degraded by endopeptidases such as neprilysin (Iwata et al. 2000), while a small subset of Aβ undergoes N-terminal truncation and modification resulting in production of AβN3pE (Cynis et al. 2008). This N-terminal processing step is a minor pathway of Aβ metabolism requiring decades for an accumulation of AβN3pE to a [^{11}C]PIB-positive level, but may be accelerated in the event of downregulated neprilysin activity (Miners et al. 2008). To test this notion, Saido and coworkers have recently established APP23 mice deficient in neprilysin, and their preliminary results indicate a selective increase in AβN3pE in these animals relative to APP23 mice. Consistent with this biochemical finding, our pilot [^{11}C]PIB-PET assays have illustrated augmented radioligand binding to the neocortex and hippocampus of the double mutant mice, supporting the views that [^{11}C]PIB preferentially binds to AβN3pE aggregates, and that manipulation of Aβ metabolism could lead to advances in the in vivo modeling of AD through the use of genetically engineered animals.

A number of researchers have developed PET ligands to visualize fibrillar tau lesions in vivo (Okamura et al. 2005; Maruyama et al. 2009; Fodero-Tavoletti et al. 2011). However, rather disappointedly, only a limited class of β-sheet ligands exhibit high-affinity for tau inclusions. An ^{18}F-labeled amyloid probe, 2-(1-{6-[(2-[^{18}F]fluoroethyl)-(methyl)amino]-2-naphthyl}ethylidene)malononitrile ([^{18}F]FDDNP), was developed to bind to both plaques and tangles in AD brains (Small et al. 2006), although it remains inconclusive whether [^{18}F]FDDNP provides a sufficiently sensitive detection of AD pathologies at an early stage (Thompson et al. 2009). In addition, compounds reported to possess high selectivity for tau lesions versus Aβ deposits (Okamura et al. 2005; Maruyama et al. 2009; Fodero-Tavoletti et al. 2011) have not been used in humans. Since the concurrence of Aβ and tau depositions in AD brains hampers separate in vivo assessments of selective binding of radioprobes into Aβ and tau pathologies, animals developing tau inclusions may serve to establish the reactivity of candidate chemicals with their specific targets. Multiple lines of mice transgenic for human tau with FTDP-17 mutations were found to harbor tau aggregates typically stained with fluorescent chemicals such as thioflavin-S and 1-fluoro-2,5-bis(3-carboxy-4-hydroxystyryl)benzene (FSB) (Fig. 3) (Lewis et al. 2000; Higuchi et al. 2002b; Santacruz et al. 2005; Yoshiyama et al. 2007). However, the majority of β-sheet ligands capable of capturing AD neurofibrillary tangles do not bind to these transgenic tau lesions, suggesting a structural distinction between tau

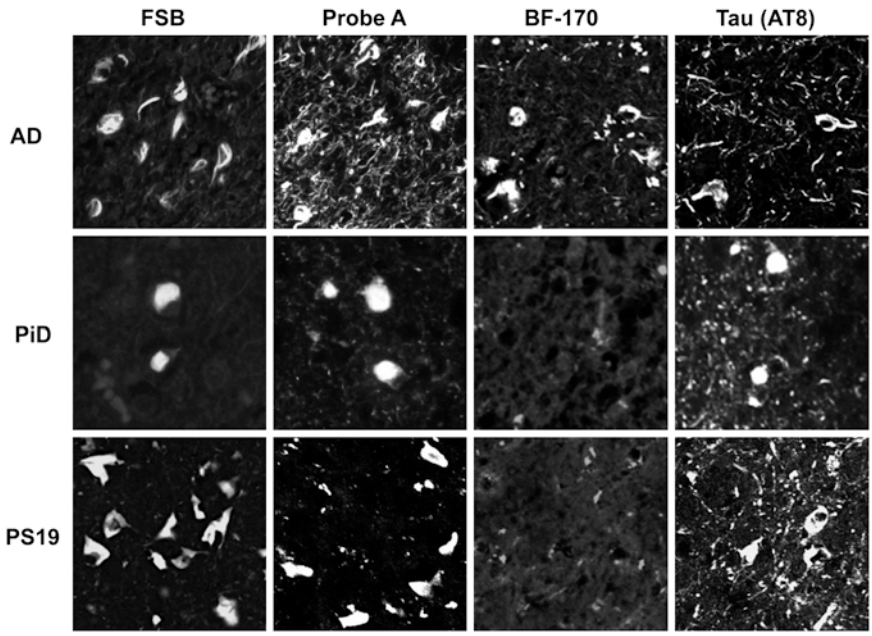

Fig. 3 Fluorescence staining of frontal cortex slices from patients with AD and Pick's disease (PiD) and brain stem slices from a PS19 tau transgenic mouse. Separate samples were reacted with FSB and a new tau ligand designated as Probe A, and were doubly labeled with a tau-selective compound, BF-170, and monoclonal antibody against phosphorylated tau, AT8. Tau inclusions in all tested samples are illuminated with FSB and Probe A, while BF-170 only recognized tau lesions in AD

assemblies generated in AD and mouse brains (Fig. 3) (Okamura et al. 2005; Maruyama et al. 2009). This discrepancy might be attributed to the composition of tau isoforms incorporated in fibrillar aggregates, as most transgenic mouse strains overexpress a single tau isoform with three or four microtubule-binding repeat domains, unlike the assembly of all six tau isoforms into fibrils in AD (Higuchi et al. 2002a). Consistent with this notion, many tangle-binding compounds do not show affinity for intraneuronal and intraglial tau inclusions in the majority of non-AD tauopathies, including progressive supranuclear palsy, Pick's disease and corticobasal degeneration (Fig. 3). Moreover, there is a marked overlap of compounds reactive with tau deposits in transgenic mouse models and non-AD neurodegenerative diseases (Maruyama et al. 2009). Hence, utilization of mice modeling tauopathies may facilitate the pharmacodynamic and pharmacokinetic evaluation of candidate imaging agents targeting a wide spectrum of tau pathologies and consequently enabling differentiation among diverse tauopathies including AD. Indeed, several tau transgenic mouse lines have been employed for evaluating putative tau-binding radioligands by PET (Maruyama et al. 2009; Fodero-Tavoletti et al. 2011). However, clinical PET studies are needed to establish in vivo homologies of tau

lesions in AD, non-AD tauopathies and transgenic mouse models. It should also be noted that analogs of tau probes could act as anti-aggregation drugs by blocking β-sheet formation (Taniguchi et al. 2005), a possibility that requires further rigorous evaluation in rodent models of tau pathology.

3 Imaging Biomarkers for Immune Responses to Neuropathologies

Immune signaling in the CNS is regulated by signaling between neurons and glia (Amor et al. 2010; Kettenmann et al. 2011). Indeed, activation of microglia and astrocytes is commonly observed in diverse neuropathological conditions (Eikelenboom et al. 2006; Block et al. 2007). The main role of glial cells appears to involve the removal of toxic proteinaceous deposits (Sawada 2009), and appear largely dependent on glial subpopulations and types, and the severity and stages of disorders (Maeda et al. 2007b; Serrano-Pozo et al. 2011). Activated microglia are known to express 18-kDa translocator protein (TSPO, also known as peripheral benzodiazepine receptor) at a high level, and different classes of low-molecular-weight chemicals have been applied to PET imaging of TSPO in living animal models and humans (Banati et al. 2000; Cagnin et al. 2001; Ouchi et al. 2005; Maeda et al. 2007a, b; Rojas et al. 2007). In addition, TSPO is upregulated in astrocytes responding to experimental neuronal injuries, such as ischemia and ethanol-induced tissue damages in rats (Chen et al. 2004; Maeda et al. 2007a, b; Rojas et al. 2007). Our recent work indicated that TSPO upregulation occurred in a profound manner in neurotoxic microglia contacting neurons and protective astrocytes with an enhanced production of glial cell line-derived neurotrophic factor (Ji et al. 2008). In line with this finding, tau transgenic PS19 mice developing significant neuronal loss display TSPO positivity in microglia (Yoshiyama et al. 2007; Ji et al. 2008). By contrast, APP23 mice, which exhibit an upregulation of astrocytic TSPO, do not develop abundant plaques and neuronal loss (Fig. 4) (Ji et al. 2008; Maeda et al. 2011). The basis for this finding likely reflects the fact that TSPO-PET signals in PS19 mice are more intense than those in APP23 mice (Fig. 4), and become detectable before the onset of neuronal loss (Yoshiyama et al. 2007; Maeda et al. 2011). Pronounced loss of neurons and high-level expression of TSPO in microglia have also been observed in a transgenic mouse line, which overexpresses the mutant human APP gene, resulting in the accumulation of oligomeric Aβ in neurons (Tomiyama et al. 2010). However, increased TSPO follows neuronal death in these mice, unlike the earlier onset of TSPO-positive microgliosis prior to the occurrence of neuronal loss in PS19 mice (Maeda et al. 2011). Moreover, suppression of TSPO-positive neuroinflammation in PS19 mice by oral administration of an immunosuppressant has been reported to attenuate the deposition of insoluble, hyperphosphorylated tau proteins and loss of neurons (Yoshiyama et al. 2007). These PET and neuropathological data imply that tau

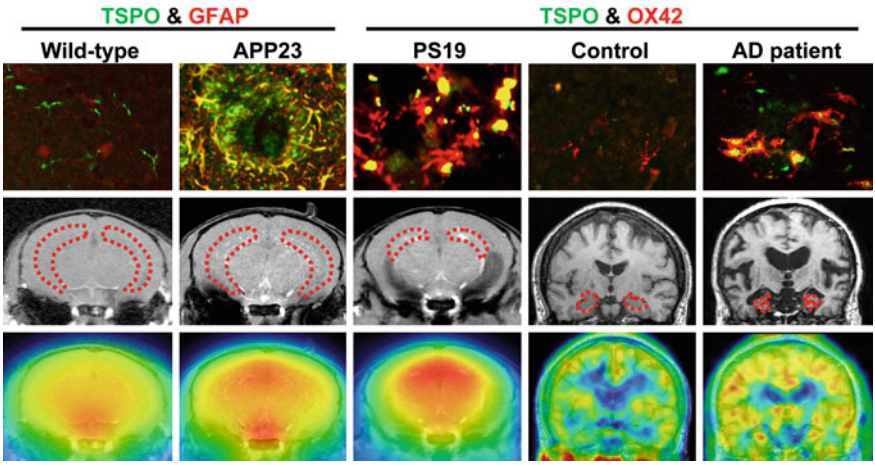

Fig. 4 Cellular localization of TSPO in neuroinflammatory conditions with or without overt neuronal loss. (*Top panels*) Double immunofluorescence staining of cortical or hippocampal sections demonstrating overlap of TSPO signals with astrocytic marker, glial fibrillary acid protein (GFAP), in an APP23 mouse and with microglial marker, OX42, in PS19 mouse and AD patient. (*Middle panels*) In vivo T1-weighted coronal magnetic resonance images containing the hippocampus (outlined by *dotted lines*). Marked hippocampal atrophy is observed in PS19 mouse and AD patient. (*Bottom panels*) Coronal PET images of TSPO superimposed on individual MR images displayed in the middle panels. Images were generated by averaging dynamic scan data at 0–60 min after injection of [^{18}F]fluoroethyl-DAA1106 in mice and at 0–90 min after injection of [^{11}C]DAA1106 in humans. PS19 mouse exhibits a notable increase in TSPO signals, exceeding the changes in APP23 mouse. TSPO is also upregulated in extensive regions of the AD brain

pathology is a strong inducer of dysfunctional microgliosis associated with TSPO upregulation.

In light of the above findings, it is conceivable that TSPO signals are intimately correlated with tau lesions in patients with neurodegenerative disorders. This view is supported by immunohistochemical localization of TSPO to microglia in the vicinity of tau aggregates in AD and non-AD tauopathy brains (Maeda et al. 2011). TSPO was also documented to increase in AD patients (Fig. 4) (Cagnin et al. 2001; Yasuno et al. 2008) and asymptomatic carriers of tau gene with an FTDP-17 mutation (Miyoshi et al. 2010) as assessed by PET with TSPO radioligands, [^{11}C]PK-11195 and [^{11}C]DAA1106. However, the elevation of binding potential for TSPO in AD was only 20–30% of the baseline level, and was detected in extensive brain regions, distinct from the spatial distribution of tau and Aβ depositions. Binding potential for TSPO in the hippocampus of aged PS19 mice was approximately 3-fold relative to normal mice imaged with an analog of DAA1106, [^{18}F]fluoroethyl-DAA1106, and 5–6-fold with a relatively new ligand, [^{11}C]Ac5216 (Maeda et al. 2011). This discrepancy of tau-induced changes of TSPO between humans and mouse models may be accounted for by the high signal in control scans. Such ‘normal’ binding of the TSPO radioprobe is also abundant in

monkeys relative to rodents, and is displaceable by co-administration of nonradioactive ligands (Maeda et al. 2004; Zhang et al. 2007). Accordingly, there is a possibility that additional cell types express TSPO in normal primate brains, and insights into TSPO induction gained from mouse models of neuroinflammation should be carefully interpreted in light of these findings.

In addition to TSPO, a number of other inflammatory biomarkers are also applicable to visualization and analysis by PET, including cyclooxygenases (de Vries et al. 2008; Shukuri et al. 2011) and type 2 cannabinoid receptors (Fujinaga et al. 2010). However, there is a paucity of information about immunohistochemical positivity for these molecules in diseased states relevant to AD. Thus, future research should be directed toward the investigation of neuroinflammatory processes using PET ligands for non-TSPO biomarkers.

4 Neurochemical Profiling and Neuronal Viability

Dysfunctional neurotransmission is the likely consequence of neurotoxic protein aggregation in neurodegenerative disorders and undoubtedly contributes to the symptomatic manifestation of such disorders. PET investigations of neurotransmitter systems in living brains support the evaluation of symptomatic treatments, which currently are the only pharmacotherapeutic approaches available to clinicians (Mangialasche et al. 2010; Gárdián and Vécsei 2010). Optimal doses of drugs acting on neuroreceptors and neurotransmitter transporters have been estimated previously by quantifying the occupancy of these targets in vivo relative to their concentration in plasma (Saijo et al. 2009). Plasma concentration of a drug yielding 50% of target occupancy in the brain (EC50) is a representative index for its potency. The utility of this approach is reflected by the high resemblance of EC50 values in humans and rodents reported recently for several radioligands (Saijo et al. 2009). However, it is noteworthy that high-affinity PET radioligands may occupy a considerable percentage of their specific binding sites, impeding accurate quantification of the occupancy by therapeutic agents (Hume et al. 1998; Klunk et al. 2005). To offset this problem it is necessary to reduce the mass of injected radiochemical by increasing the specific radioactivity coupled with the use of highly sensitive PET cameras.

Although in its infancy previous PET research in animals provides a platform for investigating neurotransmitter function in animal models of neurodegenerative disorders. Such work has gained momentum from in vitro autoradiographic imaging of brain slices, as exemplified by our studies in mice heterozygously deficient in calcium-calmodulin-dependent protein kinase IIα (Yamasaki et al. 2008). Such mice display aggressive behaviors and exaggerated infradian rhythms together with altered levels of dopamine, serotonin and glutamate receptors in the hippocampus and other brain regions (Yamasaki et al. 2008). Changes in the synaptic concentration of neurotransmitters are detectable if occupancy of binding sites by endogenous ligands can be displaced by PET radioligands with appropriate affinity

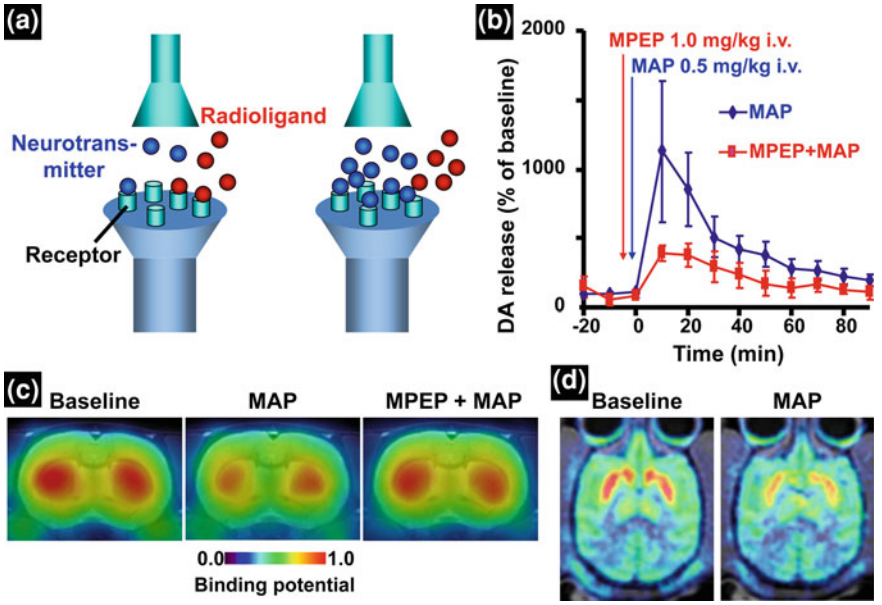

Fig. 5 PET detection of neurotransmitter release and signaling between different neurotransmitter systems. **a** Availability of neuroreceptors at baseline (*left*) and following stimulation of neurotransmitter release (*right*). These endogenous ligands compete with exogenous radiotracers for agonistic binding sites. **b** Extracellular dopamine concentrations measured in microdialysis samples obtained through a probe inserted into rat striata. The 10-fold increase in dopamine induced by the intravenous injection of methamphetamine (MAP) is profoundly attenuated by pretreatment with an antagonist for the type 5 metabotropic glutamate receptor, MPEP. **c** Coronal PET images of D2 DA receptors in a conscious rat under experimental conditions identical to the microdialysis assays. The binding of the D2 receptor agonist radioligand, [^{11}C]MNPA, is noticeably suppressed by endogenous competitors following MAP injection, an effect reversible by administration of MPEP prior to the MAP treatment. PET images are superimposed on an MRI template. **d** Horizontal [^{11}C]MNPA-PET data showing binding potential for D2 DA receptors in a conscious monkey at baseline and following administration of MAP. PET images are superimposed on individual MR images

(Fig. 5a) (Laruelle 2000). This 'endogenous blocking' effect can be validated by measuring extracellular levels of neurotransmitters with in vivo microdialysis under experimental conditions similar to those used for PET scanning. We have shown following administration of the psychostimulants amphetamine and methamphetamine in rats and monkeys that there is an approximately 10-fold increase in extracellular dopamine concentration in the striatum (Fig. 5b), which is accompanied by a near 20% decrease in the binding potential of the D2 receptor agonist radioligand, (R)-2-[^{11}C]methoxyl-N–n-propylnorapomorphine ([^{11}C]MNPA) (Fig. 5c, d) (Seneca et al. 2008; Tokunaga et al. 2009). Notably, the decline is less prominent with the use of dopamine receptor antagonists such as [^{11}C]raclopride since endogenous dopamine is less effective at competing at dopamine receptor

antagonist binding sites (Seneca et al. 2008). Physiological stimuli may lead to less manifest changes in synaptic dopamine levels, and resultant alterations of PET ligand binding might be below test–retest variabilities of the assay setting. Despite this limitation, relatively small increases in neurotransmitter release are likely to trigger a notable reduction in radiotracer binding potentials by promoting internalization of neuroreceptors, rendering binding components less accessible by the exogenous ligands (Laruelle 2000).

Combined PET, microdialysis and electrophysiological studies in experimental animals also enables an assessment of 'crosstalk' between different neurotransmitter systems. Consistent with this view, the aforementioned decline of radioligand binding to D2 receptors induced by methamphetamine in monkeys and rats was found to be reversed by the co-administration of the type 5 metabotropic glutamate receptor (mGluR5) antagonist, 6-methyl-2-(phenylethynyl)pyridine (MPEP) (Tokunaga et al. 2009), suggesting a modulatory role of glutamate and mGluR5 in the regulation of dopamine neurotransmission. To circumvent the effects of a general anesthetic agent on such processes, we have recently developed a PET protocol to investigate neurotransmitter function in awake primates and rodents (Tokunaga et al. 2009). This approach has now been advanced using a skull-mounted PET system to monitor behavior in awake, freely moving animals (Schulz et al. 2011). Such developments will undoubtedly have a major bearing on the coupling of PET with animal behavioral models of neurodegenerative and neuroinflammatory diseases.

In addition to imaging of neurotransmitter release, neuroreceptor and neurotransmitter transporters, PET can be used to investigate regional brain glucose metabolism using with the tracer [^{18}F]fluorodeoxyglucose (FDG). Uptake of FDG in the brain and its regionality are profoundly affected by the use of general anesthetic agents (Toyama et al. 2004), Nevertheless, current methodologies allow PET assays of monkeys, rats and mice without anesthesia (Obayashi et al. 2001; Tokunaga et al. 2009; Mizuma et al. 2010). However, it is yet to be clarified whether neuronal activity in a resting state with minimal stimuli can be robustly evaluated in animal models by FDG-PET. Our preliminary work has indicated that glucose metabolism remains unchanged in the hippocampus of PS19 tau transgenic mice until atrophy of this region becomes observable by volumetric MRI scans (Hattori et al. 2009). Thus, FDG uptake at baseline may reflect neuronal viability and the number of surviving neurons and there may be considerable compensation in this capacity. Similarly, diminution of glucose metabolism is less pronounced than the increases in neuroinflammatory TSPO signals in a rat model of traumatic brain injury (Yu et al. 2010). A parsimonious explanation for the lack of sensitivity of FDG-PET in such models is that attendant increases in glucose utilization by activated astrocytes may override more subtle reductions in metabolism caused by neuronal loss (Magistretti and Pallerin 1996; 1999).

5 Conclusion

The entire pathological cascade in neurological disorders can be monitored in living animal models by visualizing the accumulation of putative culprit proteins and peptides, dysregulation of neurotransmission, and intermediate molecular events linking proteinopathy, synaptopathy and neuronal death. PET offers the significant advantage of performing multiple scans in the same subject to reveal the time course of pathogenesis as well as the onset of disease-related behavioral and cognitive phenotypes. The close face and construct validity of animal models of AD provides an additional impetus for comparative interspecies PET research. The investigation of neuroreceptors by PET to infer changes about neurotransmitter release is a promising avenue of research to discover anciliary biomarkers and therapeutic targets relevant to AD and other neurodegenerative disorders. However, work is needed to establish whether PET-visible markers related to neurotransmission are robust endophenotypes in this context. The parallel application of invasive (e.g. in vivo microdialysis) and non-invasive (e.g. PET) neurochemical imaging techniques in awake, freely moving animals is expected to reveal new insights into the neurochemical basis of synaptic pathophysiology and its pharmacotherapeutic modification. Application of this experimental paradigm to genetically engineered animal models should eventually lead to the identification of pathological mechanisms upstream of psychiatric disease symptoms.

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Neural and Behavioral Endophenotypes in ADHD

Natalia del Campo, Ulrich Müller and Barbara J. Sahakian

Abstract In recent years, descriptive symptom-based approaches of attention deficit hyperactivity disorder (ADHD) have been increasingly replaced by more sophisticated endophenotype-based strategies, better suited to investigate its pathophysiological basis, which is inherently heterogeneous. Measurements derived from neuroimaging techniques such as positron emission tomography (PET) and magnetic resonance imaging (MRI) constitute endophenotypes of growing interest, capable of providing unprecedented windows on neurochemical and neuroanatomical components of psychiatric conditions. This chapter reviews the current state of knowledge regarding putative neural and behavioral endophenotypes of ADHD, across the lifespan. To this end, recent evidence drawn from molecular and structural neuroimaging studies are discussed in the light of widely accepted neuropsychological and pharmacological models of ADHD.

Keywords ADHD · Endophenotypes · Dopamine · Positron emission tomography · Magnetic resonance imaging

Contents

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1 Introduction

Attention deficit/hyperactivity disorder (ADHD) is an early onset neurobehavioral disorder characterized by symptoms of inattention, impulsivity and/or hyperactivity (Biederman 2005). The presence of at least six symptoms in either domain can result in different phenomenological subtypes, namely predominantly inattentive type, predominantly hyperactive/impulsive type and combined type. With about 3–10% of children affected worldwide, ADHD is the most prevalent pediatric disorder (Burd et al. 2003; Faraone et al. 2003; Ford et al. 2003; Solanto 2001). Follow-up studies have shown that some symptoms persist into adulthood in 30–60% of affected children (Faraone and Biederman 2005), leading to the recent reconceptualization of the disorder as a lifespan condition (Barkley 1998). In adulthood, ADHD can be highly debilitating, being often associated with substance abuse, depression, unemployment and criminal offenses when left untreated (Biederman et al. 2006; Faraone and Biederman 2005; Kessler et al. 2006; McGough et al. 2005; Molina et al. 2009; Murphy and Barkley 1996).

Without necessary or sufficient symptoms, behavioral heterogeneity is a defining characteristic of ADHD. This heterogeneity might result either from genetic heterogeneity in pathogenetic mechanisms, or from differences across patients in severity of symptoms along an underlying continuum. In recent years, descriptive symptom-based approaches in ADHD have been increasingly replaced by more sophisticated models based on endophenotypes, better suited to encapsulate this heterogeneity and investigate its hypothesized pathophysiological basis (Castellanos and Tannock 2002; Doyle et al. 2005). The term "endophenotype" as it is commonly used in psychiatry refers to a phenotype that (a) is more proximal to the etiology of a disorder than its clinical symptoms and (b) shares one or more of the same susceptibility genes with the condition it subtends (Almasy and Blangero 2001; Gottesman and Gould 2003). One of the key features of biologically based endophenotypes is that their genetic basis is less complex than that of the disorder itself, due to their relative proximity to gene actions. As a result, they confer greater statistical power to identify genetically driven neurobiological mechanisms underlying the clinical manifestation of psychiatric conditions.

Neuroimaging measurements constitute endophenotypes of great interest, capable of providing unprecedented windows on the neurobiology of psychiatric conditions. Different neuroimaging techniques enable different types of research questions to be investigated in vivo: For example, magnetic resonance imaging (MRI) provides information about morphological aspects of the brain, such as brain tissue density or connectivity, while positron emission tomography (PET) and single photon emission computed tomography (SPECT) allow the direct assessment of neurotransmitter systems in vivo, through the application of radioactively labeled tracers that bind to or are metabolized by specific neurochemical markers. Objective neuropsychological measurements constitute a further type of endophenotypes, with the added advantage that they are cheaper and easier to implement relative to neuroimaging paradigms.

The interest in identifying and characterizing ADHD endophenotypes of behavioral, anatomical and neurochemical nature is two-fold. Firstly, this approach may prove fruitful in promoting our understanding of the precise neurobiological mechanisms underlying the disorder and its treatment, thus opening the possibility of developing optimized behavioral and pharmacological treatment solutions for affected patients. Secondly, compared to clinical symptoms, endophenotypes are better suited to be used as quantitative trait loci in future ADHD linkage and association studies, given their relative proximity to the underlying biology in the chain of events leading from gene to behavior. The latter point is particularly stimulating because decades of effort to map the genetic underpinnings of ADHD (conceived as the collection of traits falling under its broad diagnostic umbrella) has yielded disappointing results. Yet, like most major psychiatric syndromes, ADHD is known to be highly heritable (see below).

This chapter reviews the present state of knowledge regarding putative neural and behavioral endophenotypes of ADHD. To this end, we integrate data from the following research areas: neuropsychology, psychopharmacology and molecular and structural neuroimaging in ADHD. Given that co-segregation with the associated illness is an inherent characteristic of endophenotype, we open the chapter with a brief overview of the literature on the genetics of ADHD.

2 Genetics

Based on family studies indicating heritability estimates of ADHD between 0.6 and 0.9 (Biederman et al. 1990; Durston 2008), genetic factors are thought to play an important role in the etiology of ADHD. Exploratory genome-wide studies have to date failed to discover one single gene contributing substantially to the manifestation of the disorder (Franke et al. 2009, 2011), implying that multiple genes are involved, each contributing only a small portion of the variance of ADHD symptoms (Kuntsi et al. 2006b).

Over the past 15 years, there has been a considerable effort to examine specific genes based on their function and perceived etiological relevance, resulting in a

large and often conflicting literature (Gizer et al. 2009). The two most frequently studied and replicated candidate genes for ADHD are the dopamine (DA) receptor type 4 (DRD4) (Brookes et al. 2006; LaHoste et al. 1996; Swanson et al. 1998) and the DA transporter gene (DAT1) (Brookes et al. 2006; Cook et al. 1995; Gill et al. 1997). Both of these candidate genes appear to represent individually only a weak risk factor whose effects are thought to unfold across development in the presence of complex interactions with other genes and adverse environmental factors (Plomp et al. 2009). Other genes for which significant associations with ADHD have been described include the noradrenergic (NA) transporter gene SLC6A2 (Kim et al. 2006) and the alpha 2 adrenergic receptor gene ADRA2A (Gizer et al. 2009), both of which are involved in the adrenergic pathway. Additionally, the ADRA2A, catechol-O-methyltransferase, DA receptor type 5 and NA transporter protein 1 genes have been significantly associated with stimulant medication response (Froehlich et al. 2010).

3 Cognitive Markers of ADHD

In their seminal comprehensive reviews, Nigg (2005) and Willcutt (2005) compared the relative magnitude of ADHD case-control differences reported in the literature on a variety of cognitive domains (reviewed in Swanson et al. 2011a). The authors concluded that: (a) only few ADHD children showed pervasive deficits across neuropsychological tests, (b) an underlying executive dysfunction was not a necessary or sufficient condition in the manifestation of ADHD. These findings contributed to the shift from the traditional core deficit view to multiple deficit theories, representing a turning point in the literature of cognitive deficits of ADHD (Swanson et al. 2011a). In recent years, computerized batteries of neuropsychological tests have been used to fully characterize the cognitive profile of ADHD, such as the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Chamberlain et al. 2010) and the Maudsley Attention and Response Suppression (MARS) (Rubia et al. 2007). A meta-analysis of case-control differences observed on prefrontal CANTAB tasks in children and adults with ADHD revealed medium-large deficits in performance in response inhibition, working memory and executive planning, and a small decrement in attentional set-shifting (Chamberlain et al. 2010), though deficits have also been observed in tests of temporal and parietal lobe function, such as pattern recognition and delayed matching to sample (Swanson et al. 2011a).

A distinct set of cognitive deficits often described in ADHD involves the abnormal processing of reward (e.g. delay aversion) and salience (Luman et al. 2005; Sonuga-Barke 2003). The simultaneous existence of deficits in top-down cognitive control processes and motivational processes (e.g. Gupta et al. 2011) supports the aforementioned multiple pathway models of ADHD pathophysiology (Nigg 2005; Sonuga-Barke 2003). Underlying alterations of distributed fronto-striato-cerebellar circuits, one of the best replicated findings in ADHD constitute a

likely neural substrate for the deficits observed in prefrontal-dependent top-down control processes, including executive functions and attention (Barkley 1997; Bush et al. 2005; Kuntsi et al. 2006a; Paloyelis et al. 2007; Pennington and Ozonoff 1996). On the other hand, a disruption of meso-cortico-limbic networks is thought to underlie motivational abnormalities in ADHD (Castellanos et al. 2006; Sonuga-Barke 2003).

4 Pharmacological Treatment

The psychostimulants methylphenidate (MPH) and amphetamine (AMPH) constitute the two main therapies of choice for the treatment of ADHD (Wilens 2008). Both drugs increase extra-synaptic DA and NA levels, albeit via different mechanisms: While both inhibit the reuptake of DA and NA, AMPH additionally increases the release of these neurotransmitters into extraneuronal space and inhibits the catabolic activity of monoamine oxidase (Kuczenski and Segal 1975).

Evidence from neuroimaging research suggests that the abuse potential of psychostimulants is mediated by subcortical DA effects (Volkow 2006). Atomoxetine, a selective noradrenaline reuptake inhibitor with limited effects on subcortical DA mechanisms, was recently approved by the FDA for the treatment of ADHD (Faraone et al. 2005). In animals, systematic administration of this drug was found to increase both cortical NA and DA levels several-fold (Bymaster et al. 2002). Due to its limited abuse potential, atomoxetine may thus offer clinical advantages, particularly for those patients who do not respond to stimulant treatment, estimated to constitute 30% of all ADHD pediatric patients (Madras et al. 2002). Modafinil, though not currently FDA-approved for the treatment of ADHD, also appears to exert therapeutic effects (Greenhill et al. 2006). The neurobiological mechanisms through which this drug exerts pro-cognitive effects remain to date poorly understood; however, aspects of its behavioral and cognitive effects appear to be contingent on the integrity of NA transmission (Minzenberg and Carter 2008). To summarize, medications used in the treatment of ADHD (including methylphenidate, dextroamphetamine and atomoxetine) act to increase brain catecholamine levels.

5 Cognitive Effects of Psychostimulants: Implications for our Understanding of ADHD

A growing body of evidence drawn from controlled studies supports the effectiveness of stimulant medications in the treatments of ADHD in children, adolescents (Faraone and Buitelaar 2010) and adults (Castells et al. 2011a, b). But what do we know about their cognitive effects?

There is some evidence that single therapeutic doses of MPH ameliorate functions in children and adults diagnosed with ADHD often reported to be sub-optimal in this condition (Clark et al. 2007; Mehta et al. 2004; Spencer et al. 2007; Turner et al. 2005). Therapeutic effects are thought to be mediated through neuromodulatory influences over fronto-striato-cerebellar circuits, although the exact underlying neurochemical mechanisms are not well understood (Del Campo et al. 2011). Analogous cognitive findings have been documented in healthy subjects, following both MPH (Elliott et al. 1997; Koelega 1993; Mehta et al. 2000; Strauss et al. 1984) and AMPH administration (Mattay et al. 1996, 2000; Rapoport et al. 1978, 1980), suggesting that stimulant effects are not pathognomic for ADHD. These findings imply that the "calming" effects of stimulants on hyperactive children, initially considered as paradoxical, are not likely to be mediated by an underlying neurochemical deficit; instead, they might be best understood through their normal actions. A further implication is that ADHD differs from health only quantitatively on normally distributed dimensions of attention, impulsivity and hyperactivity, with the criterion of six symptoms representing an arbitrary cut-off point at the top end of a normal distribution, rather than a true categorical boundary between ADHD vs. non-ADHD conditions.

It is noteworthy, however, that a non-neglectable number of controlled studies investigating pro-cognitive effects of stimulants have also yielded negative or null results (e.g. Rhodes et al. 2006). Recent reviews of cognitive stimulant effects in children with ADHD (Pietrzak et al. 2006) and healthy volunteers (mostly adults) (Faraone and Glatt 2010; Smith and Farah 2011) conclude that the pattern of stimulant-induced enhancement is far from clear. Overall, it appears that stimulants do not improve cognition across the board but rather selectively: In both ADHD patients and healthy volunteers, the neurocognitive processes affected most prominently by stimulants are in the domains of impulse control, working memory and attention (Chamberlain et al. 2010; DeVito et al. 2009; Finke et al. 2010; Naylor et al. 1985; Rhodes et al. 2006; Turner et al. 2003), and less so in executive function (Advokat 2010; Rhodes et al. 2006; Swanson et al. 2011b). Importantly, stimulants appear to interact with these domains in a baseline performance-dependent manner (Clatworthy et al. 2009; DeVito et al. 2009; Naylor et al. 1985; Robbins and Sahakian 1979; Rogers et al. 1999; Sahakian and Robbins 1977; Turner et al. 2003).

The contingency of stimulant effects to baseline performance has been interpreted in accordance with a hypothesized inverted u-shaped function, whereby optimal catecholamine levels determine optimal performance, and catecholamine levels along the curve at either side of the optimum are associated with impaired performance (Arnsten and Goldman-Rakic 1998; Mattay et al. 2003; Williams and Goldman-Rakic 1995). This model accounts for the more consistent drug effects found in patients with ADHD, who have relatively low baseline performance and putatively impaired catecholamine transmission. For example, improvements in attention following stimulant treatment of ADHD are substantially greater than those caused by non-medical use of stimulants by healthy individuals (Swanson et al. 2011b). Moreover, among a group of adults referred for an ADHD

assessment, those individuals whose elevated ADHD symptom severity leads to a clinical diagnosis of adult ADHD manifested a more pervasive benefit of MPH across computerized cognitive tasks (Turner et al. 2005). Also among healthy volunteers, stimulant-induced cognitive enhancement appears to be largest in those individuals with the highest scores on ADHD-like behaviors and traits (Smith and Farah 2011). This difference in baseline catecholamine levels may also underlie the differential response to stimulant medications observed between controls and patients with ADHD using functional MRI (Vaidya et al. 2005).

The inverted u-shaped function relating catecholamine levels to performance was originally formulated with respect to the chemical neuromodulation of the prefrontal cortex (Arnsten and Goldman-Rakic 1998) but it is likely to also apply to other structures within the same circuitry, including the striatum (Clatworthy et al. 2009; Robbins 2010). Studies in animals (Chudasama et al. 2005; Chudasama and Robbins 2004; Collins et al. 1998; Roberts et al. 1994) and humans (Clatworthy et al. 2009; Cools et al. 2001; Swainson et al. 2000) provide evidence for the existence of multiple inverted u-shaped functions associated with distinct cognitive processes. Indeed, stimulant medications appear to have different effects not only depending on baseline performance, but also on the context of administration and the type of cognitive domain probed. For example, in a recent functional MRI study in healthy controls, MPH exerted differential effects at prefrontal cortical and striatal sites to affect distinct cognitive components within a single task of reversal learning (Dodds et al. 2008).

6 Molecular Neuroimaging Studies in ADHD

6.1 Status of DA Markers in ADHD

Insofar as PET and SPECT allow quantification of DA receptor availability (indicative of DA receptor levels) with great specificity, both molecular neuroimaging techniques represent an attractive approach to investigate, in vivo, the role of dopaminergic abnormalities in the pathophysiology of ADHD. A number of DA markers have been compared between ADHD patients and healthy controls, including D_2/D_3 receptors, DA synthesis and DA transporters (DAT). Table 1 summarizes these findings; studies revealing decreased, increased or unchanged DA marker status in patients are highlighted in orange, blue and yellow, respectively (note that only studies including a control group are illustrated). Overall, results regarding DA parameter status in ADHD have been highly inconsistent, regarding both the direction of the observed abnormality and its magnitude. The first marker studies focused on DAT, given its role in mediating stimulant actions. At the time, a wealth of evidence appeared to indicate that ADHD patients had increased DAT density (Krause 2008; Larisch et al. 2006; Spencer et al. 2005), a finding that reached wide acceptance for almost a decade (Madras et al. 2006).

Table 1 DA marker status in patients with ADHD vs. healthy controls, as measured with PET and SPECT

Authors	Radiotracer	Age group	N (patients-controls)	Treatment history	Regions examined	Behavioral correlates
D_2/D_3 receptors						
Volkow et al. (2010)	[^{11}C]raclopride	A	45–51	Naïve	Accumbens, midbrain	MPQ (Achievement scale)
Volkow et al. (2009)	[^{11}C]raclopride	A	53–44	Naïve	Whole brain analysis followed by confirmatory template ROI analysis	SWAN
*Volkow et al. 2007a, b)	[^{11}C]raclopride	A	19–24	Naïve	Whole brain analysis and L/R CAU and PUT	CAARS
Jucaite et al. (2005)	[^{11}C]raclopride	T	12–10 (not age-matched)	n = 3 (MPH)	L/R CAU and PUT	DSM-IV scores, CPT, Motor measurements
*Ilgin et al. (2001)	[^{123}I]IBZM	C	9-published control data	Naïve	L/R CAU and PUT	CTRS, DSM-IV scores
FDOPA (brain decarboxylase activity, i.e. DA synthesis)						
Ludolph et al. (2008)	[^{18}F]F-DOPA	A	20–18	n = 12 (MPH)	Whole brain analysis, with small volume correction in midbrain, R/L CAU and PUT and amygdala	n/a
Forssberg et al. (2006)	L-[^{11}C]DOPA	T	8–6	n = 8 (MPH)	Twenty-eight manually adjusted template ROIs, including midbrain, CAU and PUT	DSM-IV scores
Ernst et al. (1999)	[^{18}F]F-DOPA	T	10–10 (healthy siblings)	n = 6 (stimulants)	L and R PFC, CAU, PUT and midbrain	DSM-III-R scores, Child Behavior Checklist, CPRS, CTRS, CPT

(continued)

Table 1 (continued)

Authors	Radiotracer	Age group	N (patients-controls)	Treatment history	Regions examined	Behavioral correlates
Ernst et al. (1998)	[^{18}F]F-DOPA	A	17–23	n = 4	L and R PFC, CAU, PUT and midbrain	DSM-III-R, CTRS (abbreviated version), Utah criteria of childhood ADHD
Dopamine transporter						
*Volkow et al. 2007a, b)	[^{11}C]cocaine	A	53–44	Naïve	Whole brain analysis and template ROIs	SWAN
Hesse et al. (2009)	[^{123}I]FP-CIT	A	17–14	Naïve	Striatum, head of CAU, PUT, thalamus, midbrain	ASRS, WURS, BDI
Volkow et al. (2007a, b)	[^{11}C]cocaine	A	20–25	Naïve	Whole brain analysis and L/R CAU and PUT	CAARS
Spencer et al. (2007)	[^{11}C]altropane	A	21–26	Naïve	L/R CAU and PUT	n/a
Larisch et al. (2006)	[^{123}I]FP-CIT	A	20–20	Naïve	L and R striatum	n/a
la Fougere et al. (2006)	[^{99}Tc]TRODAT-1	A	22–14	Naïve	Basal ganglia template ROIs	CGI-I, CGI-S
Jucaite et al. (2005)	[^{11}C]PET2I	T	12–10 (not age-matched)	n = 3 (MPH)	CAU, PUT and midbrain	CPT, motor measurements
Spencer et al. (2005)	[^{11}C]altropane	A	6–6	n = 1 (stimulant treatment)	L/R striatum	n/a
Cheon et al. (2003)	[^{123}I]IPT	C	9–6	Naïve	L/R basal ganglia	ARS

(continued)

Table 1 (continued)

Authors	Radiotracer	Age group	N (patients-controls)	Treatment history	Regions examined	Behavioral correlates
van Dyck et al. (2002)	[^{123}I]β-CIT	A	9–9	n = 1 (stimulant treatment)	Manually adjusted template ROIs for striatum, diencephalon, brainstem	ARS
Krause et al. (2000)	[^{99}Tc]TRODAT-1	A	10–10	n/a	Manually adjusted template ROIs for striatum	n/a
Dresel et al. (2000)	[^{99}Tc]TRODAT-1	A	17–14	n/a	Manually adjusted template ROIs for striatum, CAU and PUT	n/a
Dougherty et al. (1999)	[^{123}I]altropane	A	6-n/a (compared to control data on 30 healthy controls)	n = 4 (stimulant treatment)	Striatum	n/a

Studies revealing decreased, increased or unchanged DA marker status in patients are highlighted in orange, blue and yellow, respectively. Studies highlighted with an asterisk are those in which the DA marker was also measured following stimulant treatment (see Table 2). Abbreviations: *A* adults, *T* adolescents, *C* children, D_2/D_3 D_2/D_3 receptor availability, *DAT* DAT availability, *n/a* not available, *MPH* methylphenidate, *ROI* region of interest, *CAU* caudate, *PUT* putamen, *PFC* prefrontal cortex, Questionnaires (in alphabetical order): *ARS* ADHD Rating scale-IV, *ASRS* Adult ADHD Self-Report Scale, *BDI* Beck Depression Inventar, *CAARS* Conners Adult ADHD Rating Scales, *CGI-I* Clinical Global Improvement Scale, *CGI-S* Clinical Global Impression, *CPRS* Conner's Parent Rating Scale, *CTRS* Conner's Teaching Rating Scales, *MPQ* Multidimensional Personality Questionnaire, *SNAP-IV* Swanson, Nolan and Pelham Scale—version IV, *SWAN* Strengths and Weaknesses of ADHD symptoms and Normal-behavior, *TOVA/CPT* continuous performance task, *WURS* Wender Utah Rating Scale

However, more recent well-powered designs in adult medication-naïve ADHD patients have consistently shown that ADHD is associated with decreased DAT as well as D_2/D_3 receptor availability in selected sub-cortical regions of the left hemisphere, including nucleus accumbens, caudate and midbrain (Volkow et al. 2007a, b; 2009).

Studies investigating D_2/D_3 receptor status have to date been restricted to the striatum due to the use of [^{11}C]raclopride, a low affinity tracer which can only be reliably quantified in brain regions with high receptor density. From the five published D_2/D_3 receptor PET imaging studies, those reporting unchanged or increased receptor availability in ADHD patients used a control group that was not age-matched to the patients (Ilgin et al. 2001; Jucaite et al. 2005).

The prefrontal cortex, a brain region in which inadequate catecholamine transmission is thought to play a key role in the pathophysiology of ADHD, has been investigated using [^{18}F]fluorodopa ([^{18}F]DOPA), an index of DA synthesis capacity. The same laboratory documented opposite findings in adults (Ernst et al. 1998) and children with ADHD (Ernst et al. 1999). More recently, two reports in adolescent and adult ADHD suggest that ADHD is associated with decreased DOPA metabolism in sub-cortical regions (Forssberg et al. 2006; Ludolph et al. 2008). Given the low dopaminergic neural density in the prefrontal cortex, it has been argued that well-powered designs are needed to resolve the question of whether DA neurotransmission in the prefrontal cortex is affected in ADHD (Ernst et al. 1998).

6.2 Synaptic DA Transmission in ADHD

The binding competition between D_2/D_3 radioligands and endogenous DA provides an imaging paradigm to measure DA transmission following an acute drug challenge (Laruelle 2000), illustrated in Figs. 1 and 2. Over the last two decades, several groups have shown that the administration of psychostimulants such as AMPH or MPH are associated with an acute reduction in non-displaceable binding potential (BP_{ND}) of the PET radiotracers [11C]raclopride (Volkow et al. 1994) (Breier et al. 1997), [^{11}C]FLB 457 (Montgomery et al. 2007), and also [^{18}F]fallypride (Cropley et al. 2008). Evidence from microdialysis studies in non-human primates suggests that the magnitude of this reduction is indeed related to the AMPH-induced increase in extracellular DA measures (Laruelle et al. 1997).

Of the five published studies documenting the magnitude of stimulant-induced increases in endogenous DA in ADHD patients (summarized in Table 2), only one included an age-matched control group (Volkow et al. 2007b). Using [^{11}C]raclopride PET, Volkow et al. found that ADHD patients showed smaller increases in DA levels in the caudate following i.v. MPH treatment (0.5 mg/kg). Moreover, a voxelwise analysis revealed that the volumes of the regions where MPH significantly reduced tracer binding were significantly smaller in ADHD patients compared to controls in bilateral caudate, hippocampus and left amygdala. However,

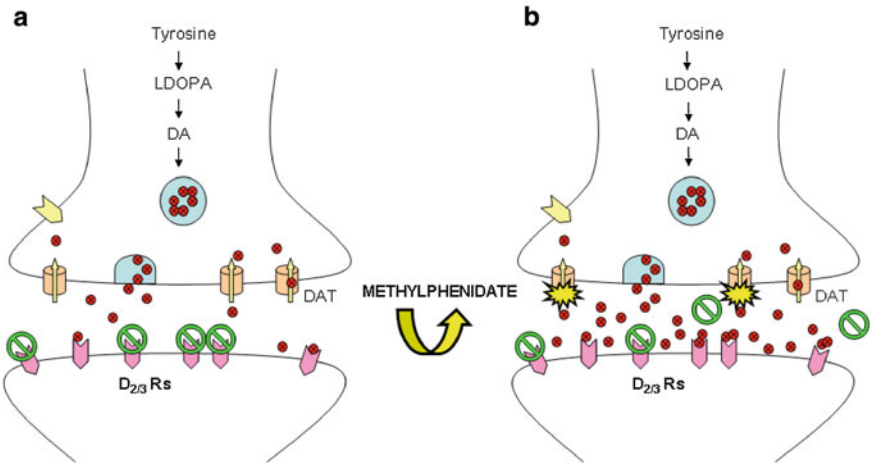

Fig. 1 Schematic illustration of a dopaminergic synapse visualized using [^{18}F]fallypride-PET or [^{11}C]raclopride PET before and after methylphenidate (MPH) administration. Dopamine (DA) is synthesized in the pre-synaptic terminal from the essential amino acid tyrosine and L-3,4 –dihydroxyphenylalanine (L-DOPA). DA is released into the synaptic cleft, where it binds to D_2/D_3 receptors found both pre- and post-synaptically. The radioactively labeled ligand binds to D_2/D_3 receptors, allowing estimation of receptor availability. MPH blocks dopamine transporters (DAT), impeding the reuptake of DA into the pre-synaptic terminal thereby increasing extra-cellular DA levels. The competition between endogenous DA and the radioligand for the binding to D_2/D_3 receptors leads to a reduction in radioactive signal

these findings are difficult to reconcile with prior evidence in adolescent ADHD patients indicating that MPH-induced increases in DA concentrations in the right striatum are associated with greater symptom severity (Rosa-Neto et al. 2005).

Initial PET evidence in healthy volunteers using tracers suitable to examine regions with low D_2/D_3 receptor density such as [^{11}C]FLB 457 and [^{18}F]fallypride suggests that stimulants increase DA levels also extra-striatally (including amygdala, temporal and orbito-frontal cortices) (Cropley et al. 2008; Montgomery et al. 2007; Riccardi et al. 2006a, b; Slifstein et al. 2010). The regional specificity and magnitude of DA changes remains to be replicated. Intriguingly, one study using AMPH yielded negative results (Aalto et al. 2009). Whether the magnitude of regional increases differs between patients and controls remains to be investigated.

6.3 Potential Confounding Factors Underlying Inconsistencies Across PET/SPECT Findings in ADHD

There are a number of factors that potentially underlie the disparities found across PET/SPECT studies assessing the status of DA markers in ADHD patients compared to controls (summarized in Table 3). Age, previous medication with

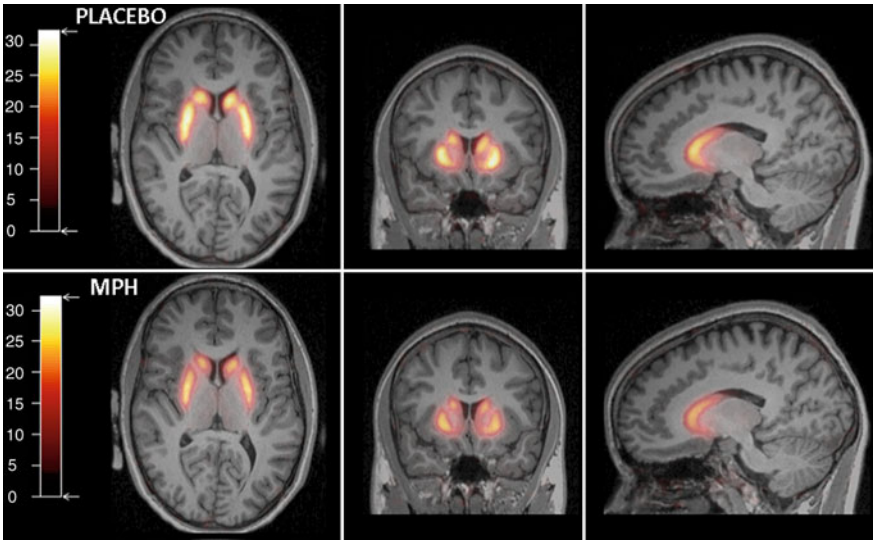

Fig. 2 [^{18}F]fallypride BP_{ND} maps of a healthy control following placebo (*top raw*) and following oral MPH (*bottom raw*). BP_{ND} maps are superimposed on a high resolution magnetic resonance image. MPH increases endogenous DA, leading to a reduction in [^{18}F]fallypride BP_{ND}

stimulants and psychiatric comorbidity seem to be the most critical factors and are highly recommended to be controlled for in future research assessing the DA system in ADHD in vivo (Del Campo et al. 2011). How the imaging data are processed and the anatomical landmarks used to define regions of interest also appear to be crucial when comparing data across studies. This appears to be particularly relevant in receptor imaging studies assessing the striatum, which has been shown to have a heterogenous distribution of dopamine receptors across its functional subregions (Martinez et al. 2003; Mawlawi et al. 2001).

7 Neuroanatomical Correlates of ADHD

The idea that fronto-striato-cerebellar circuits are altered in ADHD has been largely supported by morphological neuroimaging studies: Volumetric reductions in the interconnected frontal lobe (particularly the dorsolateral prefrontal cortex, DLPC and anterior cingulate cortex, ACC), basal ganglia and cerebellum are often reported by MRI studies (Nakao et al. 2011; Seidman et al. 2005). Abnormalities in these circuits are thought to sub-serve the deficits often observed in ADHD patients in prefrontal-dependent cognitive functions (see 3.Cognitive markers of ADHD). The cerebellum has recently attracted attention in ADHD research owing to its relevance in a wide range of other functions also reported to be abnormal in ADHD patients, such as motor timing and time estimation (Ackermann et al. 1999; Jueptner et al. 1995; Maquet et al. 1996; Rubia and Smith 2004; Smith et al. 2003).

Table 2 Changes in D_2/D_3 receptor availability following stimulant medication in ADHD patients

Authors	Radiotracer	Age group	N (patients-controls)	Treatment history	Drug challenge	Drug adminis-tration	Regions examined	Behavioral correlates
Endogenous DA levels								
*Volkow et al. 2007a, b)	[^{11}C]raclopride	A	19–24	Naïve	MPH (single dose)	I.v. (0.5 mg/kg)	Whole brain analysis and L/R CAU and PUT	CAARS
Rosa-Neto et al. (2005)	[^{11}C]raclopride	T	9-n/a	Naïve	MPH (single dose)	Oral (0.3 mg/kg)	L/R striatum	TOVA
Rosa-Neto et al. (2002)	[^{11}C]raclopride	T	6-n/a	No current stimulant treatment	MPH (single dose)	Oral (0.3 mg/kg)	L/R striatum	TOVA
Ilgin et al. (2001)	[^{123}I] IBZM	C	9-n/a	Naïve	MPH (3 month therapy)	Oral (0.5– 1.5 mg/kg.day)	L/R CAU and PUT	CTRS

Note that only the first study included an age-matched control group, allowing the comparison of the magnitude of the change in endogenous DA between patients and controls. The study is highlighted in red, since patients were found to have a smaller stimulant-induced increase in endogenous DA relative to controls. Abbreviations: *A* adults, *T* adolescents, *C* children, *D_2/D_3* D_2/D_3 receptor availability, *DAT* DAT availability, *n/a* not available, *MPH* methylphenidate, *ROI* region of interest, *CAU* caudate, *PUT* putamen, *PFC* prefrontal cortex, Questionnaires (in alphabetical order): *CAARS* Conners Adult ADHD Rating Scales, *TOVA/CPT* continuous performance task, *CTRS* Conner's Teaching Rating Scales

Table 3 Confounding factors potentially underlying the heterogeneity found across DA/PET studies in ADHD

Study population
• Age group
• Reduced sample size (insufficient power)
• Matched controls
• Comorbidity
• Medication history
• History of nicotine exposure (smoking)
• Genetic factors (e.g. DAT polymorphisms)
Methodology
• Imaging technique (PET vs. SPECT)
• Radiotracer
• Co-registration with structural MRI
• Brain regions examined/anatomical landmarks used

Accumulating evidence suggests that some patients also show alterations in meso-cortico-limbic circuits (Brieber et al. 2007; Carmona et al. 2005; Del Campo et al. under submission; Sowell et al. 2003; van't Ent et al. 2007; Wang et al. 2007). These abnormalities are thought to underlie difficulties in reward and emotional processes which often dominate the clinical profile of ADHD patients, particularly at adulthood (Hesslinger et al. 2003; Richards et al. 2006). Supporting evidence comes also from the aforementioned PET studies documenting catecholaminergic abnormalities in reward pathways (Volkow et al. 2007b; 2010). According to a recent meta-analysis, alterations in limbic regions are more pronounced in non-treated populations (Frodl and Skokauskas 2011).

7.1 Continuity of Morphological Abnormalities Across the Lifespan

The manifestation of ADHD throughout the lifespan evolves such that there is symptomatic improvement in 30–40% of children from late adolescence onwards (Mannuzza et al. 1991). Moreover, in those patients who continue to have a diagnosis at adulthood there is an age-dependent decline of hyperactivity symptoms, while the inattention symptoms often persist (Biederman et al. 2000). These observations raise the question of whether and how the neural signatures of ADHD evolve across the lifespan. While the molecular neuroimaging literature cannot address this question due to the lack of data in childhood ADHD (given regulations preventing exposure to radiation in children), MRI studies have been carried out in patients across all age ranges.

A few longitudinal studies have addressed whether there is neurobiological continuity between children and adolescents with ADHD, and whether the improvement of cognitive impairment and clinical outcome can be associated with

a normalization of the neuroanatomical correlates (Casey et al. 2007; Castellanos et al. 2002; Mackie et al. 2007; Shaw et al. 2006; Sowell et al. 2003).

The first study of this nature, carried out on 152 children and adolescents with ADHD and 139 controls scanned four times over a period of 10 years, found that all volumetric abnormalities in ADHD (decreased total cerebral and cerebellar volumes, as well as decreased overall WM) remained unchanged over time, except for the caudate nucleus volume, which normalized through adolescence (Castellanos et al. 2002). Normalization of this subcortical structure was interpreted in the light of the decreasing motor hyperactivity with age. Moreover, developmental trajectories of cerebellar and cerebral volumes were observed to be parallel in both groups, and longitudinal growth curves appeared to be unrelated to prior stimulant treatment.

This finding was largely supported by Shaw et al. (2006, 2007), who found cortical growth trajectories, which are typically characterized by cortical increase at childhood followed by adolescent decrease, to be parallel in children affected by the disorder and healthy controls. However, ADHD children attained peak thickness with a marked delay throughout most of the brain, most prominently in the prefrontal cortex (Shaw et al. 2007). Specific regional differences in the cortical maturation were associated with clinical outcome; while children with better clinical outcome presented normalization in portions of the right parietal cortex over time, a fixed non-progressive deficit of the medial prefrontal and cingulate regions was documented in children with a worse outcome (Shaw et al. 2006). The recent application of refined segmentation techniques has made it possible to identify different growth trajectories in sub-regions of the cerebellum (Mackie et al. 2007). Whereas a fixed, non-progressive volume decrease was reported in the superior cerebellar vermis of ADHD children, abnormalities in the cerebral hemispheres developed differently in children with better versus worse clinical outcome. Thus, the dissipation of ADHD symptoms throughout development may be associated with a normalization of certain neuroanatomical correlates of the disorder.

These findings contribute to a growing body of evidence suggesting that a delay in brain maturation (rather than a deviation from typical neurodevelopmental processes) is implicated in the pathophysiology of ADHD (Nakao et al. 2011; Shaw et al. 2006, 2007). Furthermore, it appears that ADHD patients may progressively catch up with their developmental delay with advancing age (Nakao et al. 2011), although more longitudinal studies are needed to confirm both observations.

What brain tissue signatures have been reported in adult ADHD patients? Initial studies by Seidman et al. (2006) and Makris et al. (2007) observed reduced cortical gray matter volume in the DLPFC and ACC, and a trend for overall increased white matter volumes in adult patients compared to controls (Seidman et al. 2006). Abnormalities in subcortical structures were not found with the exception of the nucleus accumbens, which was marginally larger in patients. The inferior parietal lobule, DLPFC and ACC, which are cortical regions thought to subserve a distinct network involved in attention, were thinner in patients, most prominently in the

right hemisphere (Makris et al. 2007). In a subsequent publication, the same authors reported a gray matter volume reduction in the left ACC in the treatment-naïve group and a reduction in the right ACC in the treated group, relative to controls (Makris et al. 2010).

Recently, other groups have also measured brain tissue volume in adult ADHD patients relative to controls (e.g. Ahrendts et al. 2011; Almeida Montes et al. 2010; Amico et al. 2011; Del Campo et al. submitted; Depue et al. 2010; Perlov et al. 2008) with few disease-related abnormalities surviving conservative statistical thresholds and whole brain analyzes. Thus, the jury is still out as to where in the brain adult ADHD patients have altered brain tissue volume, and how these abnormalities are associated with the clinical profile.

An important question that needs to be addressed when considering the continuity of neuroanatomical alterations into adulthood in ADHD is whether pharmacological treatment has an impact on structural changes across the life cycle. Two recent meta-analyzes exploring gray matter volume in ADHD agree that treatment may to be associated with the normalization of structural abnormalities (Frodl and Skokauskas 2011; Nakao et al. 2011). However, this conclusion was drawn from cross-sectional data and thus will need to be confirmed by longitudinal study designs.

7.2 Evidence for Abnormal Structural Connectivity in ADHD

Diffusion tensor imaging (DTI) is an MRI technique based on the property of water molecules to diffuse along fiber tracts which enables exploration of microstructural integrity of white matter (Assaf and Pasternak 2008). Because it is still an emerging technique, only few ADHD case-control studies of this nature exist (for a review see Konrad and Eickhoff 2010; Liston et al. 2011). The age and medication history of participants across studies, as well as the approaches used to analyze the DTI data were highly variable (whole brain voxel-wise comparisons, manually selected regions of interest or regions of interest specified by automated tractography algorithms and tract-based spatial statistical techniques), making the integration of results somehow difficult. Nevertheless, reduced white matter integrity in the superior longitudinal fasciculus and the anterior corona radiata, which carry fronto-striatal projections, are among the most consistently replicated findings. Indeed, out of the eight DTI studies that examined connectivity in fronto-striatal projections, seven report reduced connectivity in patients compared to controls (Liston et al. 2011). Connectivity between limbic regions has been comparatively less explored.

8 Conclusions

A large body of evidence from neuropsychological and neuroimaging case-control studies points chiefly toward an underlying dysfunction of distributed fronto-striato-cerebellar circuits in ADHD, although more recent studies suggest that

limbic circuits may also be implicated. Through neuromodulation of these circuits, catecholamines are thought to play a critical role in prefrontal-dependent cognitive functions, as well as motivational and emotional processes often reported to be altered in ADHD patients, representing the key target for pharmacotherapy in ADHD.

The psychostimulants methylphenidate and amphetamine, but also atomoxetine increase DA levels in prefrontal circuits and are clearly effective in improving clinical symptoms of ADHD across the lifespan. Those findings do, however, not correspond to equally clear findings from neurocognitive and neuroimaging studies of the DA system investigating single stimulant doses. Reports in both ADHD patients and healthy individuals suggest that the cognitive effects of psychostimulants are complex, being driven by two aspects: (a) baseline performance (mediated in turn by baseline catecholamine levels) and (b) the cognitive domain assessed. PET and SPECT studies in ADHD have to date focused on the DA system with ligands for the DA transporter, DA receptors and DA synthesis. The best-powered PET study, one of the most ambitious molecular neuroimaging studies ever performed in a psychiatric population, found reduced DAT and $D_{2/3}$ availability in never medicated patients with ADHD (Volkow et al. 2009). However, there was considerable overlap between patients and controls, so that dopaminergic PET imaging does not have the potential for a diagnostic assessment.

As reviewed in this chapter, the search for neural markers of ADHD has no doubt yielded important findings. Advancement in the field will be reliant on the development of improved neuroimaging techniques (e.g. allowing a finer level of spatial resolution) and analytic methods, but also on the implementation of longitudinal study designs addressing isolated symptom dimensions and their underlying mechanisms. The latter will be key to further our understanding of the pathophysiology of ADHD, which is inherently heterogeneous, and elucidate how its behavioral and neural signatures evolve across the lifespan. One fruitful approach to characterize neural markers of the disorder may be to enrich the neuroimaging data analyzes with information obtained from the same patients on well-validated cognitive tests. A further promising approach will be the development and application of radioligands aimed at hitherto unexplored neurochemical targets hypothesized to be affected in ADHD. One example is the in vivo investigation of the noradrenergic system with radioligands such as [^{11}C]methylreboxetine, which has been successfully deployed in initial human studies but is yet to be used in ADHD patients.

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Experimental Protocols for Behavioral Imaging: Seeing Animal Models of Drug Abuse in a New Light

Alexandra R. Aarons, Amanda Talan and Wynne K. Schiffer

Abstract Behavioral neuroimaging is a rapidly evolving discipline that represents a marriage between the fields of behavioral neuroscience and preclinical molecular imaging. This union highlights the changing role of imaging in translational research. Techniques developed for humans are now widely applied in the study of animal models of brain disorders such as drug addiction. Small animal or preclinical imaging allows us to interrogate core features of addiction from both behavioral and biological endpoints. Snapshots of brain activity allow us to better understand changes in brain function and behavior associated with initial drug exposure, the emergence of drug escalation, and repeated bouts of drug withdrawal and relapse. Here we review the development and validation of new behavioral imaging paradigms and several clinically relevant radiotracers used to capture dynamic molecular events in behaving animals. We will discuss ways in which behavioral imaging protocols can be optimized to increase throughput and quantitative methods. Finally, we discuss our experience with the practical aspects of behavioral neuroimaging, so investigators can utilize effective animal models to better understand the addicted brain and behavior.

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Contents

1 Behavioral Neuroimaging in Animal Models of Addiction

Animal behavioral models have been instrumental in elucidating key biochemical and physiological mechanisms underlying the progression from drug use to drug abuse and addiction. Drug self-administration and conditioned place preference (CPP) constitute the major preclinical behavioral tools used to screen new therapies before clinical testing. Although these behavioral models are useful in predicting addictive liability and effective drug treatments, the power of these models to determine clinical efficacy is a matter of dispute. Preclinical neuroimaging, coupled with real-time assessment of addictive behavior, offers a powerful model for testing addictive liability and pharmacotherapy development.

The overall objective of preclinical behavioral neuroimaging is distinctly different from existing neuroimaging paradigms. This dual paradigm takes advantage of the higher resolution and noninvasive imaging technology that combines measures of brain function with behavior, two relevant endpoints that are more informative and accurate together, rather than alone. This allows for exciting research that investigates whole brain systems in the context of drug abuse within a robust and reproducible experimental setting that reflects real-time behavioral states.

In essence, behavioral neuroimaging helps investigators compare animal behavior to human behavior. If a drug attenuates behavior in an animal paradigm but fails to do so for humans, researchers must question whether they are measuring the same phenomena across different species. The identification of independent, biological markers—patterns of brain function—of the addictive state that are homologous in both animals and humans would serve to validate behavioral models. The promise of common biological measures rests on preclinical imaging, and by

coupling behavioral models with imaging; the validity of animal models increases substantially.

Preclinical behavioral imaging modalities allow us to examine the neuroanatomical and neurochemical impairments produced by a specific substance, and examine neurochemically-specific treatment regimens that can minimize the addictive liability of drugs. Behavioral functional imaging in small animals provides a comprehensive view of the progression from drug use to drug abuse that comprises both behavioral and biological measures with strong clinical relevance. For example, limitations of drug reinstatement models for screening therapeutic medications are suggested by the generally poor agreement of findings with clinical studies in humans (Katz and Higgins 2003; Shaham et al. 2003). Ideally, the addition of imaging will lead to more effective therapies that enter into Phase I and Phase II clinical trials (McArthur 2011). The net result could be the development of more effective pharmacotherapy treatments, which would greatly enhance the therapeutic availability for individuals seeking addiction treatment. In sum, preclinical imaging offers a translational tool to study drug abuse, with continual improvement in the predictability of animal models (Borsook et al. 2006; McArthur 2011).

Our aim is to strengthen the understanding of methodological issues in behavioral imaging studies, specifically in the context of translational models of drug abuse. In this review, we will briefly cover different imaging methods with an emphasis on functional positron emission tomograph (PET) techniques, as PET offers the unique opportunity to image brain function in animals that are free to move in any experimental environment, outside of the scanner itself. We will discuss optimal study designs for preclinical behavioral imaging. We will emphasize the strengths and weaknesses of various approaches, and highlight some of the issues associated with neuroimaging across species and modalities. Finally, we will critically evaluate the potential of behavioral imaging as a translational tool to align preclinical models with clinical manifestations of drug abuse.

2 Behavioral Imaging Modalities: Strengths and Weaknesses

There are several types of neuroimaging approaches common to both clinical and preclinical fields. Our focus will be the strengths and weaknesses of behavioral imaging with PET, and we will also discuss functional magnetic resonance imaging (fMRI). Both behavior imaging modalities can provide insight on drug use and abuse, disease progression and successful treatment development.

For fMRI, there are significant challenges in imaging functional activity in animals that are not necessarily applicable to PET methods. In the main, fMRI studies require that animal subjects be inside the magnet during image acquisition, which means that they must be anesthetized, immobilized, restrained, or trained—this limits behavioral output to all but the simplest behaviors. The use of anesthetics is a significant confound to functional imaging studies not only because of obvious limitations on behavior, but also because of alterations in brain function

associated with anesthetic agents (see below). Further, the need to adopt special stimulation systems within the magnet, the intense noise levels produced by the magnet and the general lack of ability to observe the animal because it is placed deep within the magnet core, all limit the repertoire of behavioral imaging studies that can be performed with functional MRI in rodents (Borsook and Becerra 2011). However, devices for conscious animal imaging have been reported (Schulz and Vaska 2011), although most require surgical implantation into the skull of animal subjects, which can introduce additional confounds on brain function (Khubchandani et al. 2003; Schiffer et al. 2006; Frumberg et al. 2007). Restraint molds and training are also options for fMRI studies where image acquisition must occur at the time of behavioral measurements (Becerra et al. 2011), however again, this precludes measurements of all but the simplest behaviors.

For the study of drug abuse and addiction, the most appropriate behavioral models require that animals be able to respond to stimuli. Functional PET studies, as described below, can be performed outside the tomograph with minimal or no effects on animal behavior, given an appropriate radiotracer. Important information has been gained from imaging studies in which behavioral measures were obtained separately from image acquisition (reviewed in Dalley et al. 2009). No matter the paradigm, parallel behavioral measures have highlighted individual responses in correlations of change in behavior with change in brain function (Dalley et al. 2007; Schiffer et al. 2009), allowing another important dimension of cross-species consistency in the study of drug abuse.

3 "Snapshot" Imaging Protocols

The strategy we discuss here is unique in that it focuses on simultaneous measurements of brain activity and behavior from the same imaging session, however as always, there are trade-offs that must be considered in the experimental design. As previously mentioned, a major benefit to the field of neuroscience is the ability to measure changes in metabolic activity with behavioral responses. With small animal PET, animal subjects receive radiotracer administration prior to image acquisition, and successively are able to freely behave in an experimental environment during their uptake period. Thus, the images captured represent brain metabolism or neurochemical changes that occur while the animal is freely behaving. These behaviors can be trained or drug induced. Many functional PET studies in humans are designed in this same way, where scans are conducted after the completion of a behavioral task and the radiotracer is akin to a photograph taken at the moment of the behavioral experience. Some qualities of optimal radiotracers are given in Table 1.

Simultaneous PET imaging and behavior measures are acquired using radiotracers, such as 2-[^{18}F]fluoro-2-deoxy-D-glucose (^{18}F-FDG or FDG), which are captured and trapped in tissue for an extended period of time post radiotracer administration, or with the use of receptor-specific radiotracers whose kinetics are predictable and well-established (Patel et al. 2008). This dual imaging and behavioral paradigm provides a solid covariate for image analysis.

Table 1 Ideal radiotracer qualities for behavioral imaging in freely moving animals[a]

- Chemical stability
- Non-toxic
- High lipophilicity and efficient extraction into the brain (high lipid solubility with ready passage of the tracer across the blood brain barrier, BBB)
- High extraction by the brain, with a distribution that is proportional to blood flow
- Specificity—a prolonged retention in brain tissue with a fixed distribution that is independent of regional blood flow variations
- Inability of radiolabeled metabolites to cross the BBB and/or rapid clearance of these metabolites from blood, unless radiolabeled metabolites in the brain can be accounted for by ratio quantitation methods
- A trapping mechanism where the mechanism itself is not altered by the disease pathology
- Radioactive half-life long enough to permit radiotracer injection during the occurrence of the behavior and subsequent anesthesia and image acquisition
- Radioactive half life that is short enough to allow decay within a few hours to days to minimize radiation exposure to the animal and to laboratory staff, and also to permit rescanning of the animal subject as its own control
- A photon energy compatible with currently used instrumentation

[a] Adapted from (Holschneider and Maarek 2004)

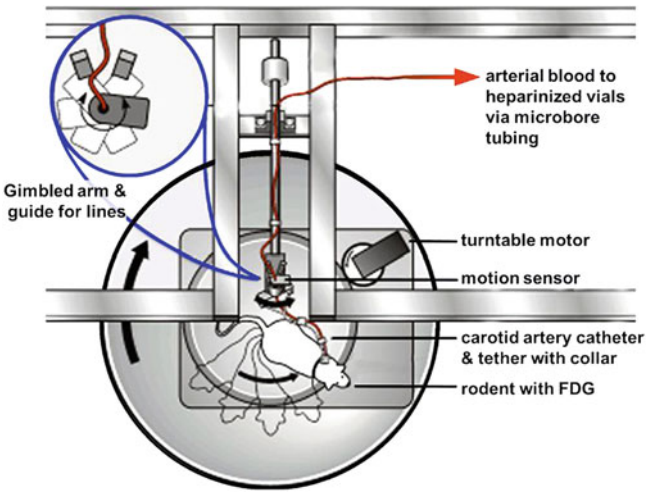


Fig. 1 Example apparatus for sampling arterial blood from freely moving animals for fully quantitative metabolic function studies. Modified BAS Raturn system (Bioanalytical Systems, Inc. [BASi] West Lafayette, IN) offer a counter-balanced arm and tethering system that allow the animal to move freely until an optical sensor is activatated, which counter-rotates the cage. This gives the animal the illusion of running when actually the cage is moving instead, while catheter lines are stabilized through the counterbalanced arm. The use of specialized microdialysis tubing minimizes dead volume to 1.2 μL/10cm, reducing the physiological burden of large volume blood samples

The compromise for performing behavioral imaging studies using these snapshot PET protocols, where radiotracer uptake occurs outside of the tomograph, is temporal resolution. Therefore, the concurrent behavioral measure must be long lasting (Schiffer et al. 2007). An example protocol is shown in Fig.1, where the

uptake and "trapping" of FDG occurs over a period of 25–45 min. Despite the relatively poor temporal resolution, the animals can move freely during radiotracer uptake in any experimental environment. Thus, animal behavior measurements can be directly related to brain activity during this period. This is fundamental to our definition of "snapshot" behavioral neuroimaging, in which measures of brain metabolism reflect the neural networks involved during the simultaneous measurement of a behavioral response.

The strengths of functional MRI and PET continue to advance the behavioral imaging field. fMRI has high temporal resolution, relative ease of application and lacks radiation exposure. In addition, fMRI provides a time course of blood oxygenation during functional imaging experiments, facilitating the use of network models such as principal or independent component analyzes (PCA or ICA, respectively). While the spatial resolution of structural MRI is orders of magnitude higher than for PET, the spatial resolution of functional MRI is roughly the same as the spatial resolution of the newest generation of small animal PET scanners. For example, a functional MRI dataset with an initial in-plane resolution of 0.8×0.8 mm smoothed with a standard filter of 1.5 voxels results in a final resolution of 1.2×1.2 mm (Steward et al. 2005), which is just over the quoted resolution of most small animal PET scanners (Magota et al. 2011).

An additional advantage of small animal PET for longitudinal behavioral imaging studies is the ability to obtain quantitative functional information from one scan to the next, such that PET outcome measures can be absolute over time. However, the variability that has plagued clinical FDG PET measurements since the initial scans were performed (Phelps et al. 1979b; Camargo et al. 1992; Alavi et al. 1994; Alkire et al. 1999) is also seen in animals; some animal subjects have higher brain metabolism than their genetically identical peers, just as some humans have higher metabolic rates than their siblings or age-matched cohorts (Bressan et al. 2004; Marsteller et al. 2006). This significant issue will be discussed below with respect to global normalization and image quantitation.

Despite the differences between PET and MRI, the literature surrounding functional MRI in rodents has been a valuable resource in the development of preclinical PET imaging protocols and procedures. It benefits any behavioral imaging study in rodents to consult both bodies of literature. For example, the important selection of a baseline task and the use of the "resting state" as a surrogate marker of disease is a compelling new direction for both modalities (see below). The success of behavioral imaging in animal models and the potential of these techniques to enable more predictive clinical treatments relies on several experimental parameters, such as the route of radiotracer administration, the imaging/anesthetic protocol, and the selection of a baseline state or condition. Next we will briefly describe the principle behind behavioral imaging with FDG PET and also with [^{11}C]-raclopride PET, another radiotracer widely employed in clinical studies of drug abuse that has recently been applied to awake imaging studies of cue-induced craving. Following, we will suggest practical experimental guidelines for behavioral imaging that can be generally applied.

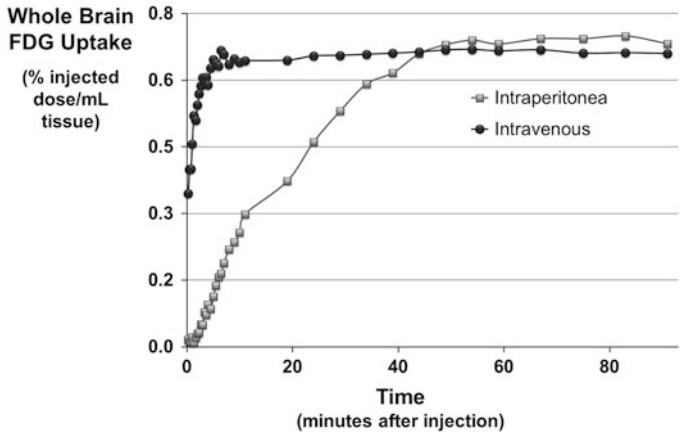


Fig. 2 Time course of intraperitoneal versus intravenous FDG uptake in the brain of the anesthetized rat. The time activity of [^{18}F] in plasma from the same animals is given in Schiffer et al. 2007

4 Behavioral Imaging of Metabolic Function with FDG

FDG is a derivative of 2-deoxyglucose that has an attached fluorine-18 isotope. 2-Deoxyglucose (DG) has been found to measure brain glucose metabolism (Sokoloff et al. 1977) and FDG a glucose analog, has similar utility. DG and FDG compete with glucose for transport sites into the brain (Phelps et al. 1979b), and when FDG is phosphorylated by hexokinase, it is transported across the membrane and becomes "trapped" intracellularly. It is important to realize that with this method, unlike functional imaging with MRI, the scanning, and the uptake period do not occur at the same time. During the uptake period, the scanning has not yet begun; the animal or the human subjects are not usually in the PET scanner, but outside performing a task. After the uptake period, the subject is placed into the tomograph for a short scan and the resulting images show the accumulated pattern of metabolic demand during the prior uptake period.

The principles underlying functional imaging with FDG PET were derived from those developed for autoradiography by Sokoloff et al. (1977). In humans, uptake of FDG and metabolic trapping in brain tissue as FDG-6-phosphate is 80–90% complete at 32 min (Phelps et al. 1979a). The eventual PET images represent the accumulated regional FDG uptake that occurred during the corresponding uptake period. Glucose metabolic rates are calculated using the deoxyglucose kinetic models of Sokoloff that were originally developed for animals, modified for humans, and now applied to animals again with small animal PET (Toyama et al. 2004b; Schiffer et al. 2006; Ravasi et al. 2011). Full quantitation of FDG requires repeated blood sampling, which can be done in freely moving animals using an in vivo microdialysis set up, as shown in Fig. 2 (Schiffer et al. 2006). However, not only does the required arterial blood sampling affect animal behavior (personal observations; animals increase locomotion and become visibly anxious), which is likely related to

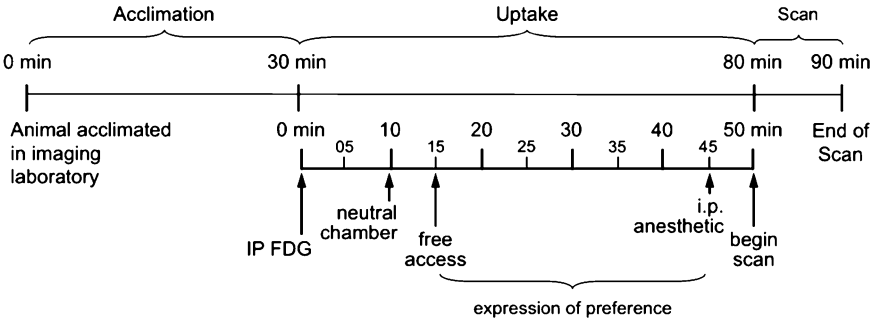


Fig. 3 Behavioral imaging protocol for FDG studies of brain function simultaneously with behavioral measurements of conditioned place preference (CPP). Animals receive an intraperitoneal (IP) injection of FDG and ten minutes later, are placed in a neutral chamber. This is based on the time course of IP FDG to reach the blood stream (Schiffer et al. 2007), which peaks 14 min after IP injection. After 10 min, animals are given free access to all chambers of the apparatus for 35 min, after which they are anesthetized and scanned for 10 min. Adapted from Carrion et al. 2009

sampling-induced activation of compensatory mechanisms such as arterial baroreceptors, it may also result in changes in blood flow or blood pressure and other physiological measurements (Kalisch et al. 2001). In addition, while microdialysis systems such as the Raturn (Bioanalytical Systems Inc., Indiana, USA) are historically referred to as "freely moving" systems (Schiffer et al. 2000), the range of available behavioral measurements is limited by the tether and the bowl (see Fig. 2).

In an extension of the experiment conducted by Sokoloff et al. (1977), it was found that the concentration of ^{18}F increases in human tissue for 90 min before plateauing (Phelps et al. 1979b). Based on previous studies and our own work with ^{18}F-FDG in rodents, it is optimal to allow at least 45 min for uptake prior to anesthesia (Kornblum et al. 2000; Schiffer et al. 2007; Wong et al. 2011). Figure 3 shows the time course of FDG in the brain with intravenous (IV) versus intraperitoneal (IP) injections. It is evident from Fig. 3 that once FDG reaches tissues and is taken up and trapped, it is fairly impenetrable for 90 min. After the 90 min period, dephosphorylation of FDG (k_4 in models of the rate of glucose metabolism) by hexokinase produces a slow decline in the time activity of FDG in these tissues. Importantly, this time period is much shorter in mice, and can be as early as 80 min (Mirrione et al. 2006, 2007; Wong et al. 2011).

Prior to image acquisition, researchers can obtain blood samples via venous sampling (i.e. tail vein). These samples provide the precise amount of ^{18}F in the bloodstream and also can be used, together with blood glucose levels, to acquire percent injected dose (Marsteller et al. 2006). We have previously shown that a single blood measure of FDG and glucose concentrations can be used to calculate a standard uptake value (SUV) that correlates to full quantitation of the rate of glucose metabolism (Schiffer et al. 2007), although this is not necessarily more reliable than using the calculated injected dose from an IP injection (Marsteller et al. 2006). Other studies have used anesthetized animals to acquire a population blood curve and demonstrated a robust and reproducible signal. However, these animals were anesthetized and not freely behaving during uptake, which must be

accounted for (Meyer et al. 2006). Similarly, image-derived input functions are also promising solutions to quantitate local metabolic rate of glucose metabolism (LMRGlu) (Huang et al. 2004; Schiffer et al. 2007), however, these animals must also be anesthetized during FDG uptake, which again, precludes simultaneous behavioral measurements.

When determining brain activations associated with a behavioral challenge, it is assumed that the changes in FDG uptake reflect an increased metabolic demand associated with changes in neuronal firing. Thus, regional changes in brain FDG uptake are only indirect reflections of changes in brain function associated with cognitive or behavioral tasks. Further, unless full kinetic modeling is used to calculate the LMRGlu in anesthetized animals, FDG uptake itself becomes the outcome measure. Thus, FDG uptake is a proxy for LMRGlu which, in turn, is a proxy for neuronal firing or inhibition. In all of this, it is critical that the fundamental principles of the FDG model are not violated by the imaging protocol or experimental design. For example, alterations in blood brain barrier permeability or changes in glucose levels during the critical period of FDG uptake can confound measurements of brain function, especially when FDG uptake is used as a proxy for LMRGlu.

FDG PET in rodent models of addiction provides an objective readout of CNS function that can inform behavioral studies of CNS disorders. This behavioral imaging paradigm introduces a novel framework for investigations concerning brain function and the effects of drug abuse on that function. This is very much an iterative process where functional measures of the physiology of drug use and abuse can be defined by neural circuitry rather than by behavior alone.

5 Behavioral Imaging of Dopaminergic Function with [^{11}C]-Raclopride

Dopaminergic neurotransmission plays a prominent role in the rewarding effects of abused drugs, such as cocaine. Binding of the radioligand, [^{11}C]-labeled raclopride, to dopamine D2 receptors is sensitive to the levels of endogenous dopamine in the synapse, which can be released either by stimulants such as cocaine or, in drug abusing patients, by the presentation of cocaine-associated cues (Volkow et al. 2006, 2008; Wong et al. 2006). In fact, behavioral imaging studies in humans have shown increased dopamine release associated with specific behavioral stimuli, such as passive reward, conditioned reward, gambling, and many others (reviewed in Egerton et al. 2009).

Several methodological and analytical approaches have been applied to [^{11}C]-raclopride studies of dopamine release following behavioral challenges in humans, each of which have different practical and methodological advantages and disadvantages. Animal studies are limited to some degree by the need to anesthetize animals for image acquisition, although our group has performed many experiments to optimize the scanning protocol such that the anesthesia has little or no effect on [^{11}C]-raclopride binding parameters (Patel et al. 2008). The basic

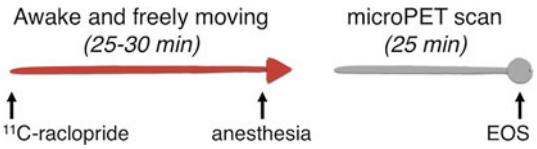


Fig. 4 Behavioral imaging protocol for [^{11}C]-raclopride studies in freely moving animals. Prior to each imaging session, polyurethane tubing is connected to a catheter port in unrestrained animals as they are allowed free movement in the home cage or test environment. [^{11}C]-raclopride is delivered as a 5–10 sec bolus and the line flushed immediately with heparinized saline. During uptake, the lines can be loosely taped to the outside of the conditioning chamber (for CPP) or routed through a swivel, as in Fig. 1. Leaving the lines in the animal permits remote administration of anesthetic without perturbing or interacting with the animal during [^{11}C]-raclopride uptake. After 30 min, ketamine/xylazine is administered through the same intravenous catheter line and animals are nonresponsive to tail pinch or eye blink in less than 10 seconds. They are then positioned in the small animal PET gantry and scanned for 25 min, after which they return to their home cage and are monitored until recovery from anesthesia

protocol is shown in Fig. 4. Following earlier studies with [^{3}H]-raclopride (Wadenberg et al. 2000, 2001), the well-established kinetic profile of [^{11}C]-raclopride and its prolonged sensitivity to changes in endogenous dopamine levels make this radiotracer an optimal ligand for behavioral imaging studies. That said, one of the most significant criticisms of [^{11}C]-raclopride as a dopamine-sensitive PET radiotracer is that the time course of the change in [^{11}C]-raclopride binding is considerably longer than the change in dopamine levels (for review, see Laruelle et al. 1997). For example, it is well known that amphetamine-induced dopamine release peaks and returns to baseline within 30 min, yet [^{11}C]-raclopride is displaced by this dopamine pulse for as long as 5 h after amphetamine administration (Houston et al. 2004). While this may be a shortcoming of the radiotracer for kinetic studies, for behavioral imaging studies in freely moving animals it provides a relatively impenetrable signal change that persists through uptake, anesthesia and scanning (Patel et al. 2008; Schiffer et al. 2009).

Importantly, with [^{11}C]-raclopride and other receptor-mediated radiotracers, the effects of specific activity (the amount of radioactivity per unit mass of unlabeled compound) is important to consider, especially when the actual image acquisition (scanning) occurs after at least one half-life of carbon-11 has passed. Contrary to intuition, high resolution PET imaging of small animals cannot be performed with tracer doses scaled down in the ratio of animal to human body mass. Indeed, it can be shown that, to a first approximation, the same absolute amount of radioactivity must be used in both subjects. The reason for this is that the size of the resolution "voxel" appropriate to the mouse or rat scales with the mass of the animal. If the statistical precision of the activity measurement in that voxel is to be equivalent to a human study, the same absolute amount of [^{11}C]-raclopride must be delivered to the small animal voxel. This requirement, in turn, implies that the same absolute amount of unlabeled raclopride must be given to the animal as to the human,since it is the raclopride that carries the radioactivity to that voxel (we will use raclopride as an example but this applies to any radiotracer with a limited number of

binding sites in the brain). However, if a human dose of [^{11}C]-raclopride is given to an animal, the concentration of raclopride in the animal may be thousands of times greater than in the human, since the animal is thousands of times less massive. The possibility exists, therefore, that such large concentrations of unlabeled raclopride could, in some cases, cause pharmacological effects in rodents rather than act as tracer doses. We have previously established the mass dose of unlabeled raclopride delivered with [^{11}C]-raclopride at a range of specific activities that causes a pharmacological effect in the brain (Schiffer et al. 2005). Simultaneous measurements of extracellular dopamine levels during small animal [^{11}C]-raclopride PET scans demonstrated that mass doses over $\sim$5 nmol/kg of unlabeled raclopride begin to influence measures of binding potential, in agreement with other reports (Hume et al. 1995; Kung and Kung 2005; Fischer et al. 2011). One way to minimize this problem is to synthesize rodent deliveries of [^{11}C]-raclopride with specific activities much higher than for human subjects. The increased sensitivity of newer generation small animal tomographs permits lower injected doses of radioactivity to obtain the same counting statistics and statistical confidence in the image. Nevertheless, with awake animal [^{11}C]-raclopride imaging, image acquisition occurs after a period of uptake that is approximately one half-life of carbon-11 (see Fig. 4), so the amount of radioactivity is at least half of that injected. Furthermore, we have shown that with awake intravenous injections of [^{11}C]-raclopride, approximately fourfold less radiotracer reaches the brain compared to [^{11}C]-raclopride delivered to an anesthetized animal (for more details, see Patel et al. 2008). This means that even more radioactivity must be injected into the animal for an awake behavioral imaging study, and further underscores the importance of high specific activity syntheses of [^{11}C]-raclopride for behavioral small animal PET. Regardless of the protocol, it is a good idea to implement a routine procedure to plot the specific activity of each injection by the measured binding potential from each scan, to ensure there are no systematic biases in radiotracer binding introduced by the mass of unlabeled ligand. Significant correlations between the mass of raclopride administered and the measured [^{11}C]-raclopride binding potential would indicate that the specific activity of the radiotracer preparation may be confounding the experimental results.

6 Technical Issues

6.1 Route of Radiotracer Administration

One major goal of preclinical behavioral imaging is to ensure that the process of imaging does not influence the animals' behavior, or is as minimally intrusive as possible. For minimal disturbance of the behavioral measure, catheterized animals are the ideal test subjects. The jugular or femoral catheter permits intravenous administration through a protracted catheter line, whose length can be extended out from an experimental test environment. This route of administration is

preferable because the injection is less of a disturbance, compared to an IP injection. However, jugular or femoral catheterization is experimentally more complicated than an IP injection, and requires flushing with heparin several times weekly to maintain the patency of the catheter.

IP injections do not require surgical catheterization, although they do involve additional animal handling during the uptake period. The IP route of administration is also problematic due to delayed and often unpredictable absorption of the radiotracer from the intraperitoneal cavity into the blood stream. This delayed absorption can result in a poorly defined temporal resolution, in addition to variable brain penetration from the IP injection. Dynamic FDG PET studies have shown that IP injections of FDG take approximately 14 min to reach the brain (Schiffer et al. 2006). Fortunately with FDG, once the radiotracer has been trapped, it is relatively impenetrable, and IP injections after 45 min of uptake do not affect the regional profile of metabolic activity (Schiffer et al. 2007; Gremese and Schulz 2011). Figure 3 shows that both IV and IP routes result in comparable brain uptake at later time points.

The question of which route of administration is "best" depends on the specific task. An IP injection of a radiotracer is optimal for experiments where behavioral measurements are performed over a long period of time. The relatively slow delivery of FDG would also be advantageous if the desired drug or task did not produce immediate effects. On the other hand, IV administration would produce a faster delivery of radiotracer or substance into the blood stream. Practically, this means that the window of imaging the drug or task effect with an IV injection should be much smaller than with an IP injection, although the temporal resolution is still limited by the duration it takes for the radiotracer to be trapped once delivered to tissues (see above). With intravenous FDG, plasma 18F concentrations peak in the blood between 3 and 5 s after FDG, whereas with IP injections, peak plasma 18F occurs 14 min after injection (Schiffer et al. 2007).

Some radiotracers, such as [^{11}C]-raclopride, are metabolized in the periphery and cannot be delivered by IP injection (personal observation). A recent study using [^{18}F]-fallypride also demonstrated limited utility of IP injections for awake animal studies of dopamine dynamics (Yoder et al. 2011).

6.2 *Effects of Anesthesia on Functional Imaging with "Snapshot" Protocols*

It should be noted that, for functional imaging with FDG and a burgeoning number of radiotracers such as [^{11}C]-raclopride, a single PET scan is taken immediately at the end of a period of tracer uptake, at a time when the animals can be immobilized by anesthesia with minimal, if any, effect on tracer distribution (Matsumura et al. 2003; Schiffer et al. 2007; Patel et al. 2008; Ravasi et al. 2011). This procedure avoids the need to use anesthesia to immobilize the animal during the critical period of tracer uptake by the brain while the animal is free to behave naturally.

The use of catheterized animals or remote infusion pumps (Holschneider et al. 2002, 2003) allow the delivery of both radiotracer and anesthetic without any interaction from the investigator. For this reason, an important avenue for functional imaging in rodents will be to further remove any interaction with the investigator, as both radiotracer and anesthetic can be delivered remotely (Schiffer et al. 2009).

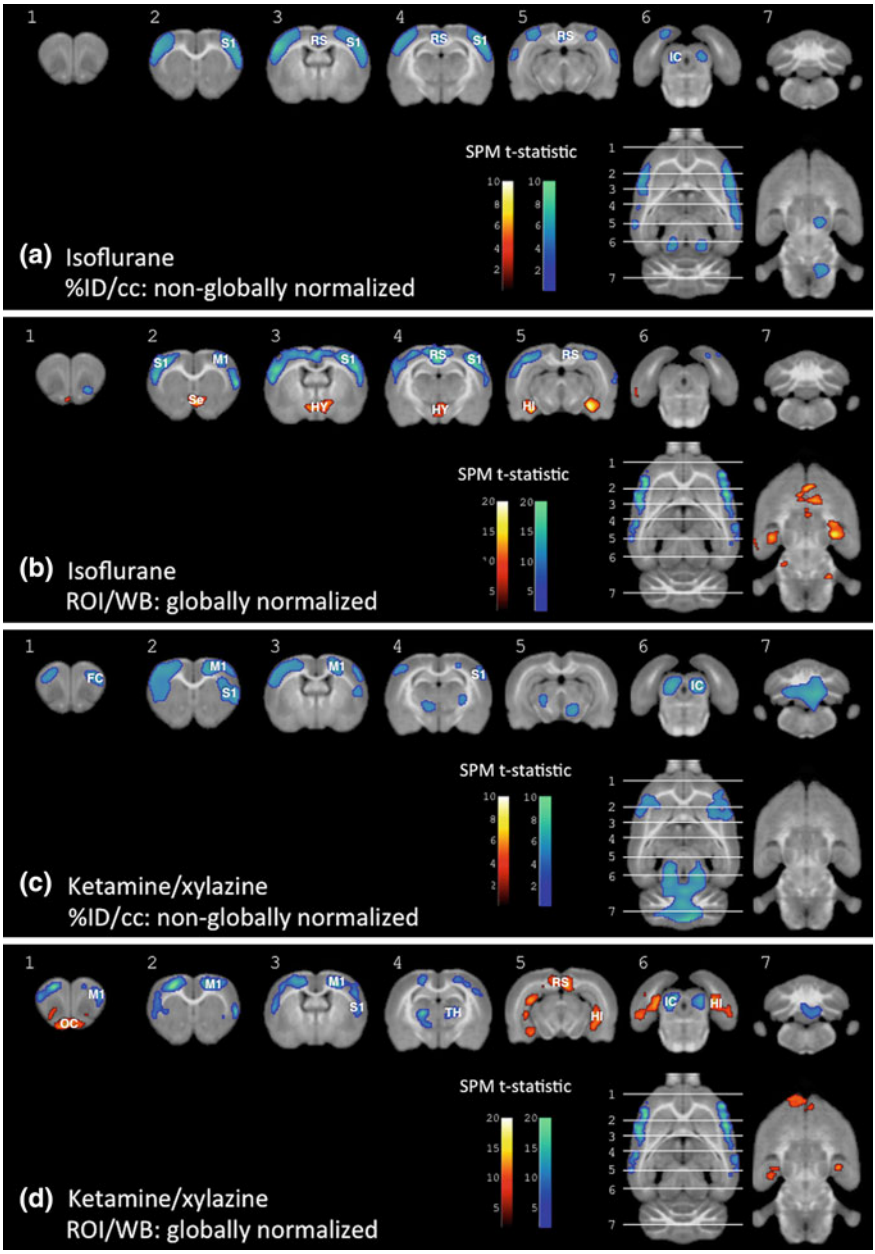
Inevitably, animals must be anesthetized for scanning. We rely on the impenetrable nature of specific radiotracers (here, FDG and [^{11}C]-raclopride) to not be affected by anesthesia at this late stage of scanning.

6.3 The Use of Anesthesia for Behavioral Imaging Studies in Animals

Many behavioral imaging research reports appropriately address the issue of anesthesia, as well as restraint stress or training, as part of their discussion. Indeed, it is our experience that journal referees and conference attendees frequently draw attention to the subject of anesthesia. However, there is very little synthesis of understanding with respect to either the use of anesthesia in these animal models or the differential effects of distinct anesthetics. Each laboratory is well able to justify their choice of anesthetic regime, training schedule, or restraint device; some because they appear to preserve normal neuronal function, others because they have minimal effects on peripheral physiological functions. Discrepancies in results between studies are commonly ascribed to differences in the anesthetic regime, training schedule or restraint device, and while these explanations are plausible, they are not illuminating. Data reported from studies using anesthetized, restrained, or trained animal models have direct implications for the design, analysis and interpretation of neuroimaging studies using awake humans. It is thus just as important to consider the effects of these experimental modifications as it is species differences. There are problems associated with movement artifacts and there is a danger of supplanting the adverse effects of anesthesia with those of restraint stress. Our "snapshot" protocol circumvents some of these limitations at the cost of temporal resolution. The awake animal models described here, though modeled as much after clinical FDG and [^{11}C]-raclopride PET studies as possible, do not provide a complete solution. It will be critical for more users to employ these types of behavioral imaging studies to both validate and push the field forward.

6.4 Defining the Baseline State

A critical challenge in behavioral neuroimaging in animals is to identify the control or baseline condition. This can greatly impact the interpretation of functional imaging studies. In a standard functional imaging design, brain activity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(a) Isoflurane
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(b) Isoflurane
ROI/WB: globally normalized
FC
(c) Ketamine/xylazine
%ID/cc: non-globally normalized
OC
TH
(d) Ketamine/xylazine
ROI/WB: globally normalized

◀**Fig. 5** Voxel-based analysis of anesthesia-induced changes in FDG uptake. Voxel values, percent change from awake, resting baseline and abbereviations for each region are given in Table 2. SPM t-maps represent changes in brain FDG uptake from rats (n = 8) tested three times: awake, resting state uptake versus anesthetized FDG uptake with first isoflurane (a and b) followed one week later by ketamine/xylazine (c and d). All SPM t-maps were corrected for multiple comparisons using False Discovery Rate (FDR) correction with a threshold of $p < 0.001$. Non-globally normalized SPM t-maps were not proportionally scaled (a and c) and represent the significance of the change in FDG uptake expressed as percent injected dose per mL brain tissue. For the globally normalized SPM t-maps (b and d), global differences were adjusted by the SPM software in each animal by scaling the voxel intensities so that the mean intensity for each brain is the same (proportional scaling). Analysis of the values in Table 2 show that increases on globally normalized SPM t-maps (for example, in the hippocampal regions of b and d) represent those regions that are less suppressed by anesthesia when non-globally normalized values are compared (a and c)

during two conditions is compared by subtraction—identifying the difference between brain areas that change activation state (increase or decrease) in the experimental condition, relative to a baseline condition. This requires great attention to matching the challenge condition to an appropriate baseline condition. Depending on the complexity of the behavioral model within a behavioral imaging study design, this may represent a formidable challenge.

In longitudinal studies, animal subjects serve as their own control, rather than comparing separate control and challenge groups. Longitudinal studies provide the advantage of using the same animals in a serial scan design, which reduces animal numbers. That said, subtle confounding factors such as repeated handling and anesthesia can complicate the interpretation of behavioral imaging data. In some cases, it is a necessary advantage to have animals that have been handled (or not handled) in exactly the same manner. For example, in longitudinal designs where the loss of a cage-mate leaves one animal singly housed, subtle effects on the behavior of the animal can impact functional imaging studies or the acquisition of a learned behavior (Langford et al. 2006, 2010).

Additional error may be introduced in longitudinal designs from repositioning errors of the subject's head within the scanner. While software for spatial pre-processing may aid in minimizing repositioning errors, detailed reference test-retest studies are needed to identify the variation between two studies on the same animal subjects under the same baseline condition (Marsteller 2006).

The ability of anesthetics to change global baseline brain function has created the opportunity to examine the relevance of global baseline (resting) brain activity in terms of region-specific cerebral processing in humans and in animals (Shulman et al. 2003; Vincent et al. 2007). Because FDG can be carried out in both awake and anesthetized animals, many of the initial small animal PET studies focused on the effects of anesthesia on brain metabolism by comparing awake and anesthetized states (for example, see Matsumura et al. 2003; Shimoji et al. 2004; Toyama et al. 2004a). A different way to use the anesthetized state is to juxtapose it to a resting baseline state, to determine which brain regions might be activated at rest

by those which are deactivated under anesthesia, and vice versa (Vincent et al. 2007). In this way, the ability of anesthetics to alter brain metabolism has been exploited as a tool to examine the contributions of global baseline or resting brain function to specific tasks controlled by discrete brain areas (Shulman et al. 2003; Vincent et al. 2007). In this regard, we have compiled data from our previous study comparing awake, freely moving uptake to the same animals under ketamine anesthesia (Schiffer et al. 2007) with new data using the same protocol in which animals were scanned in a freely moving state followed 1 week later by scanning under ketamine anesthesia, and 1 week later under isoflurane anesthesia (Fig. 5). Together, this data highlights several important points both about the resting state in animals, as well as the potentially misleading presentation of globally normalized data analyzed with voxel-based analyzes.

Furthermore, the baseline condition or basal brain function can possibly drive experimental results where resting state scans are obtained from the same animals over time. However, preclinical resting state scans are increasing in popularity because of their coherence to clinical resting state scans. Clinical studies have shown, for instance, that there is a distinct pattern of functional connections in the resting state that distinguish both Alzheimer's disease patients and Parkinson's patients from age-matched healthy controls (Huang et al. 2007; Horwitz and Rowe 2011). This study design is appealing to small animal functional imaging for several reasons. First, no training is required and no task performance necessary during image acquisition; all imaging is performed during the resting state. Second, and related, is that no interpretation of behavioral results are necessary to determine how relevant the animal model is to the clinical disease; the brain scans provide an independent, biological measure of the similarities and differences between the animal model and human resting state scans. For these reasons, resting state studies hold promise for determining the clinical relevance of genetically modified animal models of human disease where there is evidence of functional connectivity alterations at rest (Ulug et al. 2011). However, global changes in brain function can confound the interpretation of differences, especially in resting state conditions. In Fig. 5, data from anesthetized animals compared to an awake, resting state baseline state show that global normalization of pixel values can be misleading. When globally normalized, positive t-scores do not represent increases in activity per se, but actually reflect those areas in which the decreases in brain FDG uptake were of less magnitude than the change in global mean. Template-based ROI values for the same data in Fig. 5 are given in Table 2, where it is evident in regions such as the hippocampus that increases in normalized values (ROI/WB) actually represent less of a decrease in absolute FDG uptake (%ID/cc).

In clinical studies of brain function, the argument has also been posed that the baseline condition is rarely predictive of the activation condition (Morcom and Fletcher 2007; Hyder and Rothman 2011). That is, basal brain function is not a predictor of brain function in activated states. Evidence in support of this notion has also recently been shown with electrophysiological studies in animals (Li et al. 2011). However, basal brain function is a significant confound to studies that have

Table 2 Regional changes in FDG uptake with (a) isoflurane anesthesia, (b) ketamine anesthesia

Brain region	%ID/cc					ROI/WB				
	Awake		Anesthetized		%Δ	Awake		Anesthetized		%Δ
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
a. Isoflurane anesthesia										
NAcc	0.52	0.07	0.44	0.09	-16^{*}	1.17	0.03	1.24	0.05	7^{*}
Amyg	0.42	0.06	0.35	0.06	-15	0.93	0.04	1.01	0.03	9^{***}
CPu	0.58	0.08	0.47	0.09	-19^{*}	1.28	0.03	1.33	0.05	4^{*}
S1	0.61	0.08	0.38	0.07	-38^{***}	1.35	0.03	1.07	0.03	-21^{***}
M1	0.64	0.09	0.44	0.08	-31^{**}	1.42	0.02	1.26	0.03	-11^{***}
ACC	0.67	0.09	0.48	0.09	-28^{**}	1.48	0.03	1.36	0.06	-8^{***}
FC	0.60	0.09	0.46	0.09	-23^{*}	1.34	0.02	1.32	0.05	-1
Ins	0.50	0.06	0.37	0.07	-25^{**}	1.10	0.03	1.06	0.02	-4
HIP_{ant}	0.44	0.05	0.37	0.07	-15	0.98	0.02	1.07	0.03	9^{***}
HIP_{post}	0.39	0.05	0.35	0.06	-10	0.86	0.03	0.99	0.03	15^{***}
HY	0.40	0.05	0.36	0.07	-11	0.89	0.04	1.02	0.02	14^{***}
Sup Coll	0.55	0.07	0.42	0.07	-25^{**}	1.23	0.03	1.19	0.05	-3
CB	0.38	0.05	0.31	0.05	-18^{*}	0.84	0.03	0.88	0.04	5
Inf Coll	0.60	0.09	0.43	0.07	-28^{**}	1.35	0.05	1.23	0.08	-9^{**}
TH	0.55	0.11	0.41	0.08	-25^{**}	1.26	0.02	1.22	0.03	-3^{*}
RSC	0.54	0.08	0.37	0.07	-31^{**}	1.20	0.02	1.07	0.03	-11^{***}
OC	0.53	0.07	0.41	0.08	-23^{**}	1.17	0.02	1.16	0.03	-2
Sept	0.41	0.06	0.36	0.08	-12	0.91	0.05	1.01	0.07	11^{*}
b. Ketamine anesthesia										
NAcc	0.52	0.07	0.41	0.11	-21	1.17	0.03	1.26	0.04	9^{*}
Amyg	0.42	0.06	0.32	0.09	-24^{**}	0.93	0.04	0.97	0.03	4^{**}
CPu	0.58	0.08	0.41	0.11	-28^{**}	1.28	0.03	1.27	0.03	-1

(continued)

Table 2 (continued)

Brain region	%ID/cc					ROI/WB				
	Awake		Anesthetized		%Δ	Awake		Anesthetized		%Δ
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
S1	0.61	0.08	0.40	0.10	-34^{***}	1.35	0.03	1.12	0.02	-17^{**}
M1	0.64	0.09	0.42	0.10	-35^{***}	1.42	0.02	1.29	0.03	-9^{***}
ACC	0.67	0.09	0.49	0.09	-26^{**}	1.48	0.03	1.50	0.05	1
FC	0.60	0.09	0.45	0.12	-25^{**}	1.34	0.02	1.37	0.03	3^{***}
Ins	0.50	0.06	0.34	0.09	-31^{***}	1.10	0.03	1.05	0.03	-5^{**}
HIP_{ant}	0.44	0.05	0.36	0.09	-19^{**}	0.98	0.02	1.10	0.03	12^{***}
HIP_{post}	0.39	0.05	0.31	0.08	-19^{**}	0.86	0.03	0.96	0.03	11^{***}
HY	0.40	0.05	0.30	0.08	-26^{**}	0.89	0.04	0.91	0.03	2
Sup Coll	0.55	0.07	0.37	0.10	-34^{***}	1.23	0.03	1.12	0.02	-8^{***}
CB	0.38	0.05	0.26	0.06	-33^{**}	0.84	0.03	0.79	0.03	-6^{***}
Inf Coll	0.60	0.09	0.37	0.10	-39^{***}	1.35	0.05	1.12	0.04	-17^{***}
TH	0.55	0.11	0.39	0.12	-30^{***}	1.26	0.02	1.20	0.02	-5^{***}
RSC	0.54	0.08	0.45	0.13	-17	1.20	0.02	1.38	0.08	14^{***}
OC	0.53	0.07	0.42	0.12	-20^{*}	1.17	0.02	1.29	0.04	10^{***}
Sept	0.41	0.06	0.31	0.09	-24^{***}	0.91	0.05	0.86	0.02	-6^{*}

NAcc Nucleus accumbens, *Amyg* Amygdala, *CPu* Caudate putamen, *S1* Primary somatosensory cortex, *M1* Motor cortex, *ACC* Cingulate cortex, *FC* Frontal cortex, *Ins* Insula, HIP_{ant} Hippocampus (anterodorsal), HIP_{post} Hippocampus (posterior), *HY* Hypothalamus, *Sup Coll* Superior colliculus, *CB* Cerebellum, *Inf Coll* Inferior colliculi, *TH* Thalamus, *RSC* Retrosplenial cortex, *OC* Olfactory cortex, *Sept* Septum

$^{***}p < 0.001$, significant difference from awake baseline

** $p < 0.01$, significant difference from awake baseline

* $p < 0.05$, significant difference from awake baseline

no challenge or perturbation and that seek to rely on serial resting state scans to distinguish pathological conditions. This is really only relevant to studies that use the resting state as a condition that is "subtracted" from the baseline state (Hyder and Rothman 2011). Nevertheless, it can significantly hamper efforts to mimic clinical resting state studies in transgenic animals.

7 Convergence or Divergence of Behavioral and Imaging Data, of Human and Animal Data

Despite research that describes a well-defined relationship between brain function and behavior, there are circumstances in which divergence between behavioral and brain functional measures occur. Even so, the absence of a concurrent behavioral effect does not mean that regional changes in brain function are insignificant. In fact, it may be that activation of neural circuits do not always manifest in a clear behavioral response (Brodie 1996). This becomes especially complicated when extrapolating animal brain/behavior relationships to human brain/behavior relationships. More generally, not enough might yet be known about the structure of rodent cognition to say how behavioral measures should constrain interpretations of brain activation, if they should at all.

The main point we wish to highlight is that there may be legitimate circumstances where behavioral and neural activation measures diverge. There is a danger in rejecting significant patterns of brain activation just because they are not mirrored behaviorally, especially in animals. In clinical studies there are many reports in which an experimental perturbation changes response at the imaging, but not the behavioral level (for review, see Wilkinson et al. 1998; Erlandsson et al. 2003). The reverse has also been shown, in which significant effects are found in the behavioral, but not the imaging data (Grady et al. 2001).

8 Conclusions

Preclinical behavioral neuroimaging offers a platform for measuring concurrent changes in animal brain function and behavior. Brain imaging, in particular, is one technique that has cross-species consistency, as do various operant conditioning procedures. Using PET and radiotracers such as [^{11}C]-raclopride or FDG, preclinical behavioral imaging studies can closely parallel functional imaging experiments in humans. Small animal behavioral imaging, like all new disciplines, must find converging support where and when it can. There are two main areas for such support. The first can be derived from concordance with analogous functional imaging experiments in humans, and the second through multiple ancillary measures that are only accessible to preclinical imaging. These include additional measurements that may not be possible from human subjects, such as simultaneous in vivo microdialysis, electrophysiology, or histological measures to corroborate the imaging itself, in addition, to the behavioral responses.

Preclinical imaging of brain systems offers exciting opportunities to better understand the neurobiology of drug abuse and addiction. Using a dual (behavior and imaging) paradigm, we are better able to interrogate how well our animal models parallel human conditions and diseases. The behavioral imaging techniques (PET and fMRI) have emerged as fundamental tools for studying brain

structure, network connectivity, and the responses to pharmacological challenges in animal models (reviewed in Borsook et al. 2006; Lancelot and Zimmer 2010; Cumming et al. 2011). Small animal PET and fMRI instrumentation have become more sophisticated, yet easier to operate. This has enabled a greater number of researchers the opportunity to conduct small animal investigations leading to more advances in the preclinical imaging field.

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Molecular Imaging and the Neuropathologies of Parkinson's Disease

Paul Cumming and Per Borghammer

Abstract The main motor symptoms of Parkinson's disease (PD) are linked to degeneration of the nigrostriatal dopamine (DA) fibers, especially those innervating the putamen. This degeneration can be assessed in molecular imaging studies with presynaptic tracers such as [^{18}F]-fluoro-*L*-DOPA (FDOPA) and ligands for DA transporter ligands. However, the pathologies of PD are by no means limited to nigrostriatal loss. Results of post mortem and molecular imaging studies reveal parallel degenerations of cortical noradrenaline (NA) and serotonin (5-HT) innervations, which may contribute to affective and cognitive changes of PD. Especially in advanced PD, cognitive impairment can come to resemble that seen in Alzheimer's dementia, as can the degeneration of acetylcholine innervations arising in the basal forebrain. The density of striatal DA D_2 receptors increases in early untreated PD, consistent with denervation upregulation, but there is an accelerated rate of DA receptor loss as the disease advances. Animal studies and post mortem investigations reveal changes in brain opioid peptide systems, but these are poorly documented in imaging studies of PD. Relatively minor changes in the binding sites for GABA are reported in cortex and striatum of PD patients. There remains some controversy about the expression of the 18 kDa translocator protein (TSPO) in activated microglia as an indicator of an active inflammatory component of neurodegeneration in PD. A wide variety of autonomic disturbances

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contribute to the clinical syndrome of PD; the degeneration of myocardial sympathetic innervation can be revealed in SPECT studies of PD patients with autonomic failure. Considerable emphasis has been placed on investigations of cerebral blood flow and energy metabolism in PD. Due to the high variance of these physiological estimates, researchers have often employed normalization procedures for the sensitive detection of perturbations in relatively small patient groups. However, a widely used normalization to the global mean must be used with caution, as it can result in spurious findings of relative hypermetabolic changes in subcortical structures. A meta-analysis of the quantitative studies to date shows that there is in fact widespread hypometabolism and cerebral blood flow in the cerebral cortex, especially in frontal cortex and parietal association areas. These changes can bias the use of global mean normalization, and probably represent the pathophysiological basis of the cognitive impairment of PD.

Keywords Parkinson's disease · PET · Dopamine · Serotonin · Noradrenaline · Receptors · TSPO · Cerebral blood flow · CMRglc · SPECT

Contents

1 Introduction

The loss of pigmentation in the substantia nigra was noted in post mortem brain of patients dying following the epidemic of post-encephalitic parkinsonism (von Economo 1931). Once the neurochemical anatomy of monoaminergic neurons had been described in human brain (Carlsson et al. 1962), the motor symptoms of

Parkinson's disease (PD) could be linked to the degeneration of the mesencephalic DA neurons, with consequent loss of DA innervation to the extended striatum. In general, the cardinal motor symptoms of PD, i.e. rigidity and bradykinesia are alleviated with DA substitution via the precursor levodopa, or with direct DA receptor agonists. However, the therapeutic response to these pharmacological treatments is ultimately marred by the emergence of motor complications as the disease advances. Furthermore, patients with advanced PD frequently develop dementia (Aarsland et al. 2005), depression, anxiety, and other symptoms which are generally unresponsive to DA substitution therapy. While the greatest emphasis has been placed on PET investigations of neurochemical pathology in relation to motor symptoms, the cognitive and affective manifestations of PD cannot be ignored. It seems imperative to understand PD not as a disease *sensu strictu* of the nigrostriatal pathway, but as a widespread systemic disease affecting multiple sites in the central nervous system and multiple organ and sensory systems. A comprehensive understanding of the protean neuropathology of PD may be a prerequisite for designing effective neuroprotective strategies, and for appreciating the spectrum of cognitive and affective changes that occur in PD, as distinct from the motor symptoms.

2 Dopamine

PET imaging of nigrostriatal degeneration with the DOPA decarboxylase substrate 6-[^{18}F]-fluoro-*L*-DOPA (FDOPA) was one of the first successes of molecular imaging of PD (Nahmias et al. 1985). In many subsequent FDOPA PET studies, the net influx to brain of FDOPA (K_{in}^{app}; ml g^{-1} min^{-1}) is calculated based upon the assumption that the [^{18}F]fluorodopamine formed in striatum is irreversibly trapped within synaptic vesicles, mainly within dopaminergic neurons. However, this trapping is reversible due to the catabolism of [^{18}F]fluorodopamine to acidic metabolites; their diffusion from brain is especially evident in FDOPA recordings lasting longer than one hour. As an alternative index of the status of DA innervations, the FDOPA steady-state binding capacity can be calculated (V_D; ml g^{-1}). This distribution volume can be defined as the ratio of FDOPA utilization in brain tissue divided by the elimination rate constant of [^{18}F]fluorodopamine, which is best calculated from dynamic FDOPA recordings lasting 2 hours or more, wherein the progressive washout of [^{18}F]fluorodopamine is clearly evident. This approach resolves a paradox in the literature that FDOPA utilization is unchanged with healthy aging (Kumakura et al. 2010b); as seen in Fig. 1a, the FDOPA K_{in}^{app} is nearly identical in groups of healthy young and old subjects, whereas there is a substantial loss in FDOPA V_D with age. The steady-state approach also shows an even greater impairment in FDOPA V_D in putamen of early PD patients (Kumakura et al. 2006), contralateral to the main symptoms, and also throughout the cerebral cortex, as also presented in Fig. 1a. We attribute these changes to declining vesicular binding capacity within the composite of monoamine innervations, i.e. DA

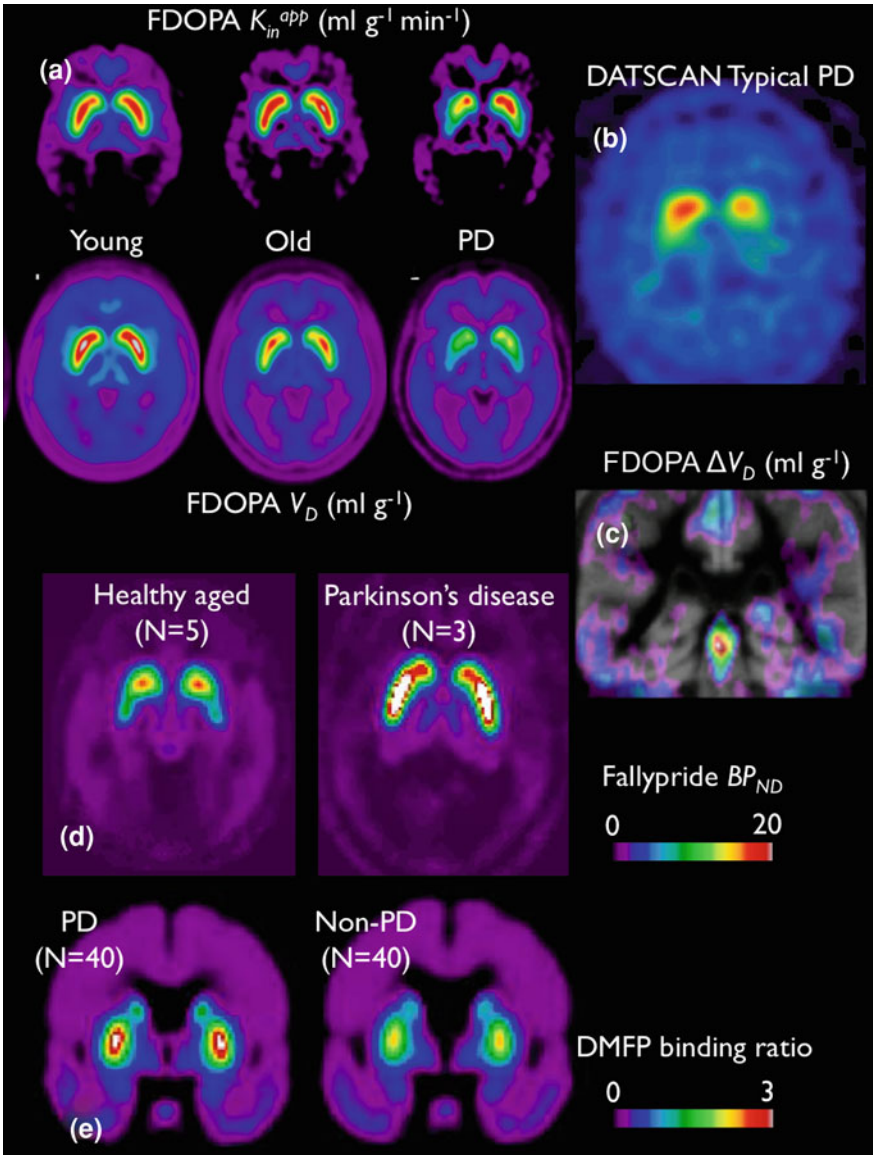

Fig. 1 **a** Mean parametric maps in groups of healthy young (<40 years), healthy old (>50 years) and Parkinson's disease (PD) patients, showing the FDOPA net blood brain clearance (K_{in}^{app}; *upper row*), and the steady-state distribution volume (V_D) [reproduced with permission from Kumakura and Cumming (2009)], **b** a representative DATSCAN image from a patient with early asymmetric PD **c** the increased FDOPA trapping (V_D) in the dorsal raphe nucleus of a group of PD patients [reproduced with permission from Kumakura et al. (2010a)], **d** binding sites for [^{18}F]-fallypride in brain of healthy elderly subjects, and untreated PD patients, and **e** the use of DMFP-PET for the discriminative diagnosis of PD and non-PD basal ganglia disease

noradrenalin and serotonin. Impaired retention of [^{18}F]fluorodopamine is also evident in FDOPA PET studies of monkeys with MPTP-induced parkinsonism (Cumming et al. 2001), consistent with the increased DA metabolite ratios measured post mortem (Pifl and Hornykiewicz 2006). Dopamine content is typically reduced by 95% in post mortem putamen from PD patients, in whom the disease has run its full course. However, the relative concentration of DA metabolites such as homovanillic acid is substantially increased in putamen from PD patients (Bokobza et al. 1984). Insofar as metabolite ratios indicate the exposure of cytosolic DA to degradation by monoamine oxidase, the *post mortem r*esults support a PET-derived model of PD in which DA storage capacity cannot accommodate the residual capacity to synthesize DA, which is relatively preserved in parkinsonian striatum.

Since the advent of FDOPA PET, diverse agents have been developed for imaging reuptake sites, vesicular transporters, and post-synaptic sites in the nigrostriatal DA system. In a multi-tracer PET study of PD patients, the relative decline in the striatal binding of [^{11}C]-methylphenidate to DA transporters exceeded the declines in the binding of [^{11}C]-dihydrotetrabenazine to DA vesicles, and of the trapping of FDOPA in nigrostriatal terminals (Lee et al. 2000). A similar phenomenon is documented in a triple-tracer PET study of aged and MPTP-lesioned monkeys (Doudet et al. 2006). These results likely reveal a compensatory decrease in the capacity for clearance of interstitial DA, which would enhance the temporal duration and spatial extension (also known as volume transmission) of DA's action in the healthy aged striatum, and to an even greater extent in PD striatum. The behavioral consequences of this altered DA signaling are poorly understood, but may manifest as declining reaction time in motor tasks.

The apparent down-regulation of DA transporters contributes to the sensitivity of molecular imaging for the detection of degeneration of nigrostriatal fibers. The tracer [^{123}I]-ioflupane, commonly known as DATSCAN, had found wide use as a SPECT agent for clinical diagnosis of PD. Figure 1b shows typical findings of asymmetric loss of DA transporters in putamen, contralateral to the main motor symptoms. However, DATSCAN does not reveal the relatively sparse extra-striatal catecholamine innervations, which can be detected with [^{11}C]-nomifensine.

In the primate, cortical DA innervations are concentrated in the motor and premotor cortex and in supplementary motor areas (Berger et al. 1986; Gaspar et al. 1991), unlike the case of rodent brain, in which the prefrontal and limbic cortices have a relatively dense DA innervation. Immunocytochemical analysis of the human motor cortex reveals that DA fibers containing only tyrosine hydroxylase (and not dopamine β-hydroxylase) are twice as abundant as are NA fibers, which contain both enzymes (Gaspar et al. 1991). Tyrosine hydroxylase fibers are 80% depleted in primary motor cortex and premotor cortex of PD patients, especially in the superficial layers. Asymmetry in cortical binding of the catecholamine transporter ligand [^{11}C]-nomifensine correlated with clinical motor symptoms (rigidity and tremor) especially in ventromedial prefrontal cortex of early PD patients with asymmetric motor symptoms (Marie et al. 1995) In healthy subjects, FDOPA uptake in caudate and putamen correlated with performance of frontal cortical tasks such as the Stroop test (Vernaleken et al. 2007). However,

PET studies with 6-[^{18}F]fluoro-*meta*-tyrosine, an alternate tracer of catecholamine synthesis, showed a complex relationship with cognition, whereby high tracer uptake in the caudate of young subjects correlated with lower performance of a frontal task (Braskie et al. 2008), in the manner of an inverted U relationship between dopamine and cognitive performance, as has bee nreported for DA D1 receptors in frontal cortex (Takahashi et al. 2008). Nonetheless, high FMT uptake in striatum predicted better performance in a working memory task (Cools et al. 2008). Pharmacological blockade of only 25% of striatal DA receptors (Mehta et al. 2008) evoked cognitive deficits in healthy young subjects similar to those seen in PD (Mehta et al. 1999). Consistent with cognitive deficits emerging before motor symptoms in early PD, deficit in performance of a delayed alternation task has been noted in MPTP-poisoned monkeys without frank motor symptoms, despite increased DA metabolite ratios in post mortem striatum (Schneider 1990). In early PD, cortical FDOPA uptake is reportedly *increased* in association with impaired performance of the Stroop task, and of a test of sustained attention (Bruck et al. 2005), perhaps reflecting an early adaptive change in cortical function in response to a primary nigrostriatal degeneration. Given the relative preservation of DA in the caudate nucleus of early PD patients, this structure has been particularly implicated in cognitive performance. Indeed, a principle component analysis of cerebral blood flow revealed greater expression of cortical networks associated with tactile discrimination of object shape in the sub-group of PD patients with better preserved FDOPA uptake in the caudate nucleus (Weder et al. 2000). Furthermore, FDOPA uptake in caudate of PD patients correlated with impaired performance of verbal memory tests, although atrophy in hippocampus emerged as a more important factor determining cognitive changes (Jokinen et al. 2009). The (inherently rather low) FDOPA uptake in hippocampus of PD patients correlated with performance of the Raven's Colored Progressive Matrices (Nagano-Saito et al. 2004). In advanced PD, the FDOPA uptake in caudate correlated with scores in tests of memory and verbal fluency (an executive function of the frontal lobe), whereas the uptake in putamen uptake correlated with mental flexibility or switching, perhaps arising from impaired motor function in the execution of tasks such as the trail making task (van Beilen et al. 2008).

In healthy subjects, FDOPA uptake specifically in the ventral striatum correlated with affective processing in the frontal cortex, as assessed by BOLD signal changes evoked by presentation of emotionally laden visual stimuli (Siessmeier et al. 2006). Loss of emotional reactivity is well established in PD. Nonetheless, this phenomenon is scarcely investigated by molecular imaging. In one such study, the binding of [^{11}C]-methylphenidate to DA transporters in left putamen correlated with emotionally evoked BOLD changes in the ventrolateral prefrontal cortex of PD patients (Lotze et al. 2009). Depression is likewise frequently encountered in PD. In one PET study, FDOPA uptake in striatum correlated specifically with the cognitive items on an inventory of depression (Koerts et al. 2007). In patients with PD, higher scores for anxiety and depression were associated with reduced DA transporter availability in the left striatum, but only in those patients with less severe PD (Weintraub et al. 2005). This finding of a link between DA loss

specifically in the left striatum and depression, as well as impaired performance of the Tower of London task, was replicated in a [^{123}I]-FP-CIT SPECT of DA transporters in PD patients (Rektorova et al. 2008). Reduced uptake of [^{11}C]-nomifensine in the right putamen correlated with impaired performance of object alternation and conditional associative learning tasks in non-demented PD patients (Marie et al. 1999). Loss of olfactory function is commonly an early presentation of PD. In healthy aged subjects, loss of olfactory sense was most pronounced in those with age-associated (i.e. non-clinical) nigrostriatal degeneration, as revealed by [^{11}C]-β-CFT (Wong et al. 2010).

The abundance of striatal DA D_2 receptors declines by 5–10% per decade of normal aging [e.g. (Antonini et al. 1993)]. Elevated binding of the DA $D_{2/3}$ antagonist ligand [^{11}C]-raclopride is reported in striatum of never-treated patients with early PD (Antonini et al. 1994), consistent with a compensatory up-regulation in response to denervation, as seen in rats with 6-hydroxydopamine lesions (Palner et al. 2010). The elevated binding of the high affinity DA $D_{2/3}$ ligand [^{18}F]-fallypride in putamen of a group of early PD patients is illustrated in Fig. 1d. Despite this early upregulation, follow-up [^{11}C]-raclopride PET studies show an accelerated rate of loss of DA receptors in patients with more advanced PD (Antonini et al. 1997), presumably occurring in parallel with the presynaptic changes described above. However, the rate of this loss is even more pronounced in patients with more aggressive parkinsonian syndromes such as progressive supranuclear palsy. Consequently, molecular imaging with the DA $D_{2/3}$ receptor ligand [^{18}F]-DMFP (Fig. 1e) can aid is the discriminative diagnosis of PD (la Fougere et al. 2010b).

3 Medullary Catecholamine Neurons

The locus coeruleus (LC) is a compact group of NA neurons located on either side of the fourth ventricle, containing approximately 15,000 neurons per side in the human (Ohm et al. 1997). The LC gives rise to NA innervations of the entire neuraxis, with some axons collateralizing to divergent targets such as the cerebellum or cerebral cortex and spinal cord (Loughlin et al. 1982). The number of pigmented neurons in the LC was reduced by 75% in PD patients (Gai et al. 1991), and this reduction has been linked to a history of depression (Frisina et al. 2009). On the other hand, the concentrations of NA and its metabolites were decreased in LC of PD patients with dementia, but were normal in non-demented PD patients, irrespective of their history of mood disorders (Cash et al. 1987). It is generally thought that LC loss is a late manifestation of PD, as supported by FDOPA-PET, which revealed reduced uptake in the LC only in advanced PD (Moore et al. 2008). However, loss of LC neurons in PD can eventually come to exceed that of DA neurons (Zarow et al. 2003). In PD patients without concurrent diagnosis of Alzheimer's dementia, the extent of LC cell loss correlated with severity of dementia

symptoms (Zweig et al. 1993). The extent of degeneration of LC neurons, most especially in PD patients who had suffered from depression, is comparable to that seen in Alzheimer's dementia (Chan-Palay and Asan 1989). LC lesions exacerbate the progression of the MPTP model of parkinsonism in marmoset monkey, (Mavridis et al. 1991), and it has been proposed that LC degeneration can predispose to the development of levodopa dyskinesia (Fornai et al. 2007). This observation led to the suggestion that specifically adrenergic aspects of PD should be the focus of new treatments, with a particular emphasis on dementia symptoms and disease progression (Rommelfanger and Weinshenker 2007).

In a PET study with the non-specific catecholamine uptake site ligand [^{11}C]-RTI, binding was especially reduced in the limbic cortex and thalamus of PD patients with notable depression symptoms (Remy et al. 2005). PET ligands selective for the NA transporter have only recently become available. In one study, age-related loss of the NA transporter was revealed in LC, hypothalamus, and thalamus (Ding et al. 2010), whereas another PET ligand has revealed loss of NA innervations in post mortem material from AD patients (Gulyas et al. 2010). However, PET findings with this class of ligands have not yet been reported in PD patients. In an early report, the densities of post-synaptic α_1 and β_1 adrenergic receptors was increased, whereas α_2 receptors were decreased in frontal cortex from PD patients, which was attributed to LC degeneration and up-regulation of post-synaptic binding sites (Cash et al. 1984). Binding of α_2 receptors in porcine cerebral cortex has been demonstrated with [^{11}C]-yohimbine (Jakobsen et al. 2006), and [^{11}C]-mirtazepine (Smith et al. 2006). The availability of binding sites for the latter compound was reduced in medication-free depressed patients who had earlier failed to benefit from antidepressant medication (Smith et al. 2009). There have been as yet no studies of pre- or post-synaptic markers of adrenergic innervations in PD patients.

Weakly melanized adrenaline neurons expressing the enzyme phenylethanolamine N-methyltransferase (PNMT) are located in the ventrolateral medulla (A1), under the nucleus of the solitary tract (A2) and in the dorsomedial medulla (A3) in human and rodent brain, but not in guinea pig (Cumming et al. 1986). These neurons give rise to adrenergic innervations especially of the hypothalamus, and also to the noradrenergic locus coeruleus and intermediolateral cell columns of the spinal cord. PNMT activity was reduced in hypothalamus of PD patients (Nagatsu et al. 1977), and the abundance of PNMT-positive A1 neurons was reduced by 50%, whereas that of the A3 was reduced by 79% relative to control values, whereas the abundance of A2 neurons was unchanged (Gai et al. 1993). In contrast, an earlier immunohistochemical study with TH antisera had reported a loss of lightly pigmented adrenaline neurons in the medial A2 (Saper et al. 1991), whereas still others had failed to find any significant loss of A1 and A2 adrenaline neurons in a group of three PD patients (Malessa et al. 1990). However, the general concurrence of findings is that brain adrenaline is deficient in PD, which could be predicted to contribute to aspects of autonomic dysfunction.

4 Autonomic Dysfunction

Autonomic dysfunction occurs in a very high percentage of PD patients, and was indeed described by James Parkinson in 1817. The most common manifestations of autonomic dysfunction in PD include male erectile dysfunction, incomplete bladder emptying, constipation, and orthostatic hypotension (Campos-Sousa et al. 2003; Singer et al. 1992). Orthostatic hypotension is most pronounced in patients with long duration of PD (Orskov et al. 1987). Lewy bodies are present in the sympathetic and parasympathetic nerves, and in the dorsal motor nucleus of the vagus nerve of PD patients (Braak et al. 2003) and in the cardiac plexus of PD patients (Iwanaga et al. 1999). The number of preganglionic sympathetic neurons in the intermediolateral cell column at the thoracic level was reduced by 70% in patients with PD; Lewy bodies were found in the remaining neurons of PD patients (Wakabayashi and Takahashi 1997). The sympathetic system is generally characterized by a cholinergic preganglionic component and by an adrenergic post-ganglionic component. In rodent, the post-ganglionic innervation of the sweat glands is atypically cholinergic, although noradrenergic markers are transiently expressed during development. However, recent studies indicate that the primate post-ganglionic sudomotor innervation co-express cholinergic and adrenergic markers (Weihe et al. 2005). Although the pathophysiological basis of the hyperhydrosis in PD is poorly understood, excessive sweating can constitute a significant psycho-social aspect of the disease (Swinn et al. 2003). The symptoms are said to be worse off-levodopa, and to correlate in severity more with other autonomic symptoms than with motor symptoms of PD. On the other hand, it has been claimed that thalamotomy was effective against tremor and also hyperhydrosis. Implying a causal relationship between the two, rather than autonomic function per se (Selby 1968).

Abnormalities in parasympathetic function have been frequently reported in PD (see (Koike and Takahashi 1997)). The baseline variation in heart rate at rest, and the variation in response to deep breathing is reduced in PD relative to healthy controls (Orskov et al. 1987), as is heart rate variability during sleep (Kallio et al. 2004), both of which findings suggest parasympathetic failure. The presence of delayed QT interval in the electrocardiogram from PD patients (Deguchi et al. 2002) suggests a basis for the increased risk of PD patients for sudden cardiac death. Sympathetic failure certainly manifests in the high incidence of orthostatic hypotension in PD, which contributes to the propensity of patients to experience falls. However, a pilot PET study with a novel nicotinic receptor ligand did not reveal any alterations in myocardium of PD patients, suggesting that the parasympathetic system in heart is intact (Bucerius et al. 2006).

The catecholamine transporters in myocardial sympathetic fibers can be measured in molecular imaging studies with [^{123}I]-*meta*-iodobenzylguanidine ([^{123}I]-MIBG), [^{18}F]-fluorodopamine, or [^{11}C]-hydroxyephedrine. The myocardial uptake of [^{18}F]-fluorodopamine was substantially reduced in PD patients with orthostatic hypotension (Goldstein et al. 2002). Uptake of this tracer was also reduced in the thyroid gland and in renal cortex of the PD patients, indicating a pervasive loss of

sympathetic fibers. The sympathetic denervation of the legs can likewise be discerned with [^{123}I]-MIGB scintigraphy of patients with PD and autonomic failure (Koike and Takahashi 1997).

The specificity of myocardial denervation for PD has been a matter of controversy; nearly a dozen imaging studies having failed to detect impaired [^{123}I]-MIBG binding in patients with multiple system atrophy or progressive supranuclear palsy (i.e. Yoshita 1998). Indeed, it was considered for a time that [^{123}I]-MIBG scintigraphy could provide discriminative diagnosis of PD from other nigrostriatal degeneration syndromes. However, the uptake of MIBG in myocardium proved to be more impaired among patients with Lewy body dementia than in PD patients (Suzuki et al. 2006). Furthermore, loss of myocardial sympathetic innervation was detected in [^{11}C]- hydroxyephedrine PET studies of some patients with multiple system atrophy or progressive supranuclear palsy (Raffel et al. 2006). The occurrence of myocardial sympathectomy only in some affected individuals indicates that these parkinsonian syndromes are highly heterogeneous with respect to the involvement of the sympathetic nervous system. Raffel et al. (2006) also investigated the movement disorder patients with [^{11}C]-dihydrotetrabenazine PET in order to measure the density of DA vesicles in striatum; the absence of any clear correlation between striatal and myocardial denervations suggested the occurrence of independent pathophysiological mechanisms in the two tissues.

Urodynamic symptoms such as incomplete voiding occur in a large proportion of PD patients, and may increase in severity with disease progression (Araki et al. 2000). Brain imaging studies with DA transporter ligands show particularly extensive loss of striatal binding sites in patients with bladder symptoms (Sakakibara et al. 2001; Winge et al. 2005). However, severity of motor symptoms likewise correlated with the decline in striatal DA transporters. Therefore, it cannot be concluded that urodynamic symptoms arise from the nigrostriatal degeneration per se, or due to independent factors such as loss of autonomic function. On the other hand, treatment with levodopa or direct DA agonists improved urodynamic symptoms (increased bladder capacity) in most PD patients, although the highly variable individual benefits suggested the presence of heterogeneous factors (Winge et al. 2004).

Constipation is another classical dysautonomic problem of PD; colonic transit time is substantially increased in PD patients due to failure of peristalsis (Sakakibara et al. 2003). Dopamine-containing neurons were nearly absent in the myenteric plexus of the majority of PD patients with chronic constipation (Singaram et al. 1995). In contrast, the enteric vasoactive intestinal peptide neurons were of normal abundance, although they frequently contained Lewy bodies.

5 Neuroendocrine Systems

The paraventricular and supraoptic nucleus of the hypothalamus contain oxytocin and vasopressin neurons, which project to the posterior lobe of the pituitary gland. Relative to age-matched control subjects, the number of oxytocin-containing

neurons was reduced by 22% (in the absence of Lewy bodies) in the paraventricular nucleus of PD patients. The abundance of vasopressin neurons were nonsignificantly reduced in the same subjects (Purba et al. 1994).

Inhibition of growth hormone secretion by somatostatin is overcome by growth-hormone releasing hormone. While this mechanism was intact in patients with PD, the release of growth hormone normally evoked by a 5-HT_1 agonist did not occur in PD patients (Volpi et al. 1997b). Fenfluramine challenge evokes a twofold increase in plasma ACTH in normal subjects, but not PD patients (Volpi et al. 1997a). However, the effect of corticotrophin releasing factor was identical in the two groups, indicating a primary defect of the hypothalamic 5-HT innervation. Fenfluramine-evoked prolactin response was also blunted in PD patients (Kostic et al. 1996). While these neuroendocrine changes might plausibly be attributed to degeneration of the 5-HT innervation (see below), hypothalamic 5-HT content was not significantly reduced in PD patients (Shannak et al. 1994). Treatment with the μ-opioid antagonist naloxone increases secretion of ACTH/cortisol and luteinizing hormone, by interfering with tonic inhibition of hypothalamic/pituitary function. However, naloxone failed to evoke increases in ACTH/cortisol or LH secretion in PD patients off levodopa; reinstatement of levodopa treatment normalized these neuroendocrine responses (Volpi et al. 1994). The activity of DOPA decarboxylase is reduced by 67% in hypothalamus of patients with PD (Lloyd and Hornykiewicz 1970), suggesting that loss of catecholamine tonus intrinsic to the hypothalamus may mediate some of the neuroendocrine changes in PD.

6 Serotonin

Serotonin transmission, like that of DA, is regulated via specific plasma membrane transporters. The binding of [^{3}H]-citalopram to 5-HT transporters (SERT) was reduced by 75% in caudate and putamen of PD patients, and by 50% in the substantia nigra and neocortical regions (Chinaglia et al. 1993). In contrast, the density of SERT in the raphe nuclei was normal in PD patients, although Lewy bodies have been reported in 5-HT neurons in that condition (Ohama and Ikuta 1976). The extent of reduced 5-HT concentration in cerebrospinal fluid from PD patients correlated inversely with Hoehn and Yahr stage and with severity of gait freezing (Tohgi et al. 1993). This finding does not indicate causality in motor symptoms, but could alternately indicate that 5-HT depletion is a surrogate marker for severe DA depletion, i.e. due to a parallel decline in monoamine systems. In another study, the occurrence of premorbid depression correlated with the extent of loss of 5-HT neurons in the dorsal raphe of patients dying with PD (Paulus and Jellinger 1991). The 5-HT metabolite 3-hydroxykynurenine was reduced by 75% in cerebrospinal fluid of PD patients, but the 5-HT concentration proved to have the higher negative correlation with motor symptoms (Tohgi et al. 1993). Serotonin levels, and also NA, were reduced by 50% in lumbar cord (Scatton et al. 1986) and in limbic cortical regions of patients dying with PD

(Scatton et al. 1983), which may have implications for the affective and pain syndromes of PD.

There is some evidence that mesencephalic DA exerts a tonic inhibition of 5-HT neurons in the raphe. An early, preclinical stage of nigrostriatal degeneration might thus entail facilitiation of 5-HT activity, at least prior to the onset of degeneration of the 5-HT neurons in late PD. In an FDOPA-PET study of early PD patients with asymmetric disease, we have observed elevated FDOPA trapping in the vicinity of the dorsal raphe nucleus (Fig. 1c), and also the ventral striatum (Kumakura et al. 2010a). This observation could predict elevated 5-HT synthesis, or production of DA as a false neurotransmitter within the 5-HT neurons in early PD treated with levodopa. In a [^{11}C]-DASB study, the availability of 5-HT transporters was reduced by some 30% in forebrain of PD patients without pronounced depression (Guttman et al. 2007), whereas [^{11}C]-DASB binding was *elevated* in some cortical regions of PD patients with depression (Boileau et al. 2008). A [^{11}C]-DASB study linked reduced 5-HT transporters to fatigue symptoms in PD patients (Pavese et al. 2010). Others found relative preservation of 5-HT transporters in specific brain regions such as amygdala and posterior cingulate cortex of depressed PD patients (Politis et al. 2010).

Post-synaptic actions of 5-HT are mediated via a large number of receptor subtypes. The concentration of 5-HT$_2$ receptors was reduced in parietal cortex of patients with PD and dementia, in whom the cortical concentration of choline acetyltransferase (ChAT) was also reduced (Perry et al. 1984). In other post mortem studies, the abundance of 5-HT$_2$ receptors labeled with [^{3}H]-ketanserin was reduced by 50% in temporal cortex from patients who had died with PD, whereas nearly normal levels were present in frontal cortex; declines in temporal lobe 5-HT$_2$ receptors did not correlate with severity of dementia in these PD patients (Maloteaux et al. 1988a). Fine anatomical analysis of cortical [^{3}H]-ketanserin binding revealed declines in all cortical layers of patients with PD, Lewy body dementia, and Alzheimer's dementia (Cheng et al. 1991). Thus, loss of 5-HT receptors is well-documented in PD, but is equally present in other neurodegenerative disorders. Declines in post-synaptic 5-HT receptors in conjunction with loss of cortical 5-HT innervation suggest that degeneration of 5-HT pathways involves pre- and post-synaptic mechanisms, rather than a simple denervation, which might be expected to result in up-regulation of some classes of 5-HT receptors. The observed changes in 5-HT markers may be especially relevant to cognitive changes associated with PD.

7 Acetylcholine

A cardinal neurochemical feature of Alzheimer's disease is the loss of cortical cholinergic innervation derived from the basal nucleus of Meynert in the basal forebrain (Coyle et al. 1983). However, reduced enzymatic activity of the cholinergic marker choline acetyltransferse (ChAT) in frontal cortex also correlated

with the extent of cognitive impairment in PD patients, as did the DA D_1 receptor density in the caudate nucleus (Mattila et al. 2001). Loss of basal forebrain cholinergic neurons is especially prevalent in PD patients with dementia that is unresponsive to levodopa therapy (Chan-Palay 1988), and reduced cortical ChAT was noted above in association with loss of cortical 5-HT receptors in demented PD patients (Perry et al. 1984). However, ChAT levels were unaffected in the substantia nigra of PD patients (Javoy-Agid et al. 1981), nor was ChAT reduced in putamen of patients dying with PD (Perry et al. 1991). In association with the cholinergic neurons of the basal nucleus of Meynert, there are to be found fusiform neurons expressing galanin peptide, which is inhibitory to cholinergic neurons. In patients with AD and PD, the density of galanin-immunoreactive terminals on soma and dendrites of the cholinergic neurons was increased (Chan-Palay 1988).

The relationship between cortical availability of nicotinic receptors and cognition (Kadir et al. 2006) and treatment effects of galantamine (Ellis et al. 2009) has been investigated in molecular imaging studies of Alzheimer's disease. There were slight (10%) reductions in nicotinic receptor binding in the striatum of PD patients, but no cortical changes were reported in that PET study (Kas et al. 2009).

8 Peptide Neurotransmitter Systems

The striatum contains two populations of GABA-ergic medium spiny neurons. The GABA-enkephalin neurons project mainly to the external pallidum, whereas the GABA-substance P neurons project to the substantia nigra and pallidal internal segment. The concentration of met-enkephalin was reduced by one half in the caudate, putamen, and substantial nigra of PD patients (Fernandez et al. 1996), and likewise in striatum of MPTP-lesioned monkeys (Perez-Otano et al. 1995), but was increased in striatum of 6-OHDA-treated rats (Thal et al. 1983). The leu-enkephalin concentration was substantially reduced in putamen and substantia nigra of PD patients (Fernandez et al. 1996), but leu-enkephalin levels were normal in striatum of MPTP-lesioned monkeys (Jenner et al. 1986). The concentration of substance P was reduced by 50% in post mortem putamen samples from PD patients (Fernandez et al. 1996); in animal studies of acquired parkinsonism, a similar reduction on Substance P concentration was reversed by levodopa treatment (Engber et al. 1991).

Opioid receptors are found on pre- and post-synaptic elements as well as on interneurons in the striatum. Opioid binding was investigated in PET studies employing [^{11}C]-diprenorphine in groups of patients with PD and other akinetic rigid syndromes (Burn et al. 1995); the striatal binding ratio in the PD patients was similar to that in control subjects, whereas the binding ratio was reduced in putamen of striatonigral degeneration patients, and in caudate and putamen of Steele–Richardson–Olszewski syndrome. However the same group subsequently reported reduced [^{11}C]-diprenorphine binding in striatum of PD patients with dyskinesias, in whom the radioligand binding was also reduced in thalamus and cingulate gyrus, but was increased in the prefrontal cortex (Piccini et al. 1997).

Substance P is also expressed in several cell populations of the mesopontine tegmentum, including the pedunculopontine tegmental nucleus and the 5-HT neurons of the dorsal raphe. Very substantial loss of these groups of peptidergic neurons is reported in PD (Gai et al. 1991). Studies in experimental animals suggest that lesions of the pedunculopontine nucleus may result in the REM sleep behavior disorder, a newly described clinical entity characterized by vivid dreaming and loss of normal atonia, resulting in simple or complex movements during sleep, even to the extent of "acting out" dreams. In a longitudinal study, 38% of cases with initial diagnosis of REM sleep behavior disorder had proceeded to develop PD, a mean of 4 years later (Schenck et al. 1996). Striatonigral degeneration has been confirmed in a [^{123}I]-FP-CIT SPECT study of PD patients with a history of REM sleep behavior disorder, as well as olfactory dysfunction (Stiasny-Kolster et al. 2005).

Within the parabrachial nucleus there is also a population of neuromelanin-containing tyrosine hydroxylase-positive neurons; the abundance of which is reduced in patients with PD (Goto and Hirano 1991). Since this structure is involved in the regulation of autonomic function via input from the amygdale, this cell loss may contribute to the autonomic dysfunction frequently occurring in PD, as described above.

9 GABA

Despite the great abundance of GABA-ergic neurons in the striatum, only moderate densities of GABA-A binding sites are to be found in the basal ganglia, whereas the densest binding in brain occurs in the cerebellum and cerebral cortex. The specific binding of [^{3}H]-flunitrazepam was unchanged in cerebral cortex, but increased in post mortem striatum from PD patients (Maloteaux et al. 1988b). Despite this finding in vitro, the binding of the SPECT ligand [^{123}I]I-iomazenil was decreased throughout cerebral cortex of PD patients, in proportion to the severity of their disease (Kawabata and Tachibana 1997). Indeed, binding of the GABA-A ligand [^{11}C]-flumazenil in cerebral cortex has been interpreted to be a surrogate indicator of cortical thickness and neuronal density (la Fougere et al. 2010a), which may be reduced in PD. However, GABA-A binding is relatively preserved in cortex of AD patients, despite the clear reductions in cerebral blood flow (Ohyama et al. 1999). This suggests that the [^{123}I]I-iomazenil results in PD may indicate an actual loss of cortical neurons, rather than a decline in cortical thickness due to atrophy of the neuropil.

10 Microglial Activation

The 18 kDa translocator protein (TSPO), formerly known as the peripheral benzodiazepine receptor, is highly expressed in the outer mitochondrial membrane, most especially in microglia, which are the resident macrophages of the

brain. As such, TSPO binding can be taken as an indicator of inflammatory reactions in the brain parenchyma. In a rat microPET study, the binding of the prototypic TSPO ligand [^{11}C]-PK11195 was persistently increased in striatum and substantia nigra following infusion of the DA neurotoxin 6-OHDA (Cicchetti et al. 2002). Indeed, a histological study with antibodies against another marker of activated microglia revealed a continuing inflammatory process in parkinsonian monkey brain several years after intoxication with MPTP (McGeer et al. 2003). A longitudinal PET study of idiopathic PD patients likewise revealed a widespread and persistent increase in [^{11}C]-PK11195 binding, which did not, however, correlate with impaired striatal FDOPA utilization or clinical severity (Gerhard et al. 2006). The authors suggested that microglia activation had occurred at an early stage of the disease process. Levels of [^{11}C]-PK11195 uptake in midbrain correlated inversely with the DA transporter marker [^{11}C]-CFT binding potentials in the putamen and correlated positively with motor symptoms of PD patients (Ouchi et al. 2005). Protective effect of non-steroidal anti-inflammatory drugs (NSAID) have been described in animal models of PD (Aubin et al. 1998; Mohanakumar et al. 2000) and epidemiological studies have reported decreased risk of developing PD with chronic intake of a non-steroidal anti-inflammatory drug (Chen et al. 2005), suggesting that activated microglia play a role in PD pathogenesis, at least as an initiator. Nonetheless, others failed to detect any elevated [^{11}C]-PK11195 binding in a small group of PD patients, and also failed to detect an effect of treatment with a COX-2 inhibitor on the cerebral binding (Bartels et al. 2010). However, the low sensitivity of [^{11}C]-PK11195 for detecting TSPO is well-known; there was only a trend toward increased binding in the brain of pigs with well-documented MPTP lesions of the nigrostriatal pathway (Cumming et al. 2006). There is currently a very active search to identify an optimal TSPO tracer with binding properties superior to those of [^{11}C]-PK11195 (e.g. Van Camp et al. 2010).

11 Blood Flow and Glucose Metabolism

The investigation of cerebral blood flow (CBF) and rate of glucose consumption (CMRglc) has had two major applications in the context of PD: to advance the understanding of neuroenergetic features of the disorder, and to develop tools for differential diagnostic purposes, treatment- and disease-monitoring. Before the era of human PET was underway, a substantial body of knowledge had already been collected using [^{14}C]-2-deoxy-glucose (2DG) autoradiography (Sokoloff et al. 1977) in animal models of PD. The findings of more than 20 such studies of parkinsonian animals were recently reviewed (Borghammer et al. 2009b; Obeso et al. 2008). Evaluation of this evidence is complicated by differences in imaging procedures, and the precise nature of the neurotoxic lesion obtained with agents such as MPTP or 6-OHDA. Importantly, the interval of time between intoxication and autoradiographic imaging ranges from a few days to many months.

Nevertheless, the evidence shows some clear consistencies. In brief, the resting state in the parkinsonian rodent and monkey seems to be characterized by unchanged or decreased CMRglc in frontal and parietal cortex. The subthalamic nucleus, in which the neurons display increased firing rate in the condition of DA denervation of the striatum (Bergman et al. 1994; Obeso et al. 2008), is unequivocally hypometabolic. In contrast, the external pallidum (GPe) exhibits decreased firing rate but is nearly always hypermetabolic. This illustrates the principle that glucose metabolism is more closely associated with the input to a given region than with its output (Attwell and Laughlin 2001). The pedunculopontine nucleus (PPN) is probably also hypermetabolic. A few studies reported hypermetabolism in the internal pallidum, striatum, the ventral-lateral (VL), and ventral-anterior (VA) thalamic nuclei, but many more studies reported no change or even decreases in these structures. In summary, the brain of parkinsonian animals is probably characterized by cortical hypometabolism and concurrent hypermetabolism in only a few discrete and very small, basal ganglia structures (PPN and GPe).

Many subsequent quantitative PET studies in human PD focused on replicating findings from the animal literature. However, the final spatial resolution of nearly all PET studies performed to date is little better than 10 mm, when taking all factors into account, i.e. intrinsic scanner resolution, head movement, post-reconstruction filtering, and less-than-perfect co-registration of emission images to a standard coordinate system. In other words, given present technical limitations, PET studies are unfit to replicate the animal findings of hypermetabolism in small structural elements of the basal ganglia. In addition, human CBF and CMRglc values display a great deal of biological variation. The relative standard deviation of these parameters is usually 14–20% in groups of normal subjects (Leenders et al. 1990; Moeller et al. 1996), and patients with neurodegenerative disorders (Berding et al. 2001; Imon et al. 1999), but can be as high as 30% in PD (Huang et al. 2007). This substantial variance hinders detection of low-magnitude signals in typical PET studies consisting of 10–20 subjects per group. Taken together, these observations probably explain why absolute subcortical hypermetabolism has not been robustly confirmed in patients with PD, even though more than 30 quantitative CBF and CMRglc studies have been performed to date [see Borghammer et al. 2010 for references].

On the other hand, nearly all of the 30 quantitative PET studies of metabolism reported significant or non-significant global and regional decreases of CBF and CMRglc in PD. Many of the studies reported significant absolute decreases of CBF and CMRglc in frontal (Eberling et al. 1994; Takahashi et al. 1999) and parieto-occipital cortical regions (Berding et al. 2001; Bohnen et al. 1999; Hu et al. 2000; Takahashi et al. 1999). Figure 2 illustrates the results of a recent meta-analysis of all available CBF and CMRglc studies, in which global mean values were specifically stated (Borghammer et al. 2010). The meta-analysis confirms the presence of decreased CBF and CMRglc in the global mean values of PD patients, although these defects are not always detected in individual studies due to their insufficient sample sizes.

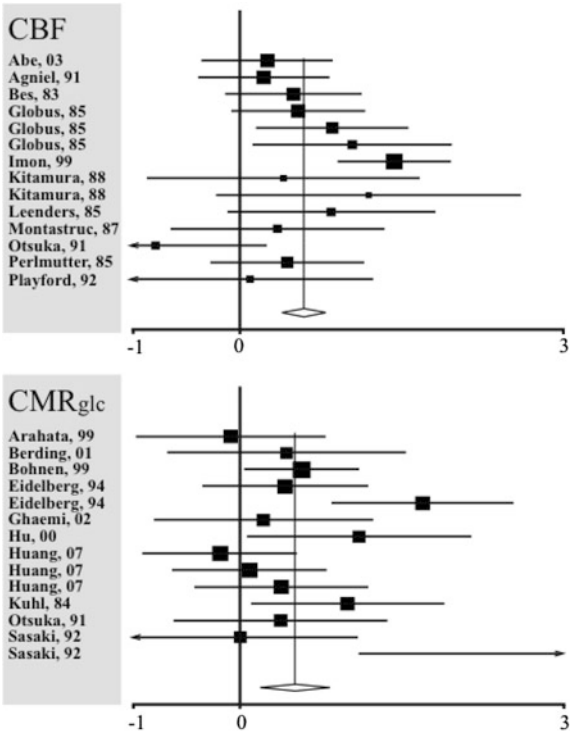

Fig. 2 Forest plots of meta-analyses of CBF (*top*) and CMRglc (*bottom*) differences between PD patients and healthy controls. *Horizontal lines* represent 95% confidence intervals around the standard mean difference (SMD) of each study. The *size of the squares* represents the relative weight assigned to that particular study in calculation of the overall SMD. The *vertical lines* signify overall SMD with 95% CI (*diamond*). The SMD is defined as the ratio (between-group difference in mean)/(pooled standard deviation with correction for small sample sizes). Reprinted with permission from Borghammer et al. (2010)

12 Hypometabolism in Normalized PET Studies

Inclusion of sufficiently large sample sizes to compensate for the considerable variation in CBF and CMRglc data is so costly that the use of data normalization was quickly introduced in PET studies (Fox et al. 1988). The most commonly used method is to simply divide the tracer uptake in each region or voxel by the global mean (GM) value of the whole brain, or the mean value of all gray matter. This kind of normalization process yields ratios with drastically decreased variation (relative SD of 4–10%) (Antonini et al. 1998; Defebvre et al. 1999; Ghaemi et al. 2002). Thus, normalization favors the detection of group differences which are focal and of small magnitude. However, valid use of this approach is predicated on the assumption of absent between-group GM differences. As shown in Fig. 2, this requirement is probably not met in PD patients, in whom the GM metabolism and blood flow are most likely decreased.

Thus, PET scans in PD and other neurodegenerative disorders should instead be normalized to other regions, which are known to be less affected than the GM. The cerebellum (Dukart et al. 2010), the pons (Minoshima et al. 1995), the somatosensory cortex (Yakushev et al. 2009), and white matter (Borghammer et al. 2008) have all been demonstrated to perform better than GM normalization in

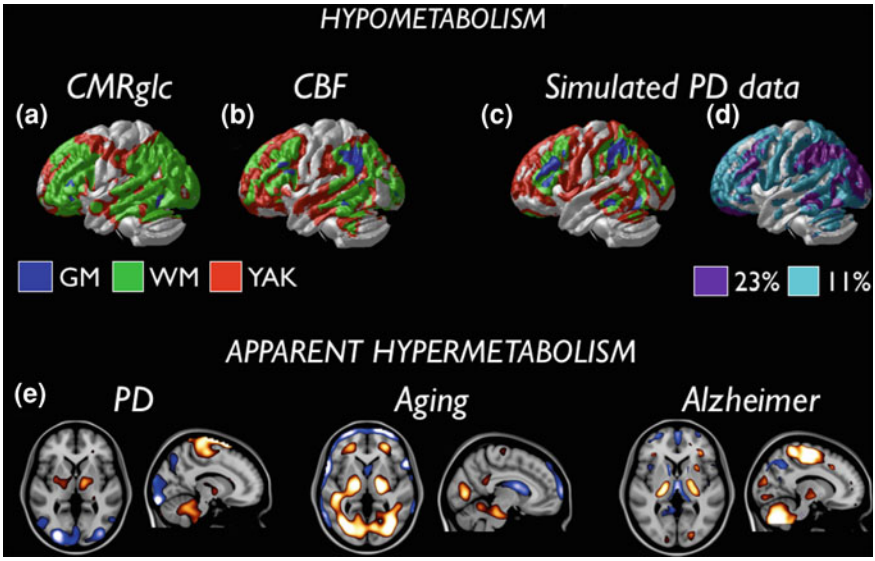

Fig. 3 **a**, **b** The patterns of relative CBF and CMRglc decreases in PD are closely matched. These patterns are only robustly detected when normalization to white matter (WM; *green*) or data-driven Yakushev (YAK; *red*) normalization is applied. **c**, **d** Using global mean (GM; *blue*) normalization only residual traces of the pattern are detected. This figure shows results of a simulation study in which isolated cortical decreases of 11% (*light blue*) and 23% (*purple*) were applied in a group of 20 subjects, and then compared to 20 unchanged control images. Here, WM and particularly YAK normalization recovered much more of the true signal than did GM normalization. **e** When GM normalization is employed (and only then), most CBF and CMRglc PET studies of PD have detected a pattern of relative increases in the thalamic-capsula internalentiform intersection, motor cortices, WM structures, pons, and central cerebellum. However, similar patterns have been reported in healthy aging, Alzheimer's disease, and other disorders. Thus, the pattern arises as a general consequence of GM normalization, and the correct interpretation of the finding is most likely that metabolism in these regions is unchanged or even decreased, but to a lesser degree than is the global mean. [Adapted from Borghammer et al. (2010, 2009b) with permission.]

neurodegenerative disorders. Recently, a data-driven normalization strategy for identification of useful normalization regions a posteriori was devised by Yakushev and colleagues (Borghammer et al. 2009a; Yakushev et al. 2009). Figure 3 summarizes the effect of different normalization strategies on the extent of the detected pattern of perturbed metabolism in PD. In brief, normalization to the cerebellum, the white matter, or Yakushev normalization yields evidence for widespread cortical hypometabolism (Fig. 3a) and hypoperfusion (Fig. 3b), notably in the lateral and medial prefrontal, and parieto-occipital regions, and also in the lateral temporal lobe. Metabolism in the medial temporal lobe, sensory-motor cortex, basal ganglia, brainstem and cerebellum is apparently unchanged in PD. For two reasons, it seems likely that this pattern of relative cortical hypometabolism is a more accurate description of true, absolute hypometabolism. First, the quantitative PET literature

supports that cortical regions display absolute decreases of CBF and CMRglc. Second, the pattern is in agreement with the animal literature, as discussed above. Interestingly, this cortical pattern, which is already present in early-stage patients (Borghammer et al. 2010) seems to coincide at least in part with the pattern of of Lewy Body deposition at later disease stages (Braak et al. 2003). This observation suggests that early perturbation of cerebral cortical metabolism could be causally involved in the subsequent deposition of inclusion bodies.

13 Hypermetabolism in Normalized PET Studies

A number of PET and SPECT studies of PD have been performed, in which GM normalization was used. However, since the GM metabolism and blood flow are most likely decreased in PD, the use of GM normalization will necessarily lead to the appearance of seemingly hypermetabolic foci in brain, relative to the undetected decline in GM (Borghammer et al. 2009b). Thus, the GM studies have without exception reported a pattern (Fig. 3e) of widespread relative hypermetabolism in the thalamic-capsula-interna-lentiform intersection, primary motor sensory cortex, white matter, pons, and central cerebellum (Eidelberg et al. 1994; Hosey et al. 2005; Huang et al. 2007; Imon et al. 1999; Nagano-Saito et al. 2004). As detailed above, this widespread pattern finds little overlap with the 2DG autoradiographic results in parkinsonian animals, in which robust hypermetabolism was seen only in a few discrete subcortical structures—structures too small to have been detected in nearly all PET studies performed to date. In addition, a very similar pattern of hypermetabolism has been reported in a range of other neurological conditions, but only when GM normalization is used (Borghammer et al. 2008). As illustrated in Fig. 3e, similar patterns were reported when aged subjects were compared to young controls (Borghammer et al. 2009b; Kalpouzos et al. 2009; Moeller et al. 1996) and also in Alzheimer's disease patients (Borghammer et al. 2009b; Dukart et al. 2010; Habeck 2010). There is no theoretical basis for expecting such subcortical hypermetabolism in healthy aging or Alzheimer's disease. The parsimonious explanation for the commonality of these patterns is therefore that it represents regions of preserved metabolism, which were similarly inflated by biased GM normalization. This interpretation was recently verified in a large quantitative MRI study in PD patients (Melzer et al. 2011). Here, Melzer and colleagues demonstrated that the apparently hypermetabolic regions demonstrated in Fig. 3e are in reality the only metabolically conserved regions in PD, whereas most of the cerebral cortex displays small or large absolute deficit in perfusion, as demonstrated in Fig. 3a and b. Moreover, we recently compared FDG uptake in a group of PD patients and healthy controls using a brain-dedicated high-resolution PET scanner with sufficient spatial resolution to investigate very small subcortical structures (Borghammer et al. 2011a). In this study, absolute hypermetabolism was suggested only in the GPe, which is also in accordance with the 2DG autoradiographic evidence in animal models of PD.

It must be noted that, although GM normalization may yield patterns that are not strictly physiologically meaningful, these patterns can nevertheless be very disease-specific and of great diagnostic utility. For instance, disease-specific pattern reliably separates PD patients from healthy controls (Ma et al. 2007) and from patients with atypical movement disorders (Tang et al. 2010). Changes in the pattern have also been successfully used to evaluate treatment effects of levodopa and deep brain stimulation in PD patients (Asanuma et al. 2006).

14 Cerebral Oxygen Consumption

The resting cerebral metabolic rate of oxygen consumption ($CMRO_2$) has been investigated in only few PET studies of PD patients. This parameter is however of some interest since decreased activity in complex I of the mitochondrial electron transport chain (ETC) has been reported in post-mortem studies of PD patients (Henchcliffe and Beal 2008; Keeney et al. 2006). Dysfunction of the ETC would under most circumstances not only lead to reduced $CMRO_2$, but also to reductions of the CMRglc according to the stoichiometric relationship for $CMRO_2$:CMRglc of 6:1 for the complete oxidation of glucose. Specific defects in the ETC would likely result in proportionally greater deficits in $CMRO_2$ than CMRglc, i.e. a shift to anaerobic respiration (Frackowiak et al. 1988). Four PET studies reported non-significant global $CMRO_2$ decreases and significant decreases in cortical regions in early- to moderate-stage PD patients (Kitamura et al. 1988; Leenders et al. 1985; Takahashi et al. 1999; Borghammer et al. 2011b). Thus, the results of these studies could be consistent with a dysfunctional ETC in PD. However, a recent study surprisingly detected significantly increased global $CMRO_2$ and near-significant increase in global CMRglc ($p = 0.056$) in 12 early-stage, drug na PD patients (Powers et al. 2008). The authors speculated that an uncoupling of ATP production from oxidative phosphorylation could potentially explain this unexpected finding. However, one of the earlier $CMRO_2$ in early-stage previously unmedicated PD patients reported a non-significant decrease in the $CMRO_2$ (Takahashi et al. 1999). So, considering that the entire quantitative PET literature reports only significant or non-significant decreases of metabolism and blood flow (Borghammer et al. 2010), the report of globally increased $CMRO_2$ in drug na patients is an isolated finding.

Deep brain stimulation (DBS) targeting the overactive subthalamic nucleus can markedly relieve the rigidity and tremor of PD. However, the net cerebrometabolic effects of DBS have been a matter of some controversy. We have predicted that effective DBS, by blocking or entraining the activity of subthalamic neurons, should rectify a putative focal hypermetabolism. However, the treatment proved to evoke no discernible change in the magnitude of $CMRO_2$ in the target structure, producing instead unexpected metabolic activations in distal cortical regions linked to the perception of motion (Vafaee et al. 2004). In FDG-PET studies of similar design, DBS evoked activation of the relative CMRglc in the

target structure and in some limbic and associative projection territories of the basal ganglia (Hilker et al. 2004), or had no effect in the target structure, while evoking increases and decreases in distal cortical regions (Le Jeune et al. 2010). These discrepant findings highlight the difficulties in interpreting PET measurements of brain energy metabolism in the basal ganglia of PD patients on accordance with heuristic models of the underlying cerebrometabolic disturbances.

15 Conclusions

The theme of this review is that nigrostriatal degeneration, while of key importance for the motor symptoms of PD, constitutes only part of PD pathology. A full understanding of the clinical presentation should be informed by knowledge about the spectrum of pathophysiological changes in the parkinsonian brain, and also in diverse peripheral tissues. Thus, the presence of widespread cortical hypometabolism in frontal and parietal cortical regions early in the disease could underlie the early onset of cognitive decline reported in PD. Newly diagnosed PD patients most often display deficits in frontal lobe cognitive domains, such as executive functions and psychomotor speed (Muslimovic et al. 2005; Rodriguez-Ferreiro et al. 2010). Other studies have reported deficits in visuoconstructive abilities (Levin et al. 1991; Stella et al. 2007; Williams-Gray et al. 2007), which are widely accepted to be reflective of parietal lobe dysfunction (Makuuchi et al. 2003). Moreover, evidence is accumulating that working memory, which is also often impaired in early PD (Koerts et al. 2009; Whittington et al. 2006), relies heavily upon superior parietal cortex (Koenigs et al. 2009). As such, the pattern of cortical hypometabolism displayed in Fig. 3a seems to overlap fairly well with cortical regions responsible for the particular cognitive functions known to be compromised in early PD. However, the causality of this link is presently uncertain; are cognitive deficits in PD due to primary cortical hypometabolism, or are both phenomena down-stream effects of progressive loss of monoaminergic cortical innervations?

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Imaging of Seasonal Affective Disorder and Seasonality Effects on Serotonin and Dopamine Function in the Human Brain

Nicole Praschak-Rieder and Matthaeus Willeit

Abstract According to current knowledge, disturbances in brain monoamine transmission play a major role in many psychiatric disorders, and many of the radioligands used for investigating these disorders bind to targets within the brain monoamine systems. However, a phylogenetically ancient and prevailing function of monoamines is to mediate the adaptation of organisms and cells to rhythmical changes in light conditions, and to other environmental rhythms, such as changes in temperature, or the availability of energy resources throughout the seasons. The physiological systems mediating these changes are highly conserved throughout species, including humans. Here we review the literature on seasonal changes in binding of monoaminergic ligands in the human brain. Moreover, we argue for the importance of considering possible effects of season when investigating brain monoamines in healthy subjects and subjects with psychiatric disorders.

Keywords PET · SPECT · monoamine transporters · season · SAD

Contents

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1 Introduction

Organisms living in temperate and polar zones undergo profound seasonal changes in their metabolism and behaviour. These oscillations are a necessary evolutionary adaptation that allows for coping with dramatic changes in temperature, lighting conditions, and food availability. Humans show a wide range of behaviors that vary with seasons. This includes variations in eating behavior and sleep, mood, and energy balance. The degree of seasonal changes in these parameters is termed "seasonality". Individuals with particularly high seasonality scores are highly vulnerable for seasonal affective disorder (Hardin et al. 1991; Winkler et al. 2002). Seasonal affective disorder (SAD), and in particular winter type SAD (the most common form of the disorder) is a special form of recurrent major depression, with regular depressive episodes during autumn and winter, alternating with euthymia or hypomanic phases in spring and summer (Rosenthal et al. 1984). Frequently, patients with SAD suffer from atypical depression, a condition characterized by an increase in sleep, hyperphagia, and subsequent weight gain. This symptom profile, together with the high proportion of female patients with SAD, has led to the hypothesis that seasonality is an adaptive response regulating reproductive cycles in populations that migrated to Northern or Southern temperate and polar zones (Davis and Levitan 2005; Pjrek et al. 2007).

Seasonality *per se* is not necessarily associated with clinical symptoms; rather, it is normally distributed in the general population (Kasper et al. 1989; Winkler et al. 2002; Hardin et al. 1991). In contrast to many animal species who show more dramatic changes in behaviour, such as hibernation or the exclusive restriction of mating behaviour to a certain period of the year, humans living in temperate or polar zones show much more subtle changes. Still, many events show a clear seasonal distribution, and most of the time, we do not know the mechanisms involved in the rhythmic expression of phenomena as diverse as suicide (see below for further discussion), metabolic changes (Au-Yong et al. 2009), activity of disorders such as multiple sclerosis (Meier et al. 2010), or the incidence of sudden infant death syndrome (Douglas et al. 1996; Osmond and Murphy 1988).

Some physiological and pathological conditions show rhythmic patterns oscillating at different discernable frequencies. A good example is sleep. Sleep follows an obvious circadian rhythm, with another seasonal rhythm that is superimposed in large parts of the population, showing a regular increase in sleep duration during winter, and reductions in sleep duration in summer (Kasper et al. 1989). At times, systems regulating circadian oscillations—some of the components are well characterized—are also involved in mediating the effects of changing seasons and varying light schedules (Balzer and Hardeland 1991; Wehr et al. 2001; Gonzalez and Aston-Jones 2006, 2008). Indoleamines (tryptophan, serotonin, melatonin) and other monoamines (e.g., dopamine, norepinephrine) are of special importance in signaling change of seasons in plants, animals, and humans (Azmitia 2001). Today, there is solid evidence that a dysregulation in brain monoamine systems contributes to the pathogenesis of SAD (for review see

Neumeister et al. 2001a; Lam and Levitan 2000; Sohn and Lam 2005). SAD is regarded as an extreme reaction to seasonal changes. Seasonal changes in mood, drive, and wellbeing also occur in the general population, although to a lesser degree (Kasper et al. 1989). As a consequence, studies on monoamine systems in psychiatric disorders may substantially profit from studies on biological rhythms, and failing to take into account the effects of season may limit the progress otherwise made possible by new brain imaging methods.

2 Brain Imaging of Seasonal Affective Disorder

There are only a limited number of studies specifically investigating SAD. Two early studies measured cerebral metabolic rates using [^{11}F]-deoxy-glucose ([^{11}F]FDG) and PET in winter SAD (Cohen et al. 1992) and summer seasonal affective disorder (Goyer et al. 1992), a rare condition where subjects suffer from depressive episodes regularly reoccurring in summer. The Cohen et al. study found globally reduced cerebral metabolic rates and relative reductions in frontal cortical areas. The Goyer et al. study described reduced overall cerebral metabolic rates, decreased rates in the left inferior parietal lobule but an increase in the orbitofrontal cortex in patients with summer depression. Our group has conducted a small study on regional cerebral blood flow (rCBF) using [^{99m}Tc]HMPAO and single photon emission computed tomography (SPECT) in patients with SAD and healthy subjects, suggesting increased left frontal rCBF in untreated patients with SAD, and subsequent normalization in rCBF after successful light therapy (Praschak-Rieder et al. 1998).

Studies using SPECT and the nonspecific monoamine transporter ligand [^{123}I]β-CIT showed decreased [^{123}I]β-CIT binding in the midbrain (Willeit et al. 2000) and striatum (Neumeister et al. 2001b) in SAD. Midbrain binding of [^{123}I]β-CIT is predominantly to serotonin transporters (SERT), while binding in the striatum—where equilibrium binding is achieved substantially later than in midbrain—is predominantly to dopamine transporters (DAT). In sum, these studies show reduced [^{123}I]β-CIT in a relatively small sample of depressed patients with SAD. However, independent replication and studies using more specific ligands are needed before firm conclusions can be drawn.

3 Seasonal Effects on the Brain Serotonin Systems

Serotonin neurotransmission has been implicated in several neuropsychiatric disorders, and the serotonergic system is certainly among the best studied transmitter systems with regard to seasonal changes and their behavioral and medical consequences. Indeed, some of the key molecules of the serotonergic system are accessible to molecular imaging studies: one of the most important molecules for

regulation of serotonin transmission is the serotonin transporter (SERT or 5-HTT; Ramamoorthy et al. 1993). By taking up serotonin into the presynaptic neuron soon after its release, SERT controls spatial and temporal spread of the serotonergic signal. Blocking SERT is one of the most important pharmacological principles in antidepressant drug therapy, and the successful clinical use of selective serotonin reuptake inhibitors (SSRIs) in the last decades suggests that SERT blockade is sufficient for antidepressant actions of a drug.

Much less is known on the role of SERT in the pathogenesis of psychiatric disorders. Indirect evidence is derived from studying genetic polymorphisms of the SERT gene. A polymorphism in the promoter region of the SERT gene (5-HTTLPR; Heils et al. 1996) has been shown to be functional in human cell lines, with the long (L) variant being associated with substantially higher 5-HTT expression than the short (S)-allele. 5-HTTLPR has been extensively studied for possible associations with personality traits (Lesch et al. 1996), brain function (Pezawas et al. 2005), suicide (Bondy et al. 2000, 2006; Anguelova et al. 2003), affective disorders (Collier et al. 1996), and their relation to traumatic life events (Caspi et al. 2003) and seasonality (Rosenthal et al. 1998; Willeit et al. 2003). Recently, another functional single nucleotide variant within the 5-HTTLPR L allele has been described, designated LA and LG (Kraft et al. 2005; Nakamura et al. 2000) Only LA is associated with high levels of SERT mRNA transcription and SERT expression in vitro, whereas LG is as low expressing as the S-allele indicating that 5-HTTLPR is functionally triallelic (Hu et al. 2006). In contrast to older studies using less refined imaging technologies (Willeit et al. 2001), three newer studies using the selective SERT ligand [11C]DASB (Wilson et al. 2000), and considering the triallelic nature of 5-HTTLPR, were able to show an association of the 5-HTTLPR LongA/LongA (LA/LA) genotype and high SERT binding in the living human brain (Praschak-Rieder et al. 2007; Reimold et al. 2007). However, a recent [^{11}C]DASB study gave negative results for both the bi- and triallelic 5-HTTLPR polymorphism (Murthy et al. 2010). Thus, the effect of the triallelic 5-HTTLPR on SERT density in the human brain seems to be rather limited, in that three positive [^{11}C]DASB PET studies describe only moderate elevations (approximately 10–20%) in SERT binding for high-expressing genotypes, and the studies disagree on the brain regions displaying higher binding. In contrast to cell lines, several conditions other than genetic ones have been shown to influence or associate with SERT binding in vivo. These include ethnicity (Praschak-Rieder et al. 2007), personality (Kalbitzer et al. 2009), biography (Miller et al. 2009), psychiatric diagnoses (Malison et al. 1998; Willeit et al. 2000, for review see Meyer 2007 and Stockmeier 2003), and season (Praschak-Rieder et al. 2008).

One of the first studies to investigate seasonal effects on brain SERT binding evaluated a small sample of healthy females ($n = 11$) using the nonspecific monoamine transporter ligand [^{123}I]β-CIT and SPECT. Evidence was found for higher binding in subjects scanned in spring and summer as compared to those scanned in autumn (Neumeister et al. 2000). However, using the more specific radioligand [^{11}C]DASB and PET in a much larger group of healthy subjects ($n = 88$), we were able to show that [^{11}C]DASB binding potential (BP_{ND}) values

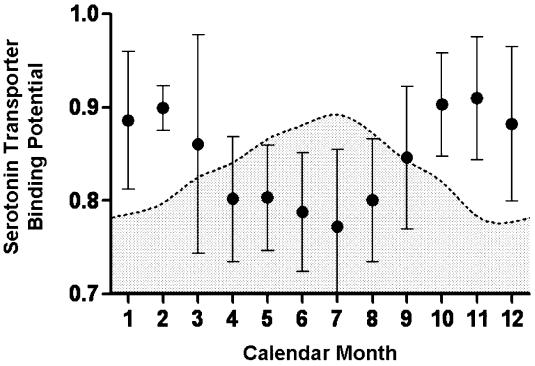

Fig. 1 Reciprocal peaks and troughs of brain serotonin transporter (SERT) binding in 88 healthy subjects and duration of sunshine in Toronto, Ontario (shaded area; range: 2.4 h (minimum) to 9.2 h (maximum) a day. SERT binding potential (BP) values were measured using the selective SERT radioligand [^{11}C]DASB and Positron Emission Tomography. *Circles* represent bimonthly moving averages of mean binding potential values derived from six predefined brain regions (prefrontal cortex, anterior cingulate, caudate, putamen, thalamus, and midbrain). Numbers on x-axis are calendar months

show considerable seasonal variation (Praschak-Rieder et al. 2008), in that [^{11}C]DASB BP_{ND} values are high in autumn and winter, and decrease with increasing duration of sunshine in spring for reaching a nadir in summer (Fig. 1).

In this study and another study on the influence of light on brain SERT binding (Kalbitzer et al. 2010), [^{11}C]DASB BP_{ND} values showed a negative correlation with average daily duration of sunshine, with higher values occurring at times of lesser sunlight. Peak differences in [^{11}C]DASB BP_{ND} values between the months with highest and lowest binding values in the Praschak-Rieder et al. were as high as 40% (Fig. 2).

Higher SERT binding in autumn/winter as compared to spring/summer has been found in several recent studies. For example, in a study involving 29 healthy subjects that examined the effects of age on SERT binding using the radioligand [^{11}]C-(+)McN5652 and PET (Buchert et al. 2006), in a [^{123}I]β-CIT SPECT study involving 98 subjects (49 of them with major depression; (Ruhe et al. 2009)), and in a recent [^{11}C]DASB-PET study involving 54 healthy subjects who were also genotyped for the 5-HTTLPR polymorphism (Kalbitzer et al. 2010). The latter study found seasonal variation in [^{11}C]DASB binding in carriers of the 5-HTTLPR s-allele only, while no significant effects of season were found in 5-HTTLPR *l*-allele homozygous subjects. A small SPECT study comparing binding of the selective SERT ligand [^{123}I]ADAM in 12 individuals assessed in winter and summer (Koskela et al. 2008) did not detect significant seasonal changes.

Thus far, higher brain SERT binding in autumn and winter as compared to spring and summer has been found in four studies using three different imaging methods A direct consequence of this finding is that studies on group differences in

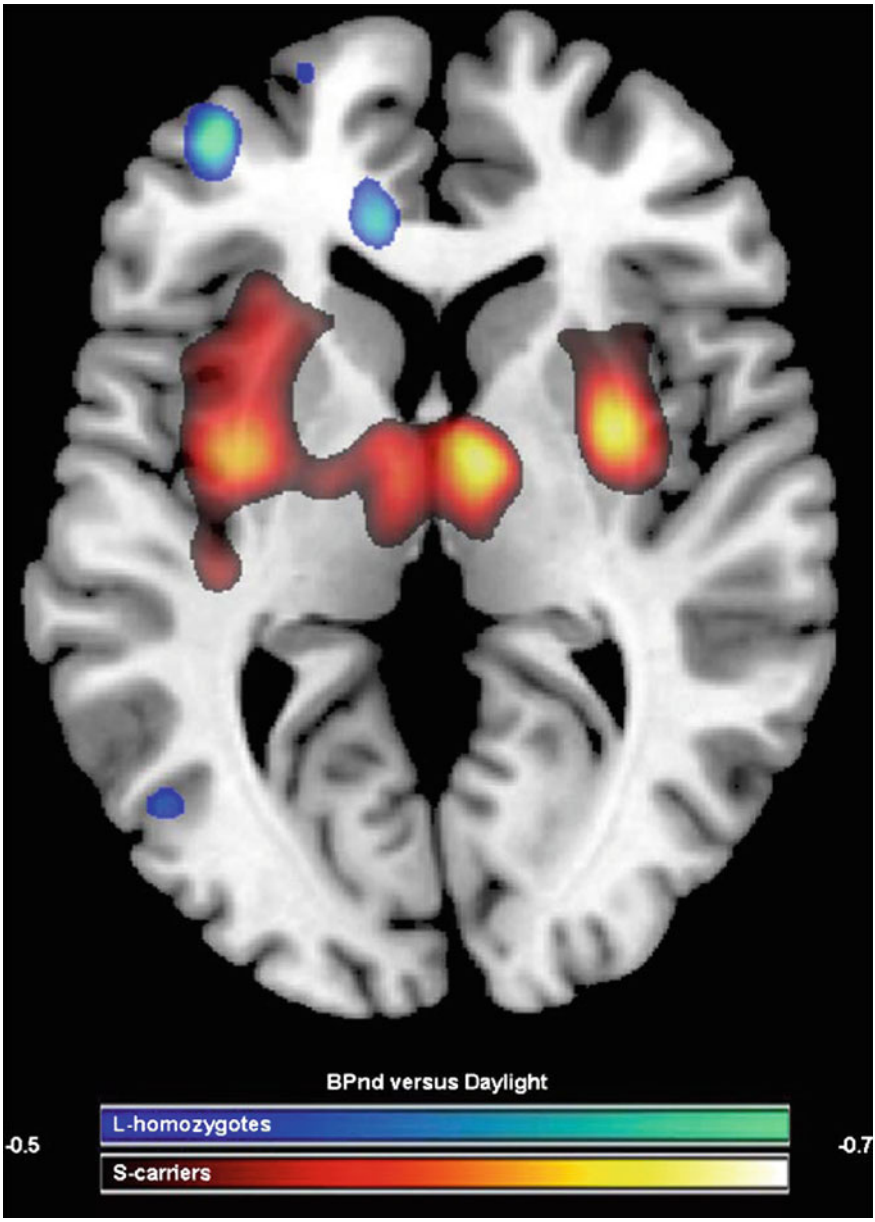

Fig. 2 Results of a voxel-based analysis of parametric images representing binding of the selective serotonin transporter (SERT) radioligand [^{11}C]DASB. The image represents a map of voxels with significant negative correlation between the local amount of daylight on the day of the positron emission tomography (PET) scan and [^{11}C]DASB nondisplaceable binding potential (BP_{ND}) values in carriers of the 5-HTTLPR S-allele. Brain structures with the strongest correlation are putamen and thalamus. Correlations between daylight and SERT binding in L-homozygotes (*blue*) failed to reach level of significance (Kalbitzer et al. 2010)

brain SERT binding, such as studies on the difference between depressed and healthy subjects, or studies on the effects of genetic polymorphisms on brain SERT binding, need to control for the effects of season. A good example is a study by Praschak-Rieder et al. (2007) that investigated the effects of 5-HTTLPR on [^{11}C]DASB binding in the brain. The study found significantly higher binding in subjects with high-expressing 5-HTTLPR variants. A post hoc analysis controlling for the effects of season as a confounding variable (Praschak-Rieder et al. 2008) found an enhanced effect of genotype as compared to the analysis that did not take into account seasonal variance in SERT binding.

While it is now well supported by evidence that there exists a seasonal variation in brain SERT ligand binding, much less is known on the behavioral consequences or molecular mechanisms associated with this finding. Still, the finding of a seasonal variation in SERT binding might help to explain seasonal variation in physiological and behavioral parameters observed in clinical and non-clinical populations. It is, for instance, a remarkable and highly replicated finding that suicide numbers in temperate and polar zones show a peak in spring (Durkheim 1897; Chew and McCleary 1995; Partonen et al. 2004; Bjorksten et al. 2005; Rocchi et al. 2007; Preti and Miotto 1998; Vyssoki et al. 2011). The peak is shifted for 6 months in the southern hemisphere (also occurring in spring; Heerlein et al. (2006)) and it seems to be absent in equatorial regions (Parker et al. 2001). Some studies relate the suicide peak to the rapid increase of environmental light in spring, with suicide numbers increasing parallel to the duration of daily sunshine (Linkowski et al. 1992; Petridou et al. 2002; Papadopoulos et al. 2005; Lambert et al. 2003; Vyssoki et al. 2011). Consistent with this perspective, suicide attempts (Praschak-Rieder et al. 1997; Haffmans et al. 1998), although rare (Lam et al. 2000), are described as an early complication of bright light therapy in SAD.

The literature offers extensive evidence for a close relationship between brain serotonin function, impulsivity, and violent suicide (see for example (Asberg et al. 1976, 1986; Coccaro et al. 1989; Mann and Malone 1997; Mann et al. 1996; Malone et al. 1996; Walderhaug et al. 2007). Post-mortem studies have found reduced SERT binding in several brain areas of suicide victims (Leake et al. 1991; Joyce et al. 1993; Laruelle et al. 1993; Arango et al. 1995; Mann et al. 2000), and the low-expressing allele of the serotonin transporter promoter polymorphism 5-HTTLPR (Heils et al. 1996) has repeatedly been associated with violent suicides (Bellivier et al. 2000; Bondy et al. 2000; Courtet et al. 2001; Lin and Tsai 2004 see Anguelova et al. 2003; Bondy et al. 2006 for review). Converging evidence from brain imaging (Tiihonen et al. 1997; Lindstrom et al. 2004; Frankle et al. 2005; Ryding et al. 2006; Sekine et al. 2006) and peripheral SERT binding studies (for example Coccaro et al. 1989; Patkar et al. 2004; Callaway et al. 2005) suggests a close relationship between reduced SERT binding and impulsivity. Our data show a uniform reduction in SERT BP values from winter and early spring towards June. This coincides remarkably with the timing of the suicide peak in late spring found in epidemiological studies (Chew and McCleary 1995; Rocchi et al. 2007; Partonen et al. 2004; Bjorksten et al. 2005; Preti and Miotto 1998; Vyssoki et al. 2011). Our findings could thus suggest that reductions in SERT binding in spring

favor a state of heightened impulsivity in some subjects, who might then be more prone to enact on suicidal thoughts than they would have been during other times of the year.

However, the evidence for seasonal variation in brain SERT binding has some caveats that should be addressed in future studies: the above-mentioned studies (Praschak-Rieder et al. 2008; Ruhe et al. 2009; Kalbitzer et al. 2010; Buchert et al. 2006) rely on a comparison of subjects who underwent one single PET scan and were then grouped according to season. None of these studies measured SERT binding repeatedly in the same subjects at various times of the year. Since brain SERT binding shows high interindividual variability, it is not possible at present to quantify the true effect size of intraindividual seasonal variation in brain SERT binding. Given that individuals differ greatly in their behavioral and psychological response to seasonal changes in the environment, it is to be expected that seasonal variation in brain SERT binding might vary from individual to individual as well. The mechanism leading to seasonal changes in brain SERT binding is another unresolved scientific question.

In our view, this problem involves three interrelated issues. First, what do the outcome parameters of these studies (BP_{ND} or specific to nonspecific binding ratio) really measure? Second, which one of the many seasonal changes in the environment influences brain SERT binding? And third, what are the molecular mechanisms that connect brain SERT binding with seasonal changes in the environment?

The BP_{ND} values used in the [^{11}C]DASB studies are calculated as B_{max}/k_d, where B_{max} is the maximal binding capacity of SERT molecules in the region of interest and k_d is the equilibrium dissociation constant between SERT and the radioligand and thus a measure for the 'affinity' of SERT for its ligand. Only saturation experiments with low versus high amounts of cold ligand can help distinguish between seasonal changes in SERT affinity for [^{11}C]DASB and seasonal changes in the amount of extracellular SERT protein. Another possible confound are changes in radioligand binding due to competition between the radioligand and endogenous or "natural" ligands. Changes in binding of dopamine $D_{2/3}$ receptor radioligands have successfully been used to obtain estimates on fluctuations in endogenous dopamine levels in the living human brain (see for example Laruelle et al. 1995; Breier et al. 1997; Abi-Dargham et al. 2000; Willeit et al. 2008a; see Laruelle 2000 for critical review of the method). In theory, it would thus be possible that seasonal changes in [^{11}C]DASB binding are secondary to changes in extracellular serotonin levels. A study in non-human primates has indeed found reductions in brain [^{11}C]DASB binding after administration of a high dose of the serotonin precursor 5-hydroxy-L-tryptophan. However, results from a study in humans combining [^{11}C]DASB PET and tryptophan depletion (Praschak-Rieder et al. 2005), a research paradigm where serotonin levels are artificially reduced by dietary measures, suggest that extracellular serotonin levels do not significantly influence [^{11}C]DASB binding in the living human brain. Ideally, seasonal variations in SERT expression levels should thus be investigated in post-mortem brain samples, where an earlier study found considerable seasonal changes

in serotonin availability and metabolism (Carlsson et al. 1980). Some preliminary evidence on possible implications of a seasonal variation in SERT binding is derived from a study on platelet SERT function in depressed patients with SAD and healthy control subjects (Willeit et al. 2008b). The study found significant seasonal variation in platelet SERT function in patients and healthy control subjects but no significant differences in SERT B_{max} or k_d values between autumn/winter and spring/summer. However, in good accordance with brain imaging studies, if a parameter analogous to the BP_{ND} (B_{max}/k_d) used for PET imaging is calculated, the parameter is significantly greater in fall/winter than in spring/summer (M. Willeit, unpublished observation). However, the increase in SERT binding (Praschak-Rieder et al. 2008; Ruhe et al. 2009; Kalbitzer et al. 2010; Buchert et al. 2006), SERT activity (Willeit et al. 2008b), and metabolic turnover of serotonin (Lambert et al. 2002), has the potential to explain the emergence of depressive symptoms during the dark season, especially in those individuals who have other predisposing factors, such as an increase in brain monoamine-oxidase activity (Meyer et al. 2006).

The second unresolved question is which of the many seasonally varying environmental factors cause the changes observed in brain SERT binding. Although it seems somewhat obvious at first sight that it is the different amount of light that leads to changes in brain SERT binding, it is methodologically quite difficult to disentangle the influence of factors such as the amount of light, temperature, day-length, rate of change in day-length, and so on. The main reason for this is that all those factors show high cross-correlation, since they all are secondary to the main astronomical changes during the course of the year. However, one study using the nonspecific SERT ligand [^{3}H]imipramine has shown higher SERT binding in the brain of rodents kept at long night–short day conditions when compared to animals kept at short night–long day conditions (i.e., lighting conditions that resemble summer). To a certain degree, the Praschak-Rieder et al. 2008 study also supports an important role of light, as duration of sunshine correlated significantly more strongly with brain [^{11}C]DASB binding than with average temperature values (Praschak-Rieder et al. 2008).

The third aspect of this problem is to propose and prove a plausible causal mechanism connecting changes in the environment with SERT binding in the brain. A solution to this problem is contingent on the above described steps, and requires animal testing with prospectively manipulated environmental conditions. There exist several potential pathways, but a first step will be to clarify whether changes in brain SERT binding are induced by a systemic factor (in this case most likely humoral) or a factor affecting the brain only, thus possibly involving neuronal signaling into the brain (for instance via the retino-hypothalamic tract). The above-mentioned parallel changes in brain and platelet B_{max}/k_d values rather point to a common, possibly humoral factor regulating SERT in the brain and periphery at the same time.

A recent study (Spindelegger et al. 2011) also suggests that brain serotonin 1_A ($5\text{-}HT1_A$) receptor binding of the selective radioligand [^{11}C]WAY-100635 might show light-dependent changes. In contrast to SERT binding, $5\text{-}HT1_A$ receptor

binding showed a positive correlation with the amount of sunlight, such that higher binding occurred in individuals exposed to more light in the week before PET scanning. However, this finding needs independent replication.

In summary, PET and SPECT imaging of serotonergic target structures has yielded fascinating results, with many of the results still waiting for replication studies. An increase in SERT binding in the dark season as compared to spring and summer has been shown by four studies using three different imaging methods. This finding is also a good example for the importance of this string of research for the entire field, as it advances our understanding of regulatory processes in serotonin neurotransmission that may be relevant in physiological and pathological processes.

4 Seasonal Effects on the Brain Dopamine Systems

Dopamine is a monoamine transmitter formed from the essential amino acid tyrosine. It is of preeminent importance for physiological functions such as motor control, motivated behavior, and implicit and explicit learning processes (Dalley and Everitt 2009). Several components of dopamine signaling show clear circadian oscillations, and some of them have been shown to be regulated by the photoperiod via a mechanism depending on the integrity of the hypothalamic suprachiasmatic nucleus (SCN), the main zeitgeber-structure in mammalians. This includes dopamine transporter (DAT) binding and expression of dopamine hydroxylase, the rate-limiting enzyme for dopamine synthesis, in the ventral-striatal nucleus accumbens (Sleipness et al. 2007b). Consistently with these effects it has been shown that primary cocaine reinforcement, which depends on the mesolimbic dopamine system (Espana et al. 2010), also shows photoperiodic regulation (Sorg et al. 2011; Sleipness et al. 2007a). Dopamine and dopamine metabolites show a clear circadian rhythm in the rodent and avian retina (Doyle et al. 2002; Lorenc-Duda et al. 2009), with dopaminergic retinal neurons being regulated by non-visual photoreception via intrinsically photosensitive retinal ganglion cells (ipRGCs; Zhang et al. 2008). In humans, polymorphisms in the dopamine D_4 receptor gene interact with season of birth to influence psychological traits and body mass index (Chotai et al. 2003; Eisenberg et al. 2007; Roussos et al. 2010; Levitan et al. 2006, 2010). In summary, there is strong evidence that dopamine neurotransmission is regulated in part by photoperiodic and light-dependent rhythms.

However, there is only limited evidence on seasonal or light-dependent changes in dopamine neurotransmission in humans. One SPECT study showed reduced [^{123}I]β-CIT binding in the DAT rich striatum in patients with SAD, suggesting that besides serotonin (Willeit et al. 2000), changes in dopamine transmission may be involved in the pathogenesis of SAD (Neumeister et al. 2001b). Two recent studies have investigated light-dependent changes in dopamine $D_{2/3}$ receptor binding (Tsai et al. 2011) and seasonal changes in uptake of the radiolabeled dopamine precursor [^{18}F]DOPA (Eisenberg et al. 2010) in the striatum of healthy subjects.

The Tsai et al. study, conducted in subtropical Taiwan, investigated a total of 68 healthy subjects and analyzed subjects in the lowest and highest quartile of average sunshine duration in the 30 days prior to their SPECT scans, leaving 35 subjects for final analysis. Subjects scanned at times of more sunshine had higher striatal [^{123}I]iodobenzamide ([^{123}I]IBZM) binding than subjects with lower mean sunshine hours during the month prior to SPECT scans. The duration of sunshine and day-length varies little during the year in Taiwan. Conducting the study at higher degrees of latitude might thus have improved the power for detecting light-dependent changes in $D_{2/3}$ receptor binding. The result emphasizes the importance of seasonal changes in dopamine transmission in humans. However, its interpretation is somewhat difficult, since it is known that [^{123}I]IBZM is sensitive towards fluctuations in endogenous dopamine levels (Innis et al. 1992; Laruelle et al. 1995, 2000). Higher [^{123}I]IBZM binding could thus indicate either higher dopamine $D_{2/3}$ receptor levels, or a decrease in extracellular dopamine levels. A way to disentangle the two possible interpretations is to decrease competition of endogenous dopamine by catecholamine-depleting procedures.

The Eisenberg et al. study investigated the effects of season on striatal [^{18}F]DOPA uptake in a large sample($n = 86$) of healthy subjects scanned during a period of several years. Subjects scanned in fall and winter ($n = 42$) had significantly higher uptake of [^{18}F]DOPA into the putamen than those scanned in spring and summer, with the difference in the rate constant K_i being approximately 5%. A voxel-wise analysis located the maximal difference in [^{18}F]DOPA uptake in the posterior putamen. The study did not relate [^{18}F]DOPA uptake to sunlight or other environmental parameters. The outcome parameter used in this study, and in most other [^{18}F]DOPA PET studies, the rate constant K_i, is generally interpreted as reflecting presynaptic L-DOPA uptake and storage. The result would thus indicate a greater amount of dopamine stored in presynaptic terminals during fall and winter as compared to spring and summer. This would be in line with the finding of lower striatal [^{123}I]IBZM binding at times of lesser light (Tsai et al. 2011), since competition at postsynaptic $D_{2/3}$ receptors would be greater with levels of synaptic dopamine being higher during fall and winter. However, further studies are needed to support this hypothesis, particularly because—as shown in patients with schizophrenia—higher dopamine synthesis rates can also be associated with reduced steady-state storage of [^{18}F]dopamine (Kumakura et al. 2007).

5 Summary

The change of seasons induces variations in several behaviors and psychological domains in humans living in temperate and polar zones. On the one hand, these variations are part of an adaptive response of the organism to the dramatic changes in temperature, lighting conditions, and food availability between warm and cold seasons. On the other hand, these variations may become maladaptive in some individuals, leading to depressive episodes in seasonal affective disorder, or to an

increase in impulsivity that might be associated with increased risk for suicide. Brain monoamine systems have a key role in modulating all behavioral and psychological domains that vary with the seasons. This, together with the fact that many radioligands for human use target monoaminergic structures in the brain, has led to an increasing number of neuroimaging studies investigating seasonal changes in human brain monoamine systems.

Some intriguing findings, such as a seasonal variation in brain dopamine $D_{2/3}$ receptor binding (Tsai et al. 2011) and striatal [^{18}F]DOPA uptake (Eisenberg et al. 2010) or the finding of light-dependent changes in brain 5-HT_{1A} receptor binding (Spindelegger et al. 2011) still need independent replication. One finding, the seasonal variation in brain SERT binding, has been shown in four studies using three different imaging technologies (Praschak-Rieder et al. 2008; Kalbitzer et al. 2010; Ruhe et al. 2009; Buchert et al. 2006). The strong correlation of SERT binding with the amount of environmental light found in two of the studies (Praschak-Rieder et al. 2008; Kalbitzer et al. 2010) further corroborates the importance of seasonal influences on brain serotonin transmission.

Today, we still lack deeper knowledge on the mechanisms mediating the adaptive—and sometimes maladaptive—changes in brain function following seasonal changes in the environment. Future research on the molecular background of seasonal changes in the human brain will need to establish suitable animal models that allow for a discrete and prospective manipulation of the multitude of environmental parameters that change during the course of a year. Only when integrating knowledge on the effects of season derived from animal models with results from human neuroimaging studies, will we be able to make significant progress in our understanding of brain monoamine function.

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Magnetic Resonance Spectroscopic Methods for the Assessment of Metabolic Functions in the Diseased Brain

Hélène Hall, Sandra Cuellar-Baena, Carina Dahlberg, René in't Zandt, Vladimir Denisov and Deniz Kirik

Abstract Magnetic resonance spectroscopy (MRS) is a non-invasive technique that can be used to detect and quantify multiple metabolites. This chapter will review some of the applications of MRS to the study of brain functions. Typically, ^{1}H-MRS can detect metabolites reflecting neuronal density and integrity, markers of energy metabolism or inflammation, as well as neurotransmitters. The complexity of the proton spectrum has however led to the development of other nuclei-based methods, such as ^{31}P- and ^{13}C-MRS, which offer a broader chemical shift range and therefore can provide more detailed information at the level of single metabolites. The versatility of MRS allows for a wide range of clinical applications, of which neurodegeneration is an interesting target for spectroscopy-based studies. In particular, MRS can identify patterns of altered brain chemistry in Alzheimer's patients and can help establish differential diagnosis in Alzheimer's and Parkinson's diseases. Using MRS to follow less abundant neurotransmitters is currently out of reach and will most likely depend on the development of methods such as hyperpolarization that can increase the sensitivity of detection. In particular, dynamic nuclear polarization has opened up a new and exciting area of medical research, with developments that could greatly impact on the real-time monitoring of in vivo metabolic processes in the brain.

Keywords Brain · Neurodegeneration · Metabolism · Alzheimer's disease · Parkinson's disease · ^{13}C · ^{15}N

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Abbreviations

ACh	Acetylcholine
AD	Alzheimer's disease
Ala	Alanine
CDR	Clinical dementia rating
DA	Dopamine
DNP	Dynamic nuclear polarization
DRS	Dementia rating scale
Glc	Glucose
Gln	Glutamine
Glu	Glutamate
Glx	Glutamate/glutamine/GABA
GPC	Glycerophosphocholine
Lac	Lactate
mIns	Myo-inositol
MCI	Mild cognitive impairment
MMSE	Mini mental state examination
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NAA	N-acetylaspartate
NMR	Nuclear magnetic resonance
PC	Phosphocholine
PCr	Phosphocreatine
PD	Parkinson's disease
PDD	Parkinsons's disease with dementia
PET	Positron emission tomography
Pi	Free phosphate
RF	Radio-frequency
SNR	Signal to noise ratio
Tau	Taurine
TCA	Tricarboxylic-acid
tCr	Total creatine
TE	Echo time

Contents

1 Introduction

Neurodegenerative diseases cover a heterogeneous group of neurological disorders presenting various clinical and pathological phenotypes. They are characterized by a selective and progressive neuronal dysfunction, which is typically followed by the degeneration of cells in specific functional and anatomical systems in the central nervous system (Aarsland and Kurz 2010; Przedborski et al. 2003). The prevalence of degenerative brain disorders is increasing worldwide, which is closely correlated with the ageing population. The resulting health-related impact on patients and caregivers along with the substantial financial cost to society makes their management a challenge and a major burden.

There is currently no cure available for neurodegenerative diseases and the main relief to patients resides in symptomatic treatments. Development of more advanced treatment modalities that can slow the progression of the disease has been defined as a priority in the scientific community. However, this goal comes with great challenges. The requirement to assess parameters that can report the progression of the disease over time on individual subjects renders the commonly used biochemical and histological post mortem analysis insufficient, as they can only provide cross-sectional information of mainly the end-stage disease. Therefore there is a critical need to develop better diagnostic methods as well as better ways to follow the patients up.

The establishment and refinement of various noninvasive imaging techniques over the last decade provide a unique potential in longitudinal assessment of patients as subjects can be submitted to examinations repeatedly. Consequently, we now have the opportunity to explore dynamic changes in the brain over time as the disease advances. Several imaging methods relying on positron emission tomography (PET) tracers and magnetic resonance imaging (MRI) are currently used in preclinical and clinical studies to assess disease-related changes in patients suffering from neurodegenerative diseases. PET is a very sensitive imaging technique based on the intravenous injection of radiolabeled ligands, their tissue uptake and subsequent binding to specific molecular targets (e.g., neurotransmitter receptors or transporters) (Kirik et al. 2005; Strome and Doudet 2007). PET can provide information about the regional distribution and affinity of receptors in the brain and enables the measurement of the local rates of chemical processes in vivo (Hutchinson et al. 2002), making it possible to detect molecules in a noninvasive

way with a very high sensitivity (10^{-9} to 10^{-12} M) in the living brain (Fumita and Innis 2002). PET has been widely used to study several neurological disorders, e.g. in applications characterizing the role of the dopaminergic system in Parkinson's disease (PD) and its involvement in motor control [for review see (Brooks 2003)], or in differential diagnosis of uncommon forms of neurodegenerative diseases, such as typical PD and other Parkinsonian syndromes or dementia (Herholz et al. 2007). It has also been applied to in vivo monitoring of disease progression in PD (Morrish et al. 1996). Finally, one of the advantages of in vivo imaging relates in the long-term evaluation of therapeutic efficacy in the living brain. For instance, PET has been applied to visualize the viability and function of striatal implants in PD [for review see (Brooks 2003; Olanow et al. 1996; Sawle and Myers 1993)] and the metabolic effects of intrastriatal grafting in Huntington's disease patients (Gaura et al. 2004; Reuter et al. 2008).

Despite these strengths, the utility of PET imaging is limited by a relatively low spatial resolution, the production of ionizing radiations and the extensive infrastructure required to synthesize the tracers, which often have to be on site due to short half-life of the radionucleotides used. Furthermore, PET cannot provide anatomical information by itself and is often combined with computed tomography in order to show both metabolic functions and accurate anatomical structures. In addition, PET cannot measure the direct levels of multiple neurotransmitters or metabolites separately. For example, using ^{18}F-DOPA as a ligand, the radioactive signal acquired does not distinguish between ^{18}F-DOPA, ^{18}F-DA and ^{18}F-DOPAC. The use of mathematically driven compartment models are required to estimate ^{18}F-DOPA uptake into dopaminergic neurons, conversion to dopamine (DA) or storage in vesicles.

Nuclear magnetic resonance (NMR), an ionizing radiation-free technique, can be used to provide complementary information that is not accessible through PET. Magnetic resonance imaging (MRI) and spectroscopy (MRS) techniques have become an essential part of the common tools available to clinicians and researchers in the assessment of normal and diseased brain. The magnetic resonance phenomenon allows a large variety of applications, including analysis of chemical composition, determination of molecular structure and dynamics. It has many advantages because of its molecular specificity, spatial selectivity, high sensitivity to molecular mobility and ability to give quantitative information at the molecular level. The NMR signal is intrinsically weak, but increases in strength with increasing gyromagnetic ratio of the nucleus being observed. Hydrogen (present in fat and water in the body) is by far the most commonly investigated nucleus, having high MR sensitivity. Other common nuclei to be studied are phosphorus, carbon, sodium and fluorine.

MRI has been used to obtain excellent images of brain structures and to identify structural alterations by using the signals from different nuclei within the brain. MRS on the other hand has the potential to detect more subtle pathological changes that cannot be seen with MRI, such as biochemical events that may precede or parallel the anatomical and histological changes (Firbank et al. 2002). Indeed, while MRI provides structural information about regions of interest in the

tissue examined, MRS provides information about the chemical nature of the tissue and can be used to determine levels of substances, which makes it a powerful method for direct and noninvasive identification of major metabolites in the brain. Furthermore, measurement of concentration and synthesis rates of specific biochemical compounds can be obtained, with spatial localization, in defined areas in the brain. This is referred to as localized MRS (Novotny et al. 2003; Rosen and Lenkinski 2007). Typically, most proton MRS studies can achieve a spatial resolution of about 1 cm^3 (Gruber et al. 2003).

This chapter will focus on MRS studies of the brain and on the chemical information currently available to assess brain metabolism in humans and animals, with a particular emphasis on the study of neurodegenerative diseases. In addition, it will cover some of the emerging spectroscopic techniques that are being developed to increase MRS sensitivity and the new research areas triggered by these advances.

2 Theoretical Basis of Magnetic Resonance Spectroscopy

Magnetic resonance is a noninvasive technique that in most cases does not require any special treatment or labeling of the subject. The equipment can be tuned to pick up signals from different chemical nuclei within the body. When positioned in an MR scanner, the magnetic moment of the nuclei in the body line up due to the experienced magnetic field. Owing to this polarization, a small rotating net magnetic moment builds up in the object when it is placed into the magnet. Subsequent to a radio-frequency (RF) pulse exposing the object at the resonance frequency of the nuclei under investigation, the net magnetic moment can be measured and transformed into signal information in one, two or three dimensions. Depending on the design of the experiment, biochemical information can be obtained from every position in the subject (Callaghan 1991; de Graaf 2007; Keeler 2005).

Experimentally, the subject is placed inside an RF coil in a highly homogeneous magnetic field of known strength. Nuclei with non-zero spin (such as ^{1}H, ^{2}H, ^{19}F, ^{13}C and ^{31}P) have a nuclear magnetic momentum and will align to some degree in the magnetic field. At thermal equilibrium, each magnetic momentum aligns with the field (lower energy state) or against the field (higher energy state). As the quantity of the magnetic momentum in the direction of the field is slightly higher than those in the opposing direction, this results in a net nuclear magnetisation. To perturb the nuclear magnetisation from thermal equilibrium, the nuclear spins are excited by an RF pulse. The precessing magnetisation after perturbation induces a small voltage in the surrounding tuned coil by the process of electromagnetic induction. It is this voltage that forms the NMR signal (Callaghan 1991; Keeler 2005).

The NMR sensitive nuclei can absorb energy at a frequency corresponding to the difference between their energy levels. The difference between higher and

lower energy states is influenced by the local environment of the nuclei, especially by the neighboring electrons, providing local magnetic fields that differ for each unique nuclear position within a molecule. Their nuclear magnetisation thereby precesses with slightly different characteristic frequencies, Larmor frequencies, $\omega_0 = \gamma B(1-\sigma)$, where γ is the gyromagnetic ratio of the nucleus being observed, B is the applied magnetic field and σ is the shielding factor associated with the chemical environment of the nuclei being observed. The difference between different shielding factors is called chemical shift. The unique resonance frequency (chemical shift) of an active nucleus is dependent on its chemical environment, which is what provides the chemical specificity of NMR (de Graaf 2007; Gadian 1995; Keeler 2005).

Since the NMR signal is the sum of all different characteristic frequencies that the spins are precessing with, the Fourier transform of the signal yields a spectrum in which the integral of each peak is proportional to the number of nuclei having a specific chemical environment.

Generally, after excitation by RF pulses, the precessing nuclear magnetisation returns to equilibrium. Often, this can be characterized by two relaxation times. T_1 is the longitudinal relaxation time, which describes the exponential recovery of the equilibrium longitudinal magnetisation that is aligned with the applied magnetic field. T_2 is the transverse relaxation time, which describes the exponential decay of the precessing component of the net magnetisation, and hence, also the decay of the signal. T_1 and T_2 strongly depend upon the local molecular environment and are sensitive to molecular motion.

3 Magnetic Resonance Spectroscopy in the Brain

Spectroscopy methods can be divided into two categories: one based on hydrogen (^{1}H) and another based on all other nuclei with a non-zero spin (referred to as X nuclei, typically ^{31}P, ^{13}C, ^{19}F, ^{23}Na or ^{15}N). Yet, ^{1}H, ^{31}P and ^{13}C account for approximately 99% of all the studies conducted thus far (Choi et al. 2007). It is beyond the scope of this chapter to give an exhaustive list of all compounds detectable through MRS. Instead we will focus on some of the metabolites and neurotransmitters that have proved useful in the study of brain metabolism in the healthy brain as well as in diseased states.

3.1 ^{1}H-MRS

Due to the high natural abundance of protons, ^{1}H-MRS has been the method of choice in most studies of the central nervous system. ^{1}H is also the most sensitive nucleus, as defined by the greater signal to noise ratio (SNR) it produces compared to other nuclei (Gadian 1995). At lower field strengths, such as 1.5 T or 3 T,

the number of metabolites available through ^{1}H-MRS is rather low and highly depends on the acquisition mode. Briefly, two types of acquisitions are possible, depending on the time interval between the excitation pulse and the detection, known as echo time (TE). At long TE, proton MR spectrum of the human brain exhibits quantifiable peaks from *N*-acetylaspartate (NAA), total creatine (tCr) (creatine and phosphocreatine (PCr)), choline containing compounds and in pathological conditions, lactate (Lac). At short TE, the spectrum is richer and signals from further metabolites such as myo-inositol (mIns), glutamate/glutamine/GABA (Glx), glucose (Glc), taurine (Tau) and lipids can be detected (Di Costanzo et al. 2003; Schaeffter and Dahnke 2008), although its interpretation and quantification become more challenging due to overlapping resonance frequencies. The relative levels of these compounds can reflect the cellular state of the tissue so that the established metabolic profile (result of the simultaneous quantification of many metabolites concentration) directly correlates to the health status of the tissue (Schaeffter and Dahnke 2008).

Due to the general complexity of the spectra (containing the resonances of numerous metabolites, as well as overlapping resonances), in most cases quantification of individual metabolites requires the use of a specific method for analysis. These spectral fitting methods, either time domain or frequency domain methods (Mierisova and Ala-Korpela 2001; Vanhamme et al. 2001), aim at calculating the metabolite peak area in a noisy MRS signal. There are different ways to report the results, referred to as absolute or relative quantifications (Brief et al. 2009; Kreis et al. 1993). One common way is to normalize the values in reference to water or creatine or to use the sum of all metabolites in situations where the amplitude of the reference peak (creatine) changes.

With the development of high field NMR (such as at 7 T in humans and 9.4 T or beyond in animals), RF coils, MR sequences, as well as automatic shim routines, it is now possible to have access to an even larger number of previously unresolved brain metabolites (Gruetter et al. 1998; Pfeuffer et al. 1999; Tkac et al. 2001; Mlynarik et al. 2008a). Studies in rats at 9.4 T with short TE (2 ms) provided a neurochemical profile of the brain consisting of 18 metabolites (Pfeuffer et al. 1999; Mlynarik et al. 2008b). The same 18 metabolites can also be identified and reliably quantified in much smaller structures such as the developing mouse cortex at 14.1 T, showing regional alterations in concentration of various metabolites at different postnatal days (Kulak et al. 2010). More remarkable is the detection at 14.1 T of 21 metabolites in the mouse hypothalamus (Lei et al. 2010), a structure challenging to study due to its small dimension and localization in deeper parts of the brain.

The identification of metabolites in the rat brain measured at 9.4 T with short TE and the measurement of their subsequent concentration (Pfeuffer et al. 1999) compose a neurochemical profile of the brain consisting of important presumed markers of energy metabolism (Cr and PCr, Glc, Lac, alanine (Ala)), neurotransmitters and metabolites [GABA, N-acetylaspartylglutamate, glutamate (Glu) and glutamine (Gln)], antioxidants (glutathione and ascorbate), osmolytes (Tau, mIns, scyllo-inositol), phosphoethanolamine, NAA, glycerophosphocholine (GPC)

and phosphocholine (PC) (Mlynarik et al. 2008b). However, only some of these compounds are frequently studied with MRS. The following metabolites are of most relevance to neurodegeneration.

The signal on a typical proton spectrum that arises at 2.0 ppm is attributed to NAA, which is a molecule present predominantly in neurons and synthesized in the mitochondria. Brain NAA is widely accepted as a marker of neuronal density and integrity (Baslow 2003; Urenjak et al. 1993). Therefore reduced NAA concentration can reasonably indicate neuronal loss in neurodegeneration. Because the enzyme involved in NAA synthesis is present in high concentration in mitochondria, altered NAA levels also reflect mitochondrial dysfunction (Clark 1998). The peak observed at 3.2 ppm is referred to as Cho and reflects in fact choline-containing compounds with overlapping resonance frequencies. It primarily consists of the sum of free choline, PC (a phosphatidylcholine precursor), GPC (a byproduct of phosphatidylcholine breakdown) and betaine (Katz-Brull et al. 2005). They play an important role in cell membrane synthesis and lipid metabolism so that Cho is considered a marker of membrane density and integrity. Thus, changes in choline-containing compounds can directly reflect altered cell membrane synthesis and degradation (Galanaud et al. 2007). The tCr signal at 3.0 ppm is assigned to the sum of two different overlapping resonances, Cr and PCr. Cr levels are thought to be very stable (Cr and PCr are in chemical equilibrium), homogeneously distributed throughout the brain and not subjected to change with age so that in clinical setting, the tCr peak is used as a common internal reference, with spectra interpreted as ratio in respect to Cr (such as Cho/Cr and NAA/Cr ratios) (Kauppinen and Williams 1994; Martin 2007). However, it seems that this should be taken with caution, as at least one study reported complete creatine deficiency in the brain of a child suffering from an extrapyramidal movement disorder and metabolic disturbances (Stockler et al. 1994). Further to the left on the proton spectrum, mIns gives rise to a signal at 3.6 ppm. It is a sugar-alcohol putative marker of neuroglia, found almost exclusively in astrocytes where it is recognized as an important cerebral osmolyte (Lien et al. 1990). Its concentrations can fluctuate up to tenfold. Increases in mIns levels have been reported following cellular proliferation (tumors) or in case of inflammation (Galanaud et al. 2007; Rosen and Lenkinski 2007; Ross and Bluml 2001). In the region 2.0-2.5 ppm, Glx gives rise to a complex peak resulting from the signals of Gln, Glu and GABA, three peaks inseparable at 1.5T. Glu is the most abundant excitatory amino acid transmitter in the CNS (Fonnum 1984) and the role of glutamate-mediated excitotoxicity in neurodegenerative disorders has been the center of many studies (Masliah et al. 1996; Cowan and Raymond 2006; Gubellini et al. 2006). It is the precursor of GABA, the major inhibitory neurotransmitter, which plays a crucial role in cerebral physiology (Ben-Ari et al. 2007; Fonnum 1984). Finally, the signal centered at 1.3 ppm is assigned to Lac, the end product of anaerobic glycolysis. Lac is present in physiological conditions in extremely low amounts in the brain so that it is not resolved under normal conditions. This makes its detection significant and suggest a pathological condition (Prichard et al. 1991), typically hypoxia (Edden et al. 2010) or brain ischemia (Vandersprenkel et al. 1988), situations

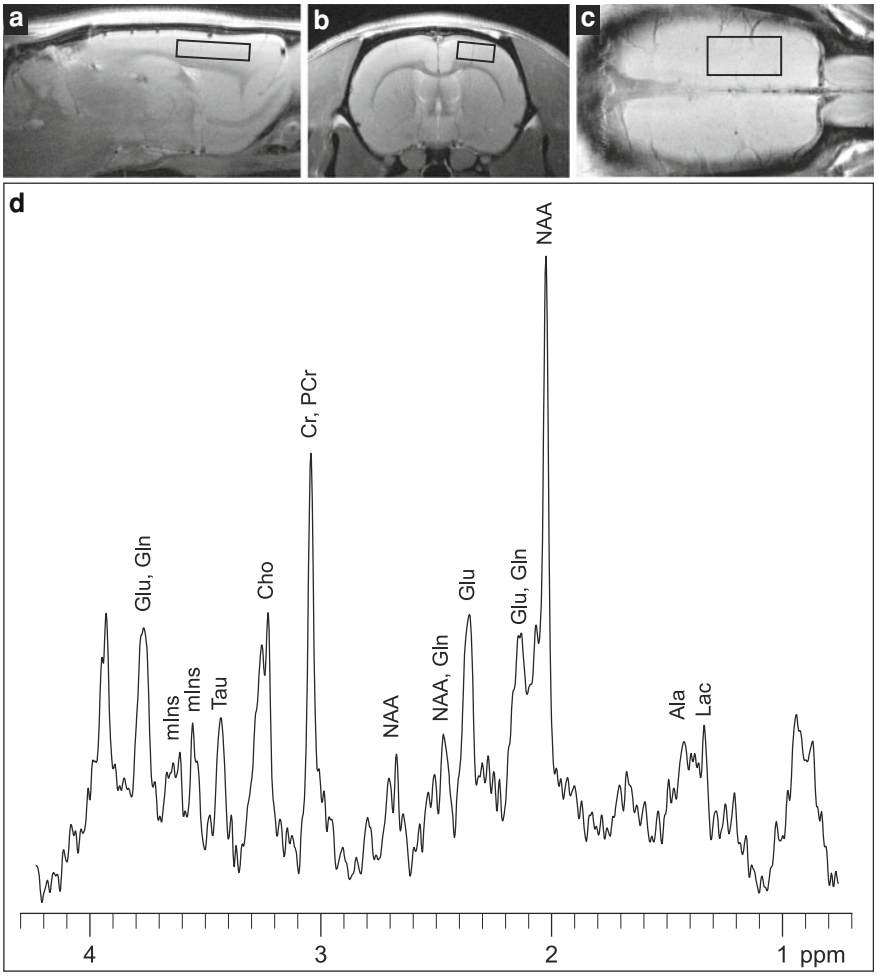

Fig. 1 Localized ^{1}H-NMR spectroscopy of a rat brain in vivo at 9.4 T. Sagittal (**a**) coronal (**b**) and axial (**c**) MRI sections indicating the location of the voxel of interest (VOI). Images were acquired using a fast spin echo sequence. (**d**) ^{1}H-MR spectrum obtained from the indicated VOI [voxel size 1.3 × 2.8 × 5 mm (= 18 μl)] using a STEAM sequence with TE = 2.5 ms, TR = 6 s and water suppression. A 4-element array coil was used and the scans were acquired using 64 averages (6 min blocks). All scans were eddy current corrected (ECC) using the jMRUI software package (http://www.mrui.uab.es/mrui/). A total of eight blocks were performed resulting in a total scan time of 50 min. A total of 32 ECC spectra were summed to give the spectrum shown and the signal was filtered for display purposes using a Lorentzian/Gaussian filter (−3Hz/6Hz) using matNMR/Matlab

where aerobic oxidation fails and anaerobic glycolysis takes over. A ^{1}H-MR spectrum recorded in a rat brain at a field strength of 9.4 T is displayed in Fig. 1 and shows the assignment of the major peaks discussed above. It should be noted that macromolecular resonances are present in the background signal of the

spectrum and therefore underlie the assigned low molecular weight metabolites. This is important to take into consideration in order to avoid the attribution of an elevated signal coming from macromolecules to the metabolites.

As shown, ^{1}H-MRS allows the simultaneous quantification of an increasing number of metabolites in the brain, thus expanding metabolic neurochemical profiling in vivo. However the difficulty in resolving the spectrum has led towards the development of other nuclei-based MRS studies.

3.2 ^{31}P-MRS

^{31}P-MRS is a heteronuclear MRS approach that has proved very useful in the investigation of tissue energy metabolism, especially in skeletal muscle (Kauppinen and Williams 1994; Radda 1986). It has become a valuable tool to evaluate the integrity of the brain through the study of its metabolism and bioenergetics (Chaumeil et al. 2009; Du et al. 2007; Shulman et al. 2004). Although ^{31}P-MRS has a lower sensitivity and spatial resolution than ^{1}H-MRS, it has the advantage of providing a broader chemical-shift range (Schaeffter and Dahnke 2008).

^{31}P-MRS is used to detect and quantify high-energy phosphate (HEP) metabolites (ATP, ADP, PCr) and free Phosphate (Pi), which reflect oxidative phosphorylation and mitochondrial function in vivo (Gadian 1995). Although ADP is not directly visible in the ^{31}P spectrum, its concentration is in fact calculated from the ATP, tCr, and PCr concentrations (Zhu et al. 2009). In addition, the chemical shift difference between the Pi and PCr peaks is used to calculate the value of intracellular pH (Henchcliffe et al. 2008; Hyder 2009). The noninvasiveness of the procedure makes it the method of choice to study the regulation of pH in vivo in the brain (Kauppinen and Williams 1994). Furthermore, it has been shown that HEP metabolites can be used to determine metabolic fluxes of ATP metabolism in the human brain. ATP metabolism is regulated by the chemical exchange network PCr $\leftrightarrow$ ATP $\leftrightarrow$ Pi, which is controlled by two enzymes. Creatine kinase (CK) catalyzes PCr $\leftrightarrow$ ATP and ATP synthase (ATPase) catalyzes Pi $\leftrightarrow$ ATP. In a study using a multiple single-site saturation method together with the solution of three Bloch equations based on the three-spin chemical exchange model, the forward and reverse rate constants for CK and ATPase reactions were calculated in the occipital lobe in humans at 7 T. First, a progressive saturation of the ATP resonance using frequency-selective RF pulses is applied and the forward CK and ATPase rate constants and fluxes calculated. Secondly, a steady-state saturation of Pi is achieved by applying frequency-selective RF pulse train over a long saturation time allowing the calculation of the CK reverse rate constant and flux. Finally, the same procedure (steady-state saturation) is applied to the PCr resonance peak to determine the ATPase reverse rate constant and flux (Du et al. 2007).

Apart from the detection of HEP metabolites, important cations can be detected by ^{31}P-NMR. Among these, magnesium is important in regulating brain energy

metabolism. Its concentration can be calculated from the chemical shift difference between α and β ATP peaks. It has been found that magnesium levels are decreased during subarachnoid hemorrhage. ^{31}P-MRS was shown to be suitable to monitor cortical intracellular magnesium to determine the reversibility of neurological alterations and the response to pharmacological treatment (Yang et al. 2008).

In order to overcome the lower sensitivity of ^{31}P-MRS, several methodological adjustments can be implemented, such as the use of high magnetic fields (Qiao et al. 2006) or polarization transfer methods (Du et al. 2007; Klomp et al. 2008; Wijnen et al. 2010). By increasing the magnetic field from 4 T to 7 T in clinical scanners, the sensitivity and spectral resolution of in vivo ^{31}P-MRS are improved and a gain of 56% in SNR in the signal of PCr can be obtained in the human brain (Qiao et al. 2006). Magnetization transfer from ^{1}H to ^{31}P uses frequency selective refocusing pulses to enhance up to 2.4 times the very low signal of phospholipids like PC, GPC, phosphorylelthanolamine and glycerol-phosphoethanolamine compared to an optimized direct ^{31}P-MRS method at 3 T (Klomp et al. 2008). Technological improvements in the design of ^{31}P head RF coils can also increase the sensitivity of the ^{31}P signal. One version of ^{31}P optimized head coil includes a quadrature birdcage resonator as a ^{1}H transmit receive coil and two free positioning surface coils operating as a ^{31}P transmit receive coil covering approximately half of the brain. In addition, this coil configuration includes ^{1}H blocking circuits to avoid electromagnetic couplings between the ^{1}H and ^{31}P elements (Klomp et al. 2008).

As described, a combination of high magnetic field strength, optimized pulse sequences and hardware development can be used to successfully overcome the lower sensitivity of ^{31}P-MRS. Following these technological developments, ^{31}P-NMR applications based on the assessment of intracellular pH, HEP metabolites and magnesium levels in the brain have been reported in multiple clinical studies. In particular, ^{31}P-MRS has proved very useful in the study of neurodegenerative disorders (Martin 2007), stroke (Schulz et al. 2009) and epilepsy (Hetherington et al. 2002).

3.3 ^{13}C-MRS

^{13}C is an MRS visible isotope, albeit with a natural abundance of only 1.1% and a low gyromagnetic ratio, making ^{13}C-MRS much less sensitive than ^{1}H-MRS and ^{31}P-MRS. The relative sensitivity of proton/phosphorus/carbon is 100:6.7:0.018 (Choi et al. 2007; Klomp et al. 2006). However, it offers a higher spectral resolution than ^{1}H-MRS and a lack of background signal. Due to this low natural abundance, most of the ^{13}C-MRS studies refer to the use of enriched substrates; an obvious advantage being that all labeled metabolites can be individually followed. Indeed, upon its administration, the ^{13}C label can incorporate into several positions in different molecules through metabolism (Ugurbil et al. 2000), which is why ^{13}C-MRS has long been used to detect the metabolism of ^{13}C-labeled compounds in vivo, with an emphasis on the use of ^{13}C-labeled glucose.

One of the first published studies describing the use of ^{13}C-glucose to assess glucose metabolism was performed in *Escherichia Coli* and demonstrated the possibility to monitor the labeling of intracellular compounds in intact cells (Ugurbil et al. 1978). Since then, it has been well established that glucose oxidation is the main mechanism for cerebral energy production under normal conditions and ^{13}C-labeled glucose can be applied to investigate glucose utilization in vivo in the brain. Following the infusion of [1-^{13}C] enriched glucose, MRS can detect the emergence of ^{13}C signal in all metabolites of glucose metabolism in the brain, from intermediates of the tricarboxylic-acid (TCA) cycle to the amino acids Glu, aspartate and Gln. After the first pass through the TCA cycle, the first detectable molecules are ^{13}C-Glu and ^{13}C-Gln labeled in the C4 position, and afterwards [3-^{13}C] and [2-^{13}C] labeled Glu and Gln, before the later appearance of Lac, GABA or NAA (Jenkins et al. 1999). This was demonstrated in ^{13}C-MRS studies in human and animal coupled to the administration of [1-^{13}C]-glucose, that indicated high labeling of [4-^{13}C]-glutamate and [4-^{13}C]-glutamine (Sibson et al. 1997; Sibson et al. 1998; Beckmann et al. 1991) It helped in establishing the glutamate-glutamine cycle between neurons and glia as a major metabolic pathway tightly coupled to glucose oxidation (de Graaf et al. 2003).

^{13}C studies can also estimate the NAA synthesis rate in vivo in humans (^{1}H-MRS can only determine NAA levels), through the infusion of [1-^{13}C]-glucose (Moreno et al. 2001). In the latter study, it was also demonstrated that NAA synthesis is directly coupled to brain glycogen metabolism, thus connecting the rate of synthesis of NAA with energy metabolism in the brain. In addition, ^{13}C-MRS is the only noninvasive way of measuring brain glycogen through incorporation of ^{13}C-labeled glucose (Choi et al. 1999), its normal levels being too low to allow natural abundance ^{13}C-NMR measurement such as in the liver (Gruetter et al. 1994).

4 Current Clinical Applications of MRS to the Study of Neurodegenerative Diseases

Since its introduction in the early 1980s in the clinics, MRS has been applied to different fields of research. Along with other imaging modalities, it is a powerful means of assessing the functionality and viability of organ transplants (e.g. kidney, liver) and is also used to establish the metabolic profile of tumors [for review see (Beckmann et al. 2000; Galanaud et al. 2007; Cox 1996)]. MRS can measure a variety of metabolic changes in the brain and this has been applied to the study of various disorders, such as brain tumors, neurodegenerative disorders, metabolic encephalopathy, ischemia, hypoxia and white matter diseases (Kauppinen and Williams 1994; Ross and Bluml 2001). Figure 2 illustrates metabolic changes detected by ^{1}H-MRS in the white matter of a patient suffering from seizures and short time hypoxia as well as traumatic subdural and subarachnoid hemorrhage.

Alzheimer's disease (AD) and PD are some of the most thoroughly studied neurodegenerative diseases, perhaps because the biochemical mechanisms underlying

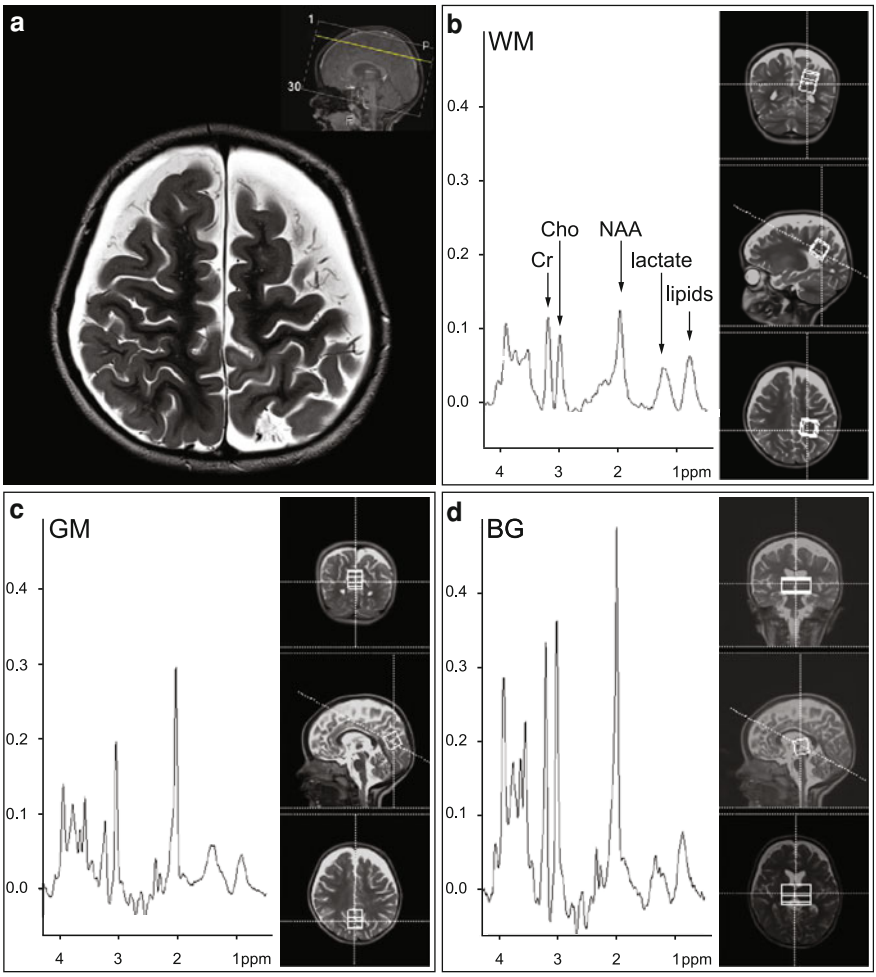

Fig. 2 Example of ^{1}H human in vivo MR spectra obtained from a patient suffering from seizures and short time hypoxia as well as traumatic subdural and subarachnoid hemorrhage. **a** Axial T2-weighted MRI displaying a subcortical lesion (hyperintense) in the medial parietal cortex and bilateral hygromas/CSF interpositions in the subarachnoid and subdural spaces. **b** Pathologic white matter (WM) spectrum with decreased NAA (N-acetyl aspartate; 1.9 ppm) and slightly increased lactate and lipids (1.3 and 0.9 ppm). **c** Normal grey matter (GM) spectrum and **d** normal spectrum from the basal ganglia (BG) in the same patient. Data were acquired in a 3 T human whole body scanner (MAGNETOM Skyra, Siemens AG, Erlangen, Germany) using a SE sequence with TE of 30 ms and TR of 1500 ms. Voxel size: 8 mm^3 for WM and GM spectra and 12 mm^3 for BG spectrum. Courtesy of I.M. Björkman-Burtscher, MD, PhD, Skåne University Hospital, Lund University

their pathology are better characterized as compared with other neurodegenerative conditions. This makes them interesting targets for spectroscopic investigations, a number of which have been reported in humans. In particular and as described

below, MRS is currently used to establish differential diagnosis of various pathologies, to follow up disease progression and more recently there has been a growing interest to apply this technology to monitoring the course of clinical treatments.

4.1 Alzheimer's Disease

AD is clinically defined by a progressive decline of cognitive functions and is considered the leading cause of dementia in the elderly. It is mainly characterized by typical neuropathological lesions in the form of two main protein aggregates: senile plaques and neurofibrillary tangles. The senile plaques are extracellular neuritic amyloid deposits primarily consisting of fibrils of the amyloid β peptide (Glenner and Wong 1984). The neurofibrillary tangles are intracellular aggregates formed mainly by the cytoskeletal microtubule-associated protein tau (Kosik et al. 1986). This is associated with the loss of cholinergic neurons within the basal forebrain (Francis et al. 1999). A noradrenergic deficiency is also described in AD patients and is characterized by a loss of noradrenergic cells in the locus coeruleus (Bondareff et al. 1981) as well as reduced levels of noradrenaline in corresponding innervated areas (Arai et al. 1984, Hoogendijk et al. 1999).

As the definitive diagnosis of AD relies on postmortem neuropathological analysis, the detection of metabolic abnormalities throughout the course of the disease could prove useful in identifying underlying mechanisms as well as following disease progression. The use of proton MRS has provided a wealth of evidence for altered metabolic profiles in different brain regions of AD patients, the most frequent finding being a reduction of NAA levels or a decrease in the NAA/Cr ratios, in both cortical and/or limbic (e.g. hippocampus) structures (Kantarci et al. 2004; Kantarci et al. 2008; Shinno et al. 2007; Ackl et al. 2005; Frederick et al. 2004; Chantal et al. 2004; Dixon et al. 2002). In a comprehensive review looking at data from reports of proton MRS in dementia, the relative decrease in NAA levels was reported to average 20% in the temporal lobe of AD patients, whereas the rest of the brain averaged a 10% drop (Firbank et al. 2002). One of the early indications that the reduction in NAA would indicate neuronal damage in AD came from studies correlating postmortem NAA levels to AD neuropathology (tangles) at necropsy (Mohanakrishnan et al. 1995). Similar conclusions were drawn from more robust studies reporting a strong correlation between reduction of MRS NAA levels and AD pathology confirmed at autopsy (higher Braak stage and neuritic plaque score) (Kantarci et al. 2008). Apart from the reduction in NAA, a consistent increase in mIns levels has been frequently reported as well (Ackl et al. 2005; Chantal et al. 2004; Kantarci et al. 2004), and would suggest that mIns is a marker of glial proliferation in AD. Some authors have shown reduced Cho levels in AD patients (Kantarci et al. 2004; Chantal et al. 2004). However, unlike the NAA and mIns findings, these results are not consistent as some researchers reported elevated Cho/Cr ratios (MacKay et al. 1996) while others failed to report any alteration in Cho measurements (Ackl et al. 2005; Schott et al. 2010), which makes the relevance of following alterations of Cho levels in AD disputable.

Based on these spectroscopic features (decrease in NAA and increase in mIns), MRS can discriminate patients with clinical AD from normal elderly subjects (Lin et al. 2005). However, alterations of NAA and mIns are not specific to AD per se. For example, decreases in NAA/Cr levels are reported in other types of dementia characterized by neuronal loss, such as frontotemporal lobar degeneration or vascular dementia (Kantarci et al. 2004). Nevertheless, the robustness of these measurements makes these two metabolites good candidates to differentiate between dementias that are attributed to AD or to other conditions. In particular, proton MRS has shown an interesting potential in predicting the evolution from mild cognitive impairment (MCI) to AD. MCI patients present memory deficits that are greater than in their normal aged counterparts but that are not severe enough to impair their daily activities (Petersen et al. 1999). MCI is considered as a transitional state between normal aging and AD (Morris et al. 2001). However, not all MCI patients will develop dementia. In terms of brain metabolic abnormalities, the available reports are somewhat conflicting. Some studies state that ^{1}H-MRS can identify a reduction of NAA levels (Chantal et al. 2004) and/or an increase in mIns (Kantarci et al. 2007) in MCI subjects. Others report that the metabolic profile of MCI subjects cannot be distinguished from normal control subjects (Garcia Santos et al. 2008), even though a trend to intermediate values between controls and AD patients can be found (Azevedo et al. 2008). Nevertheless, MRS may have some predictive value in MCI individuals. It has been suggested that baseline NAA/Cr could predict the conversion from MCI to AD, such as in the study from Modrego and colleagues which looked at metabolite ratios in hippocampal, occipital and parietal volumes. The occipital NAA/Cr was significantly lower in MCI converters compared to non-converters over a 3-year follow-up (Modrego et al. 2005). Other longitudinal studies investigating additional cortical areas have indicated similar findings [for review see (Griffith et al. 2009)]. These studies suggest that ^{1}H-MRS can have an impact on monitoring early signs of AD and that the detection of metabolic alterations as the first cognitive symptoms develop can help in the follow-up of elderly subjects with MCI.

Before the postmortem neuropathological evaluation can confirm the AD diagnosis, neuropsychological measures of cognitive functions are used to classify patients as probable AD, based on clinical rating scales such as the Mini Mental State Examination (MMSE) (Folstein et al. 1975). Once dementia is diagnosed, other scales [such as the Dementia Rating Scale (DRS) or the Clinical Dementia Rating (CDR)] can assess progression and severity of the symptoms (Hughes et al. 1982; Marson et al. 1997). Multiple studies have looked for a parallel between metabolite concentrations and MMSE, DRS or CDR scores. Several of them have reported a significant correlation between levels of NAA and/or mIns and the severity of cognitive deficit in AD (Waldman and Rai 2003; Dixon et al. 2002; Doraiswamy et al. 1998; Kantarci et al. 2007), although it should be mentioned that not all of them reached that conclusion (Jessen et al. 2005). The fact that some of these studies were based on longitudinal rather than cross-sectional assessments adds power to the correlation.

The optimal method to assess the efficacy of a therapeutic treatment would be to look at a direct effect at a cellular level in order to determine neuronal survival and/or function. However, this has so far not been possible to do in patients and clinical evaluation remains the main outcome marker. Clinical outcomes of a treatment will only look at purely symptomatic functional effects and cannot distinguish disease-modifying drug effects. This has prompted the development of surrogate markers to derive a correlate of clinical measures of disease severity. As stated above, ^{1}H-MRS offers a way to noninvasively measure markers of neuronal and glial metabolism that are of relevance in the process of AD. Several longitudinal studies have looked at the opportunity to use MRS in monitoring the treatment effect by looking at metabolic changes in AD patients during therapeutic trials with cholinergic agents. Donepezil is an acetylcholinesterase inhibitor (AChEI) shown to provide beneficial (though modest) effects on cognition in AD patients. It is currently used for symptomatic relief in mild to moderate cases along with other AChEI, such as galantamine and rivastigmine (Aderinwale et al. 2010). In a 24-week randomized, double-blind, placebo-controlled study, treatment with donepezil was associated with an increase in NAA levels in two brain regions and an improvement in performance in cognitive tasks, compared with placebo treated patients (Krishnan et al. 2003). In addition to supporting these data, another study demonstrated that NAA/Cr in the parietal lobe of patients at baseline predicted a positive treatment response: a low ratio correlated to an improvement in cognition, while a high ratio indicated no improvement in the patients (Jessen et al. 2006). Modest but favorable changes in metabolite concentrations (mainly an increase in NAA in the frontal cortex) were also shown to correlate with a modest clinical change following rivastigmine treatment for 4 months. However, the authors speculated that these changes might be transient (Modrego et al. 2006), which was in fact demonstrated by Krishnan and colleagues (Krishnan et al. 2003).

As examined, spectroscopic measures may provide good markers of altered brain chemistry in AD. In particular, markers of neuronal loss (NAA) and gliosis (mIns) that are biologically relevant to the pathology of AD can be followed by proton MRS. This method constitutes an interesting tool for the follow-up of disease progression and for the recognition of early signs of the disease as well as for the differential diagnosis of dementia.

4.2 Parkinson's Disease

PD is a disorder of the extrapyramidal motor system clinically defined by resting tremor, bradykinesia, akinesia, rigidity and postural imbalance. It is characterized by the progressive degeneration of nigrostriatal dopaminergic neurons. This leads to a severe dopamine depletion at the striatal level, responsible for most of the motor disturbances (Hirsch et al. 1988; Fearnley and Lees 1991; Nyberg et al. 1983).

Many studies using proton MRS have investigated brain metabolic profiles of PD patients in an effort to detect pathogenic markers of the disease that could broaden the understanding of the pathology as well as provide help in early diagnosis. However, it appears that quantitative ^{1}H-MRS studies in PD have yielded variable results. Due to the dramatic loss of dopaminergic neurons characteristic of the disease, a substantial loss of NAA (marker of neuronal density) could be anticipated. However, the majority of studies looking at NAA/Cr or NAA/Cho either reported a minor decrease in these ratios or failed to report any significant difference between PD cases and controls [for review see (Clarke and Lowry 2001; Firbank et al. 2002)]. In addition, an increase in NAA/Cr has also been reported in the SN of PD patients (Choe et al. 1998). However, a study using absolute quantification of metabolites showed only a decrease in the Cr level in the SN of PD patients compared to healthy age-matched controls (O'Neill et al. 2002), suggesting that the previously reported increase in NAA/Cr could be due to lower Cr rather than higher NAA. Similarly, the reduction in NAA/Cho ratios reported in some studies might in fact not reflect a reduction in NAA itself as an increase in absolute Cho levels has been observed (Clarke and Lowry 2000; Ellis et al. 1997). In this case, the biological significance of changes in Cho levels remains uncertain. Apart from the more commonly studied metabolites (NAA, Cr, Cho), Lac levels have also been measured by ^{1}H-MRS and found to be elevated in PD patients, suggesting a mitochondrial energy metabolism defect (Henchcliffe et al. 2008). A combined quantitative ^{1}H/^{31}P-MRS study investigating brain energy metabolism measured the concentration of Cr, Pi, ATP, ADP in the midbrain and putamen of PD patients. The depletion of HEP found in the mesostriatal region was in accordance with a mitochondrial dysfunction (Hattingen et al. 2009).

As it appears the results obtained in PD patients are somewhat variable, with studies reporting small changes identified with MRS while others show significant differences between PD and controls. In order to facilitate the interpretation of such data, it is of great importance to determine the origin of such variation. The disparity in the results is more likely to stem from methodological differences. The choice of absolute quantification versus the more popular use of relative ratio to an apparently stable endogenous metabolite like Cr (that has proven to vary in some situations) could be an important contributing factor (Martin 2007). The size and placement of the voxels could also account for some of the variation, especially in the SN, a challenging area to target due to its small size and location in the midbrain. The field strength could also play a major role in the variability of the results. An increasing number of studies operate at high fields, i.e. 3 T and above. Compared to 1.5 T, spectral resolution and SNR are expected to improve considerably, providing a more accurate identification and quantification of the metabolites (Di Costanzo et al. 2003). This is reflected by a study performed at 4 T that demonstrated the possibility to detect and measure GABA in the SN, a metabolite whose resonance usually overlaps with the Glu and Gln peaks. GABA quantification though required the use of a special editing sequence. The same study reported a unique profile in the SN of PD patients compared to the cortex, with high GABA and low Glu levels (Oz et al. 2006).

The relevance of ^{1}H-MRS to the study of PD extends to the investigation of dementia as well. Although PD is primarily defined as a motor syndrome, the clinical expression of the disease is more heterogeneous. Many patients express significant non-motor symptoms including depression, dementia, neuropsychiatric disturbances, olfactory deficits and autonomic dysfunction (Bosboom et al. 2004; Owen et al. 1992). Parkinson's disease with dementia (PDD) is characterized by a progressive dysexecutive syndrome and it is estimated that 30 to 40% of PD patients develop dementia (Aarsland and Kurz 2010). Studies comparing brain MRS profile obtained from PDD patients can indicate an altered brain metabolism compared to non-demented PD patients and healthy age-matched individuals. For example, a study reported occipital NAA values to be significantly reduced in PDD patients compared to PD cases and controls. The NAA levels in this region correlated with the cognitive status of the PDD patients. The authors concluded that cortical NAA level may be used as a biological marker for the severity of cognitive decline in PDD patients (Summerfield et al. 2002). Despite some similarities and overlap in the neuropathological basis of PDD and AD, increased evidence suggests that these two pathologies differ [for review see (Farlow and Cummings 2008)] and information obtained through ^{1}H-MRS tends to support this view. In fact, proton MRS has been suggested to distinguish between AD and PDD. Griffith and colleagues showed a significant reduction in NAA/Cr in the posterior cingulate gyrus of both AD and PDD patients compared to controls (Griffith et al. 2008). However, Glu/Cr was significantly lower in PDD patients versus AD patients and could discriminate between the two conditions, suggesting that these metabolic disturbances could reflect distinct neuropathologies.

Apart from the use of MRS metabolic quantification and profiling to assess disease mechanisms and progression, some studies are further exploring the use of MRS as a tool to evaluate therapeutic efficacy of pharmacological or surgical interventions. Lucetti and colleagues investigated the power of ^{1}H-MRS to assess the influence of dopaminergic treatment that is capable of improving motor functions on motor cortex metabolism. After 6 months of treatment, pergolide was shown to increase previously abnormally low Cho/Cr to normal levels, in the motor cortex of de novo PD patients (Lucetti et al. 2007). In another experiment, the survival and cellular composition of dopaminergic fetal cells transplanted into the striatum of PD patients were followed with ^{1}H-MRS. Restoration of NAA concentration at the graft level (NAA is normally absent from fetal neurons) provided evidence of the neuronal survival and maturation of the transplant (Ross et al. 1999).

Despite the extensive use of ^{1}H-MRS in the investigation of metabolic abnormalities in PD, results have been rather inconclusive when comparing PD patients with controls. Proton MRS might however have an interesting value in differential diagnosis, discriminating PDD from non-demented PD cases and controls, as well as from AD. The refinement of the existing MR methods has already allowed improvement in metabolite separation and quantification. Yet, further developments might be required to allow the detection of metabolites that would be more specific to a particular neurodegenerative disorder.

5 Emerging MRS Methods Based on the Use of Hyperpolarized Tracers: Potential Applications to the Study of Brain Function

Today, MRS has become a powerful technique and one of the most informative tools for direct and noninvasive identification of endogenous and exogenous substances in the body. It is also capable of providing a map of multiple metabolites in various tissues, and giving information about complex metabolic processes in the brain. Its potential applications have so far been hindered mainly by its intrinsically low sensitivity, precluding in vivo quantification of less abundant metabolites. Significant efforts have therefore been made to increase MRS sensitivity by utilizing higher field strength, advanced RF coil designs and sequence development, which helped to increase the list of neurochemicals detected by this method. However, the low abundant true neurotransmitters [such as DA or acetylcholine (ACh)] are far below detection by conventional ^{1}H- and ^{13}C-MRS methods performed at thermal equilibrium. In clinical examination of the brain, the NMR detection limit in terms of concentration of protons is around 0.5−1.0 mM (Ross and Bluml 2001). The low sensitivity of NMR is due to the low degree of polarization of the nuclear spins by experimentally achievable magnetic fields at thermal equilibrium, amounting to just a few parts per million. In addition, if the nucleus of interest is at low natural abundance (such as ^{13}C), MR sensitivity is then severely reduced (Mansson et al. 2006). This intrinsic low sensitivity has motivated the development of new methods, which could enable the detection of less abundant metabolites, including neurotransmitters.

Powerful novel techniques to enhance weak NMR signals from molecular tracers have recently been developed and are referred to as hyperpolarization methods. These techniques can considerably enhance the polarization of nuclear spins, and thereby provide a dramatic increase in the sensitivity of subsequently performed MRS investigations (Golman et al. 2003). One of the methods used to create hyperpolarized (non-equilibrium) nuclear spin states in probe molecules is the solid-state dynamic nuclear polarization (DNP). To use the DNP method for liquid probes (e.g., aqueous solutions of molecular tracers), the probe is doped with free radicals in a glass-forming solvent (e.g. glycerol), frozen to a very low temperature (1 K) inside a magnetic field, and irradiated with microwaves to transfer a high polarization of electron spins residing in low-concentrated free radicals to the surrounding nuclear spins. After creating a “hyper-polarized” nuclear spin state in a frozen probe, the molecular sample is quickly dissolved in water (Ardenkjaer-Larsen et al. 2003). The liquid probe can then be administrated in the body and used as a tracer so that one can visualize its biodistribution and metabolism in vivo.

This novel technology allows amplifying the baseline sensitivity of e.g., ^{13}C-MRS by a factor of up to 10,000 in vitro (Ardenkjaer-Larsen et al. 2003). The so-called DNP polarizer can be used for both in vitro and in vivo MRS and ultra-sensitive molecular imaging, including neurological applications

(Ardenkjaer-Larsen et al. 2003; Ross et al. 2010). The major limitation of the technique arises from the short lifetimes of hyperpolarized spin states in liquids, determined by the relaxation times T_1. This becomes especially critical when considering in vivo applications. Since protons in solutions of biomolecules have too short relaxation times to allow for transport and in vivo injection of hyperpolarized compounds, most applications of the technique have focused on ^{13}C-MRS. Nonprotonated carbons in ^{13}C-enriched tracers have relatively longer relaxation times (20–40 s), and thus the artificially prepared hyper-polarization decays back to the thermal-equilibrium level over a time course of a few minutes. Thus, the injection of the hyperpolarized probe and subsequent MR examination has to be performed rapidly. Despite this time limitation, the huge gain in signal intensity substitutes the need for signal accumulation, thus allowing complete data collection within the time window needed for imaging applications (Golman et al. 2006a; Mansson et al. 2006; Golman et al. 2003).

Several important application areas of metabolic imaging by ^{13}C DNP-MRS have been demonstrated by Golman's group, in particular for investigation of cardiac metabolism (Golman et al. 2008), metabolism in muscle (Golman et al. 2006a) and tumor diagnosis (Golman et al. 2006b; Day et al. 2007). Most of these studies involved ^{13}C-pyruvate as a molecular probe, but the great potential of the ^{13}C DNP-MRS method for metabolic imaging has also been demonstrated with other molecules [e.g. (Gallagher et al. 2008)]. Although no successful clinical applications have been developed yet, current applications of DNP at the pre-clinical level are very encouraging and range from angiography (Svensson et al. 2003) to metabolic imaging of healthy and diseased tissue (Gabellieri et al. 2008; Golman et al. 2006b; Golman et al. 2006a).

In addition to ^{13}C, molecular probes containing other magnetic nuclei with long relaxation times, such as ^{15}N, can be assessed using DNP-MRS, which may allow more time for metabolic conversion and accumulation of products. One of such probes that has gained recent attention is the hyperpolarization of ^{15}N labeled choline. Apart from its role in cellular phospholipid synthesis and metabolism, choline is also the precursor of the neurotransmitter ACh and therefore plays a major role in cholinergic neurotransmission (Cooper et al. 2003). Besides, alterations of the cholinergic neurotransmission are well-recognized features of neurodegenerative diseases such as AD and PD (Nakano and Hirano 1984; Perry et al. 1985; Whitehouse et al. 1982). Monitoring the physiological metabolism of choline and ACh in the brain through in vivo spectroscopy might be of great importance in the study of these diseases and may as well provide a diagnostic tool to detect biomarkers relevant to these conditions. Since ^{15}N relaxation time in choline is relatively long (100−200 s), the use of hyperpolarized ^{15}N-choline bears an exciting potential in reaching such goals. Gabellieri and colleagues first described a method to monitor the in vitro metabolic conversion of hyperpolarized ^{15}N-choline into phosphocholine by choline kinase using ^{15}N-NMR (Gabellieri et al. 2008). In vivo measurements using this method may, however, be hampered by insufficient ^{15}N peak separation of choline metabolites ($\sim$0.2 ppm for phosphocholine vs choline) and by poor sensitivity of ^{15}N-NMR. In an in vivo study the

successful detection of hyperpolarized ^{15}N-choline in a rat head after administration through the rat femoral vein has been reported, but none of the choline metabolites could in fact be detected (the choline signal was discernible above the noise level for about 100 s) (Cudalbu et al. 2010). Bringing the sensitivity of MRS detection of hyperpolarized ^{15}N choline to a level more compatible with in vivo measurements appears essential and is in the center of several studies. An alternative approach based on DNP-enhanced ^{1}H-NMR was developed, in which hyperpolarized ^{15}N choline was detected in vitro by ^{1}H-NMR after polarization transfer from ^{15}N to the distant methylene CH_2O protons, using a reversed INEPT pulse sequence (Sarkar et al. 2009). This method was shown to provide better sensitivity and an increased spectral dispersion of choline metabolites (ACh and phosphocholine) compared to direct ^{15}N detection. A selective polarization transfer strategy has been described to further increase sensitivity of this method for ^{15}N-choline (Pfeilsticker et al. 2010), whereby the polarization is transferred from ^{15}N exclusively to the methyl protons. In another study, the potential of the polarization transfer approach to track metabolic processes was explored in an in vitro experiment that applied the concept of spatially selective transfer to monitor the kinetics of two enzymatic reactions catalyzed by purified enzymes (Harris et al. 2010). The first reaction targeted was the phosphorylation of ^{15}N-choline into PC by choline kinase, also described by Gabellieri and colleagues with direct ^{15}N-NMR (Gabellieri et al. 2008). The second reaction, perhaps more interesting due to its significance of neuronal activity, was the hydrolysis of ^{15}N-ACh into choline by acetylcholine esterase. One of the advantages of using ^{1}H-NMR in that case is the good spectral resolution of the methylene group (close to the reaction hydroxyl group) between the two species. This offers the possibility to follow the kinetics of the reaction, here in a time window of 4 min.

Moving away from ^{15}N-choline, another hyperpolarized labeled choline molecular probe emerged to monitor ACh synthesis (Allouche-Arnon et al. 2010). The metabolic acetylation of a [1,1,2,2-D_4,2-^{13}C] choline chloride by a carnitine acetyltransferase was followed in vitro by ^{13}C-NMR and yielded interesting results. At the hyperpolarized state, [1,1,2,2-D_4,2-^{13}C] ACh was already detectable within 15 s. Kinetics parameters such as the reaction rate constant k were measured and the T_1 relaxation time of the product was found to be sufficiently long (34 s), which would be a clear advantage for in vivo application. Although encouraging, the metabolic conversion monitored was catalyzed by a carnitine acetyltransferase extracted from pigeon breast muscle, an enzyme primarily dedicated to carnitine acetylation. For this reason, extrapolation of the conclusions of this study to any application using choline acetyltransferase (the enzyme catalyzing the synthesis of ACh in the brain) should be made with caution.

Since their introduction in preclinical and clinical examinations, MRS techniques have developed greatly and today they represent an important part of the tools used to investigate metabolic processes noninvasively. In particular, they can provide information about brain functions through measurements of a wide range of neurochemicals. Neurodegenerative diseases in which brain metabolic changes are observed constitute interesting targets for MRS analysis. In pathologies such as

AD or PD, MR spectroscopy can detect altered metabolite levels and has therefore been applied to identify specific pathological markers, monitor longitudinal disease progression or perform differential diagnosis, with mixed outcome. Methods capable of increasing MRS sensitivity in vitro are now receiving increased attention for in vivo application. In particular, the strong signal enhancement provided by hyperpolarization methods applied to the detection of heteronuclei in MRS may have an interest in the measurement of less abundant metabolites, such as brain neurotransmitters. Great methodological advances have already been achieved in the development of DNP-enhanced MRS-based techniques that may further facilitate the detection of important brain metabolic processes in vivo. The future of these methods will depend on the possibility to transfer them to in vivo studies in living biological systems.

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MR Spectroscopic Studies of the Brain in Psychiatric Disorders

Richard J. Maddock and Michael H. Buonocore

Abstract The measurement of brain metabolites with magnetic resonance spectroscopy (MRS) provides a unique perspective on the brain bases of neuropsychiatric disorders. As a context for interpreting MRS studies of neuropsychiatric disorders, we review the characteristic MRS signals, the metabolic dynamics, and the neurobiological significance of the major brain metabolites that can be measured using clinical MRS systems. These metabolites include *N*-acetylaspartate (NAA), creatine, choline-containing compounds, myo-inositol, glutamate and glutamine, lactate, and gamma-amino butyric acid (GABA). For the major adult neuropsychiatric disorders (schizophrenia, bipolar disorder, major depression, and the anxiety disorders), we highlight the most consistent MRS findings, with an emphasis on those with potential clinical or translational significance. Reduced NAA in specific brain regions in schizophrenia, bipolar disorder, post-traumatic stress disorder, and obsessive–compulsive disorder corroborate findings of reduced brain volumes in the same regions. Future MRS studies may help determine the extent to which the neuronal dysfunction suggested by these findings is reversible in these disorders. Elevated glutamate and glutamine (Glx) in patients with bipolar disorder and reduced Glx in patients with unipolar major depression support models of increased and decreased glutamatergic function, respectively, in those conditions. Reduced phosphomonoesters and intracellular pH in bipolar disorder and elevated dynamic lactate responses in panic disorder are consistent with metabolic models of pathogenesis in those disorders. Preliminary findings of an increased glutamine/glutamate ratio and decreased GABA in patients with schizophrenia are consistent with a model of NMDA hypofunction in that disorder.

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As MRS methods continue to improve, future studies may further advance our understanding of the natural history of psychiatric illnesses, improve our ability to test translational models of pathogenesis, clarify therapeutic mechanisms of action, and allow clinical monitoring of the effects of interventions on brain metabolic markers.

Keywords Frontal · Limbic · Cortex · Neural · Glial · Metabolism

Abbreviations

1H-MRS	Proton magnetic resonance spectroscopy
2D	Two dimensional
31P-MRS	Phosphorous magnetic resonance spectroscopy
ADP	Adenosine diphosphate
AGAT	Arginine-glycine aminotransferase
ASICs	Acid sensing ion channels
ASPA	Aspartoacylase
Asp-NAT	Aspartate N-acetyltransferase
ATP	Adenosine triphosphate
CK	Creatine kinase
CNS	Central nervous system
CO2	Carbon dioxide
CSF	Cerebrospinal fluid
CSI	Chemical shift imaging
EAAT1	Excitatory amino acid transporter 1
EAAT2	Excitatory amino acid transporter 2
ECF	Extracellular fluid
EEG	Electroencephalogram
GAA	Guanidinoacetate
GABA	Gamma aminobutyric acid
GABA-T	Gamma aminobutyric acid transaminase
GAD	Glutamic acid decarboxylase
GAD65	65 kilodalton form of GAD
GAD67	67 kilodalton form of GAD
GAMT	Guanidinoacetate methyltransferase
GAT	GABA transporter
Glx	The combined signal from glutamate and glutamine
GPCho	Glycerophosphorylcholine
H+	Hydrogen ions
Hz	Hertz, or cycles per second
Km	Michaelis-Menten constant
MCT	Monocarboxylate transporter
MEGA	Mescher-Garwood
mM	Millimoles
MR	Magnetic resonance

MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MRS	Magnetic resonance spectroscopy
MRSI	Magnetic resonance spectroscopic imaging
MRUI	Magnetic Resonance User Interface
ms	Milliseconds
NAA	N-acetylaspartate
NAAG	N-acetylaspartylglutamate
NMDA	N-methyl-D-aspartic acid
OCD	Obsessive compulsive disorder
PCho	Phosphorylcholine
PEPSI	Proton echoplanar spectroscopic imaging
pH	Negative logarithm of hydrogen ion concentration
PMEs	Phosphomonoesters
ppm	Parts per million
PRESS	Point resolved spectroscopic sequence
PTSD	Post traumatic stress disorder
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricarboxylic acid
TE	Echo time
VGluT	Vesicular glutamate transporter

Contents

1 Introduction

Approximately 60% of the human body is water. Most clinical applications of magnetic resonance phenomena involve creating images based primarily on the magnetic properties of the nuclei of hydrogen atoms in water molecules. In contrast, magnetic resonance spectroscopy (MRS) provides information based on the magnetic properties of atomic nuclei present in other molecules in addition to water. Generally, this information is in the form of MR spectra, which display a series of resonance signals. The strength of each signal is proportional to the concentration of molecules containing nuclei that resonate at the indicated frequency. Although MR spectra from the atomic nuclei of several different elements in the body can be measured, most MRS studies using clinical MR systems measure spectra from the nucleus of the hydrogen atom. In this review, the MR spectra from hydrogen nuclei are referred to as 1H-MRS. MRS information can also be displayed as low-resolution images [chemical shift imaging (CSI) or magnetic resonance spectroscopic imaging (MRSI)], in which image contrast is based on regional differences in the concentration of a specific molecule.

The substance of this review is divided into two sections. The first section reviews the molecules most commonly studied with MRS in the human brain. It describes the pattern of MRS resonance peaks arising from each such molecule, the pathways for biosynthesis and degradation of each molecule, and reviews current understandings of the neurobiological function and the significance of abnormal concentrations of each molecule. This section is intended to provide the metabolic and neurobiologic background for interpreting MRS observations about each of the major metabolites studied with 1H-MRS in the human brain. In discussing each metabolite, special emphasis is given to metabolic and signaling functions that may be relevant to translational models of psychiatric disorders. The second section reviews and summarizes the scientific literature on brain MRS studies of major psychiatric disorders, including schizophrenia, bipolar disorder, unipolar major depression, and anxiety disorders. In order to provide a context for interpreting these MRS findings, this section also provides an overview of the literature on brain structure and function in each disorder and current concepts of the pathophysiology of each condition. While the MRS literature in psychiatric disorders has grown quite large, our review will attempt to identify the most consistently replicated experimental observations and will give priority to findings that address specific translational questions of theoretical or clinical importance. In addition, a discussion of the physics of MRS and a technical description of MRS methods commonly used in neuropsychiatric research today is provided as supplementary material (link below). The supplementary material assumes a basic familiarity with MR principles and concepts such as longitudinal and transverse magnetization, nutation of magnetization by radiofrequency pulses, and precession of transverse magnetization by the application of the main magnetic field and fields due to the gradient pulses. For the reader equipped with this background, this material offers an in-depth introduction to the unique physical principles

underlying MRS experiments. Supplement: http://ucdirc.ucdavis.edu/CLR327bgt/maddock-buonocore-CTBNsuppl.pdf.

2 Metabolites Observable in Normal Brain

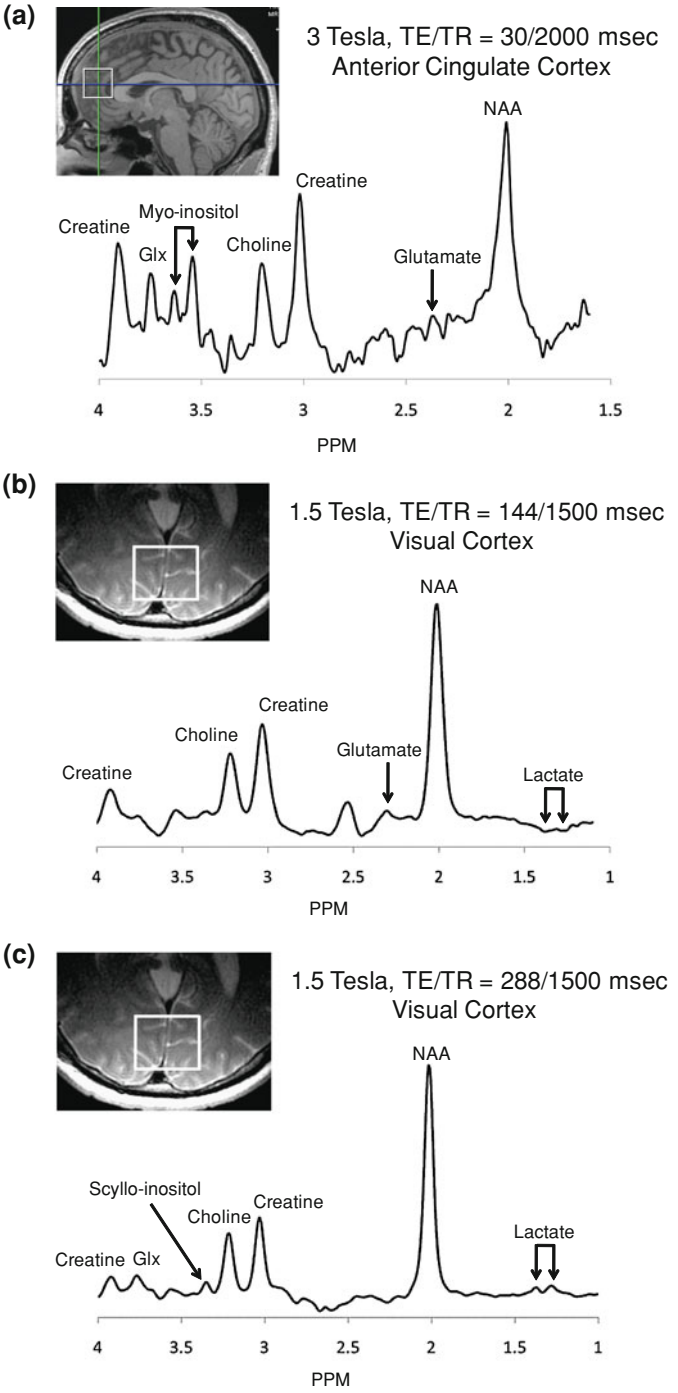
For a brain metabolite to be reliably measured with MRS methods currently available on clinical MRI systems, its concentration must be in the millimolar range and it must be in a freely mobile form (not anchored to a membrane or organelle). Molecules that are not free to rotate rapidly in solution generally do not generate a resonance that can be detected with clinical MRI systems. The brain is a densely cellular organ with a high rate of resting energy consumption. Its primary functions require complex signaling mechanisms for communication both within and between cells. Accordingly, many of the mobile molecules present in sufficiently high concentration to be reliably observed with MRS are involved in energy metabolism, signaling, and cell membrane metabolism. Figure 1 portrays examples of 1H-MRS data acquired from 3 and 1.5 T scanners using several different echo times (TEs). Each of the metabolites commonly studied with 1H-MRS in patients with neuropsychiatric disorders is discussed below in detail.

2.1 NAA

The molecular structure of *N*-acetylaspartate (NAA) is shown in Fig. 2. Other than water, the most prominent peak in the 1H-MRS spectrum of brain tissue is the singlet peak of NAA at about 2.01 ppm (Fig. 1). This large peak arises from the three hydrogen nuclei in the methyl group within the acetyl moiety of NAA. Hydrogen nuclei from the aspartate moiety of NAA give rise to several other much smaller peaks, but only the multiplet with peaks at about 2.49 and 2.67 ppm is generally visible in in vivo spectra (Govindaraju et al. 2000).

NAA is often considered to be a marker of the density of viable neuronal tissue in the brain region under study (Meyerhoff et al. 1993). However, there is accumulating evidence that NAA levels also reflect reversible changes in neuronal health (Clark 1998; Gasparovic et al. 2001; Demougeot et al. 2004). For example, reduced NAA levels are observed in the context of acute brain injury or illness, or chronic methamphetamine abuse. However, a normalization of NAA levels can be observed following a period of recovery, treatment, or extended abstinence from drug abuse (De Stefano et al. 1995; Kalra et al. 1998; Narayanan et al. 2001; Salo et al. 2010; Yoon et al. 2010b). Thus, reduced NAA is more accurately interpreted as reflecting *either* permanent loss *or* reversible dysfunction of neuronal tissue (Moffett et al. 2007).

NAA is synthesized from aspartate and acetyl-coenzyme A in a reaction catalyzed by aspartate *N*-acetyltransferase (Asp-NAT) (Moffett et al. 2007;


(a)
3 Tesla, TE/TR = 30/2000 msec
Anterior Cingulate Cortex
NAA
Creatine
Myo-inositol
Creatine
Glx
Choline
Glutamate
4
3.5
3
2.5
2
1.5
PPM
(b)
1.5 Tesla, TE/TR = 144/1500 msec
Visual Cortex
NAA
Creatine
Choline
Creatine
Glutamate
Lactate
4
3.5
3
2.5
2
1.5
1
PPM
(c)
1.5 Tesla, TE/TR = 288/1500 msec
Visual Cortex
NAA
Scyllo-inositol
Choline
Creatine
Lactate
Creatine
Glx
4
3.5
3
2.5
2
1.5
1
PPM

◀**Fig. 1** Representative 1H-MRS spectra acquired from human brain using three different TEs are shown. The spectrum in (**a**) was acquired at TE = 30 ms from the anterior cingulate cortex at 3 Tesla. The spectra in (**b** and **c**) were acquired at TE = 144 and 288 ms respectively from the primary visual cortex at 1.5 Tesla. Selected metabolite peaks are indicated. Note that the ppm value on the horizontal axis increases to the left, not the right. Spectral peaks that appear on the *right side* of the graph arise from nuclei that are relatively more shielded from the main magnetic field by nearby electrons. Spectral peaks on the *left side* of the graph arise from relatively less shielded nuclei (discussed in Supplement Sect. 4.2)

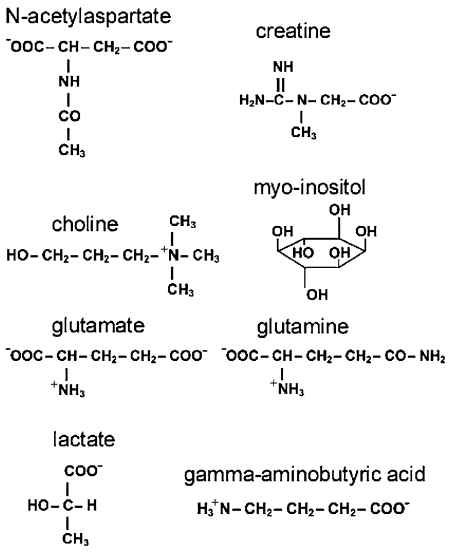


Fig. 2 The molecular structures of eight brain metabolites commonly studied with 1H-MRS are shown

Ariyannur et al. 2010). There is not yet a consensus about the subcellular localization of NAA synthesis or about the physiological functions of NAA. However, there is general agreement that NAA is synthesized predominantly in neurons, and that its substrates are found together primarily within mitochondria. Therefore, it is likely that most NAA is synthesized in neuronal mitochondria, although there may be some Asp-NAT and NAA synthesis in neuronal cytoplasm. Many investigations have shown that NAA synthesis is coupled to the capacity of neuronal mitochondria for oxidative metabolism and ATP synthesis (Bates et al. 1996; Clark 1998; Moffett et al. 2007). Animal studies of experimental brain trauma show that the acute decrease and later recovery of ATP and other indicators of mitochondrial energy metabolism were temporally correlated with changes in NAA levels (Gasparovic et al. 2001; Signoretti et al. 2010). This evidence supports the use of brain 1H-MRS NAA levels as a marker for the integrity and functional capacity of neuronal mitochondria.

Aspartoacylase (ASPA) is the enzyme that catalyzes the hydrolysis of NAA to aspartate and acetate in human brain (Bitto et al. 2007). ASPA is found predominantly in oligodendrocytes, the glial cells that constitute the myelin sheaths around axons. The important role of acetate in the synthesis of myelin and converging evidence from a wide range of studies support the hypothesis that one

important function of NAA is to transport acetate from neuronal mitochondria to oligodendrocytes for use in myelin synthesis (Moffett et al. 2007). NAA may also contribute to other aspects of oligodendrocyte lipid and energy metabolism. Several other proposed neurobiological functions of NAA have been the subject of experimental study, including participation in an alternate pathway of neuronal mitochondrial respiration in which glutamine substitutes for glucose, providing a reservoir for glutamate, functioning as an organic osmolyte for regulating cell volume, and serving as an anion to ameliorate the "anion deficit" within neurons (Clark et al. 2006; Moffett et al. 2007).

NAA is an immediate precursor for the biosynthesis of the neuronal dipeptide *N*-actelyaspartylglutamate (NAAG). NAAG is the most highly concentrated peptide in the human brain and may serve a cell-signaling function (Neale et al. 2000). It generates a small peak in the brain 1H-MRS spectrum that is difficult to distinguish from the NAA peak (Edden et al. 2007). Measures of the percent contribution of NAAG to the combined signal from NAA and NAAG range from about 9% in gray matter to about 30% in white matter (Pouwels and Frahm 1997; Edden et al. 2007). NAAG is synthesized in neurons from NAA and glutamate. It is stored in vesicles and released from neurons by a calcium-dependent mechanism, and it is hydrolyzed to glutamate and NAA by the enzyme NAAG peptidase, which resides on the extracellular surface of astrocytes (Baslow 2007; Chopra et al. 2009). Considerable evidence suggests that NAAG interacts with group II metabotropic glutamate receptors prior to hydrolysis. However, the nature and significance of this interaction is not yet clear (Neale et al. 2000; Chopra et al. 2009).

In summary, the NAA singlet at 2.02 ppm is the most prominent peak in normal brain 1H-MRS spectra. In most cases, this NAA signal represents "total NAA," as it includes the combined signals from both NAA and NAAG. The 1H-MRS signal arising from the total pool of NAA + NAAG can be interpreted as a marker for the health, viability and/or number of neurons, and it may more specifically reflect the functional capacity of neuronal mitochondria.

2.2 Creatine

Together, creatine and phosphocreatine give rise to a prominent singlet peak at approximately 3.03 ppm (Fig. 1). This peak arises from the three hydrogen nuclei in the methyl group of the creatine moiety (Fig. 2). Another smaller but distinct peak is evident at approximately 3.91 ppm. This singlet peak arises from the methylene hydrogen nuclei of the creatine moiety (Govindaraju et al. 2000). In general, creatine and phosphocreatine cannot be reliably distinguished by 1H-MRS. In this review, the term "creatine" used in the context of 1H-MRS measurements refers to the combined signal from creatine and phosphocreatine.

Creatine and phosphocreatine are present in both gray matter and white matter, and in all of the major cell types of brain parenchyma, including neurons, astrocytes, and oligodendrocytes. The pool of creatine in the body is maintained by a

combination of dietary uptake and endogenous synthesis. Although it was previously thought that the brain's supply of creatine was primarily maintained by uptake from the blood, it now appears that local synthesis within the brain may contribute substantially to its supply (Andres et al. 2008; Beard and Braissant 2010). Two enzymes are required for the synthesis of creatine. Arginine–glycine aminotransferase (AGAT) generates ornithine and guanidinoacetate (GAA), the immediate precursor of creatine. GAA is then methylated by guanidinoacetate methyltransferase (GAMT) to produce creatine. AGAT and GAMT are widely expressed throughout the brain, but it appears that they are not often co-expressed in the same cells. This suggests that the transporter for GAA and creatine is also required for the final synthesis and distribution of creatine throughout the brain (Braissant et al. 2010). Some studies have found high levels of GAMT in glial cells and suggest that the final step in creatine synthesis may occur mainly in glia. However, this and other questions regarding the precise compartmentation of creatine synthesis and transport remain unresolved (Andres et al. 2008; Beard and Braissant 2010).

Creatine has an essential role in CNS energy homeostasis. In the presence of ATP, creatine can be phosphorylated by the enzyme creatine kinase (CK). This reaction is reversible, so that ATP can be regenerated from phosphocreatine, in the presence of ADP. The creatine/phosphocreatine system has two essential functions in brain energetics. It provides a buffer, or storage mechanism, for high-energy phosphate bonds generated in subcellular regions where ATP production is high, and it provides a means for transport of high-energy phosphate bonds from subcellular regions of net energy production to subcellular regions of net energy consumption. Unlike ATP and ADP, phosphocreatine and creatine can diffuse rapidly across subcellular regions (Andres et al. 2008). This relatively rapid rate of diffusion makes the creatine/phosphocreatine system an efficient mechanism for shuttling high-energy phosphate bonds between subcellular compartments.

In addition to its central role in energetics, creatine appears to have important functions in other fundamental aspects of cellular metabolism in brain parenchyma. In combination with the CK isoform expressed in brain mitochondria, creatine has an important antiapoptotic effect by stabilizing mitochondrial membrane pores (Dolder et al. 2003). Creatine also helps suppress free radical (reactive oxygen species) formation within mitochondria by facilitating the recycling of ADP during periods of increased glucose utilization (Meyer et al. 2006). Furthermore, creatine appears to be released from neurons by a depolarization-induced, calcium-dependent mechanism (Almeida et al. 2006) suggesting that it functions as a neuromodulator. In this regard, there have been reports that creatine may act as a partial agonist at the GABA-A receptor (Koga et al. 2005; Almeida et al. 2006) and may interact with the NMDA receptor (Royes et al. 2008).

The 1H-MRS signal attributable to creatine and phosphocreatine (total creatine) is generally interpreted as a measure of the global health of brain parenchyma, with reductions indicative of impairment of function or integrity. While a measurement of the ratio of phosphocreatine to creatine would provide information about the current status of energy metabolism and high-energy phosphate bonds in the brain, this ratio generally cannot be reliably measured by 1H-MRS alone, but

requires additional measurements with phosphorous MRS (31P-MRS). In studies of patients with multiple sclerosis, increased creatine (as observed, for example, in normal-appearing white matter) has been interpreted as an indication of the proliferation of astrocytes (Caramanos et al. 2005). This interpretation is supported, in part, by in vitro studies of cultured neuronal and glial cells suggesting that the concentration of creatine in astrocytes is higher than in neurons (Urenjak et al. 1993; Bhakoo et al. 1996). However, a subsequent in vitro study did not confirm this (Griffin et al. 2002) and uncertainty remains about the relative concentration of creatine in different brain cell types.

In general, the concentration of total creatine is relatively similar throughout the brain and tends to be stable over time in the absence of major pathology. For these reasons, the 1H-MRS signal from creatine is commonly used as an "internal standard" to normalize the signals from other metabolites measured within the same voxel. There are several advantages of this approach. It partially corrects for some of the variation in metabolite signal intensity that is due to the location of the voxel, such as the proportion of cerebrospinal fluid (CSF) within the voxel and the sensitivity of the coil to signal from a specific location within the brain. The main disadvantage of this approach is that the creatine signal may increase or decrease in association with a pathologic condition, as has been demonstrated for ischemic stroke and brain trauma (Lei et al. 2009; Signoretti et al. 2010).

In summary, the combined signals from creatine and phosphocreatine give rise to singlet peaks at 3.03 and 3.91 in 1H-MRS spectra. Creatine and phosphocreatine are present in all types of brain cells. The total creatine signal is relatively similar across brain regions and reflects the global health of the underlying tissue. Creatine signal intensity is often used for within-voxel normalization of the signals arising from other metabolites of interest.

2.3 Choline-Containing Compounds

Many molecular compounds in the brain contain a choline moiety. The nine hydrogen nuclei associated with the trimethylammonium group within the choline moiety of choline-containing compounds (Fig. 2) give rise to a prominent singlet peak at about 3.21 ppm (Fig. 1) (Govindaraju et al. 2000). In brain, phosphorylcholine (PCho) and glycerophosphorylcholine (GPCho) are the primary sources of this resonance peak. Choline-containing phospholipids in myelin and cell membranes (primarily phosphotidylcholine) are present in brain parenchyma in higher concentration than PCho and GPCho (Boulanger et al. 2000). However, these compounds are not freely mobile and therefore cannot generate a measurable magnetic resonance signal during a 1H-MRS acquisition. Thus, these choline-containing phospholipids do not directly contribute to the choline resonance at 3.21 ppm. Free choline, acetylcholine, and cytidine diphosphate choline are mobile choline-containing compounds present at much lower concentrations than PCho and GPCho (Boulanger et al. 2000). They contribute directly, but to a

minor degree, to the choline peak at about 3.21 ppm. Betaine is produced by the oxidation of choline. Like choline, betaine contains nine hydrogen nuclei within a trimethylammonium group. Betaine makes a minor contribution to the total choline signal. Phosphorylethanolamine, another precursor of membrane phospholipids, also contributes in a minor way to the choline signal at 3.21 ppm.

Although 1H-MRS does not directly measure the concentration of membrane phospholipids, the observed choline peak is influenced by both the density of cell membranes and the rate of cell membrane and myelin turnover. PCho is a precursor of the synthesis of membrane phospholipids. Both GPCho and, to a lesser extent, PCho are generated during the breakdown of membrane phospholipids. Thus, an increase in either the synthesis or the breakdown of membrane phospholipids can be associated with an increase in the concentrations of PCho and/or GPCho (Geddes et al. 1997; Boulanger et al. 2000). For this reason, increases in the breakdown or turnover rate of membrane or myelin phospholipids are believed to be associated with increases in the 1H-MRS choline signal. Furthermore, at a constant rate of turnover of phospholipids, the concentrations of PCho and GPCho vary in proportion to the density of cell membranes within the voxel (Yue et al. 2009). Thus, the 1H-MRS choline signal is often interpreted as a measure of overall cell density and/or the rate of membrane turnover. Increased choline signal can also result from the accumulation of myelin breakdown products, as occurs during active demyelination.

2.4 Myo-Inositol

Inositol is a six-carbon ring sugar with an alcohol group attached to each carbon (a six-fold alcohol of cyclohexane) (Fig. 2). Myo-inositol is the most abundant stereoisomer of inositol in mammalian systems. In the brain, about 90% of the inositol is myo-inositol, less than 10% is scyllo-inositol, and trace amounts of other stereoisomers are also present (Govindaraju et al. 2000; Fisher et al. 2002). The most prominent 1H-MRS signal from myo-inositol is a pair of multiplet peaks arising at about 3.52 and 3.61 ppm (Fig. 1a). The myo-inositol peaks are generally not observable in long TE 1H-MRS acquisitions (Fig. 1b, c). The scyllo-inositol singlet peak is variably present at about 3.3 ppm. It can often be observed when using a long TE (Fig. 1c).

The myo-inositol content of brain cells is governed by several physiological mechanisms, including the recycling of inositol phosphate second messengers, de novo synthesis of inositol from glucose, carrier-mediated energy-coupled transport of inositol into cells against a concentration gradient, and efflux of inositol out of cells during hypotonic stress as part of cell volume regulation (Fisher et al. 2002). In healthy brain tissue under normal osmotic conditions, the former two mechanisms predominate (Williams et al. 2002). Myo-inositol is synthesized from glucose-6-phosphate in two steps. The final step is catalyzed by inositol monophosphatase. This same enzyme is responsible for generating free myo-inositol during the recycling of

inositol phosphate second messengers. Free inositol is required to regenerate phosphotidylinositol, a key component of the second messenger system. Interestingly, inositol monophosphatase is inhibited by lithium. Indeed lithium, valproate and carbamazepine all alter inositol phosphate metabolism to cause a reduction in intraneuronal free inositol levels (Williams et al. 2002).

In addition to its key role as a precursor for the regeneration of phosphotidylinositol in the inositol phosphate second messenger system, myo-inositol has other important functions in the brain. Free myo-inositol serves as a "non-perturbing" osmolyte that is normally maintained at a manyfold higher concentration within brain cells than in CSF (>25:1) or blood (>50:1). In response to hypotonic stress (e.g. hyponatremia), myo-inositol can efflux from brain cells (or enter brain cells in the case of hypertonic stress) to preserve cell volume without altering the function of intracellular processes (Fisher et al. 2002). Additionally, myo-inositol, similar to choline, is an intermediate in the metabolism of membrane and myelin phospholipids.

Although the myo-inositol signal in brain 1H-MRS is often considered to be a glial marker, its distribution across brain cell types is more complex than is suggested by that characterization. Currently, the extent to which myo-inositol may be preferentially concentrated within neuronal or glial cell types remains uncertain (Fisher et al. 2002). One of the strongest assertions of an exclusively glial source for the 1H-MRS myo-inositol signal comes from a 1H-MRS study of cultured brain cells that showed a high concentration of myo-inositol in astrocytes and negligible myo-inositol in neurons (Brand et al. 1993). The conclusions of this study are widely cited in support of the characterization of myo-inositol as a glial marker. Thus, it is worthwhile to examine their limitations. The cultured neurons studied were in an embryonic stage of development, as evidenced by the observation they contained only trace amounts of NAA. In contrast, the cultured astrocytes studied were in a more mature stage of development and exhibited a spectral pattern more similar to in vivo brain 1H-MRS studies (more prominent Cr and Choline peaks than observed in the embryonic neurons). Neither type of cultured cell (neurons or astrocytes) were able to synthesize myo-inositol from glucose (Brand et al. 1993), although this is known to occur in mature neurons (Schmidt et al. 2005). Since the cultured cells could not synthesize it, myo-inositol was added to the culture medium. Thus, the Brand et al. results reflect the relative uptake of myo-inositol into mature astrocytes compared to its uptake into embryonic neurons in a cell culture environment. Mature neurons and glia are both known to express myo-inositol transport proteins. Two such transporters have been described. One is present in both cell types and the other is observed only in astrocytes. Under acidic conditions, the former is less active while the exclusively astrocytic transporter is more active (Fisher et al. 2002). Acidic conditions (10% CO_2) in the culture media may have favored preferential uptake of myo-inositol by the cultured astrocytes in the Brand et al. experiment. The comparison of relatively immature neurons to relatively mature astrocytes, the absence of normal neuronal myo-inositol synthesis, and cell culture conditions favoring selective myo-inositol uptake by astrocytes limit the generalizability of their findings. In their systematic

review, Fisher et al. (2002) summarized findings from seven prior experiments on neuronal cells and five prior experiments on glial cells. There was no significant difference in the estimated myo-inositol concentrations in glia compared to neurons, but the median estimated concentration was 38% lower in the neuronal cells than in the glial cells.

Although myo-inositol may not be a specific glial marker, clinical observations often support an association between elevated 1H-MRS myo-inositol signal and gliosis in neurodegenerative disorders (e.g. multiple sclerosis and Alzheimer's disease) (Bitsch et al. 1999; Yang et al. 2010). However, elevated inositol is found in a range of pathological conditions not involving gliosis, including Down's syndrome, increased myelin breakdown, and hypertonic stress (Fisher et al. 2002). It is important to note that high concentrations of myo-inositol are observed in some types of cultured neurons, and that myo-inositol is actively taken up into most types of mature brain cells, including neurons and glia (Fisher et al. 2002). Furthermore, neurons can both synthesize inositol from glucose and regenerate it during the recycling of inositol phosphates (Schmidt et al. 2005), Thus, there is little evidence to support the characterization of myo-inositol as a specific glial marker, and increases or decreases in the brain inositol 1H-MRS signal must be interpreted in the context of the specific condition under study.

2.5 Glutamate and Glutamine

The amino acid neurotransmitter glutamate is one of the most abundant mobile metabolites present in the brain, being second only to NAA in concentration (Govindaraju et al. 2000). However, it lacks methyl groups, and the J-coupled signals from its methylene and methine groups (Fig. 2) produce broad complex peaks. For these reasons, glutamate does not generate a prominent single peak in the 1H-MRS spectrum of the brain. A multiplet peak centered at about 2.34 ppm arises from the methylene protons near the carboxy terminal of glutamate and is often the most readily recognized glutamate peak in brain 1H-MRS spectra (Fig. 1). A second methylene multiplet centered at about 2.08 ppm is typically obscured by the large NAA peak at 2.01 ppm. A third complex glutamate peak arises from its methine proton at about 3.74 ppm (Fig. 1) (Govindaraju et al. 2000).

These signals from glutamate are difficult to distinguish from the analogous peaks arising from glutamine at about 2.44, 2.12, and 3.75 ppm. The concentration of brain glutamine is estimated to be about 40% to 60% of the concentration of glutamate (Govindaraju et al. 2000; Jensen et al. 2009), thus signal arising from glutamine often confounds measures of glutamate. Hancu recently compared a range of specialized 1H-MRS methods for measuring brain glutamate on a 3 T scanner. A conventional short TE point resolved spectroscopic sequence (PRESS) and the specialized Carr-Purcell PRESS sequence provided measurements with the best repeatability. J-resolved PRESS was the most accurate for measuring absolute values of glutamate, but at the cost of reduced repeatability (Hancu 2009)

(see Sects. 4.3.1, and 4.5.3 of the Supplement for discussions of PRESS and J-resolved MRS techniques). Unless optimized 1H-MRS methods are used (e.g. a high-field scanner with a short echo time and long acquisition time, or a specialized J-editing or J-resolved sequence), the measurements obtained are generally considered to reflect the combined signal from glutamate and glutamine, with minor contributions from glutathione and GABA. This combined signal measurement is often abbreviated as "Glx."

In the resting awake state, up to 20% of brain glucose metabolism is directed toward the de novo synthesis of glutamate, which occurs primarily in astrocytes (Hertz 2006). Pyruvate carboxylase, which is located exclusively in glial cells (probably within their mitochondria), has a key role in directing pyruvate toward de novo glutamate synthesis. Thus astrocytes, unlike glutamatergic neurons, are capable of net synthesis of glutamate without depletion of tricarboxylic acid (TCA) cycle intermediates (Hertz 2004; Waagepetersen et al. 2007). The TCA cycle intermediate, alpha ketoglutarate, is the immediate precursor of glutamate via exchange reactions such as transamination (by aspartate aminotransferase) and possibly by reductive amination (by glutamate dehydrogenase). Once synthesized in astrocytes, some glutamate are used as a metabolic intermediate and some are directed toward the synthesis of glutathione, a major intracellular antioxidant in the brain that is present in much higher concentrations in glia than in neurons (Janaky et al. 2007). However, most astrocytic glutamate is converted to glutamine by the astrocyte-specific enzyme, glutamine synthase, and released for uptake into glutamatergic neurons. These neurons then convert glutamine back to glutamate (via phosphate-activated glutaminase) (Waagepetersen et al. 2007). In neurons, glutamate has both metabolic and neurotransmitter functions. Glutamate can reenter the TCA cycle for oxidative energy production or be used in the synthesis of other amino acids, including GABA. Glutamate is the most abundant excitatory neurotransmitter in the brain. For use in neurotransmission, it is first transported into synaptic vesicles, where concentrations are about 10-fold higher than whole-brain glutamate concentrations. Vesicular glutamate can then be exocytotically released into the synaptic cleft during neurotransmission. The neurotransmitter action of glutamate is quickly terminated by its rapid uptake from the synaptic zone into astrocytes. Most of the glutamate taken up by astrocytes reenters the glutamate–glutamine cycle to be returned to neurons and reused in neurotransmission (Waagepetersen et al. 2007). However, some glutamate is directed toward other metabolic fates and is lost from this cycle, necessitating the continuous de novo synthesis of glutamate in astrocytes. Possible additional components of the cycling of glutamate and glutamine between neurons and astrocytes are under investigation (Maciejewski and Rothman 2008). Recent studies suggest that astrocytes also store glutamate in vesicles for exocytotic release in the service of intercellular communication (Hertz 2006; Waagepetersen et al. 2007).

Current models of the compartmentation of brain glutamate metabolism suggest a time-limited segregation into two cellular pools: a smaller astrocytic pool (comprising about 20% of total glutamate), in which glutamate is rapidly converted to glutamine, and a larger neuronal pool (about 80% of total glutamate),

which has a slower turnover time (Waagepetersen et al. 2007). Glutamate is further compartmentalized into cytosolic, mitochondrial, and vesicular subcellular compartments. It is important to note that only about 80% of glutamate in brain tissue appears to be observable by 1H-MRS. It is possible that low MRS visibility of glutamate in the vesicular compartment accounts for this finding (Kauppinen and Williams 1991). A small amount of glutamate is present in the extracellular fluid (ECF) of the brain. However, elevated ECF concentrations of glutamate can have excitotoxic effects. Because of the rapid clearance of glutamate from the ECF, primarily by astrocytes, ECF glutamate concentration in healthy brain is maintained three to four orders of magnitude less than whole-brain concentrations (Waagepetersen et al. 2007). Some studies suggest that most glutamate observable by MRS is in rapid exchange across compartments on a timescale of seconds to minutes (Rothman et al. 2003; Hertz 2004). If this is so, then glutamate as measured over several minutes by MRS may represent a single, integrated pool of the metabolite in ongoing exchange between neuronal and glial cytoplasm.

Glutamine's primary role in the brain is as a non-neuroactive intermediate in the recycling of amino acid neurotransmitters, most abundantly glutamate and GABA. In addition, it has an important role in the regulation of brain ammonia metabolism (Waagepetersen et al. 2007). However, the synthesis and catabolism of brain glutamine are strictly yoked to glutamate metabolism. All brain glutamine synthesis is via glutamate and takes place within astrocytes. Brain glutamine participates in no metabolic pathways other than via its initial conversion back to glutamate. Thus, the 1H-MRS measure of Glx represents a good approximation of the total glutamate–glutamine pool available for the integrated metabolic and neurotransmitter functions of glutamate in the brain (Rothman et al. 2003; Yuksel and Ongur 2010).

Glutamate is one of several brain metabolites that exhibit acute changes in MRS signal strength in response to sensory, cognitive, or pharmacological manipulations. The general paradigm of measuring dynamic changes in brain metabolites in response to behavioral or drug conditions is known as dynamic MRS or functional MRS. An extensive animal literature demonstrates that changes in local cortical glutamate and glutamine concentrations are activity dependent, meaning that they increase or decrease according to the degree of local neural activity (Carder and Hendry 1994; Arckens et al. 2000; Qu et al. 2003; Hertz 2004). Dynamic 1H-MRS studies in normal human volunteers have similarly found local activity-dependent increases in cortical glutamate. Mullins et al. (2005) observed a 9% increase in glutamate in the anterior cingulate cortex during cold pressor pain. Gussaw et al. (2010) subsequently showed an 18% increase in anterior insular cortex glutamate during thermal pain. Using a 7 T system, Mangia et al. (2007) reported a small but statistically significant increase in glutamate in the visual cortex while subjects viewed a flickering checkerboard stimulus. Our laboratory has observed a similar significant 5% increase in visual cortex Glx during visual stimulation (Maddock et al., unpublished data). We recently found that vigorous aerobic exercise, which is known to cause a widespread brain metabolic activation (Fukuyama et al. 1997; Delp et al. 2001), leads to an 18% increase in Glx in the visual cortex (Maddock et al. 2011).

1H-MRS measures of glutamate or Glx arise from both neuronal and glial cells and primarily reflect cytoplasmic concentrations. Measures of glutamate or Glx can provide information about both activity-dependent changes in the size of the MRS-visible metabolite pool and about the enduring integrity of glutaminergic neurons and astrocytes that sustain this pool of glutamate and glutamine. Brain MRS measures of glutamate, glutamine, and Glx may have particular value in testing translational hypotheses about dysfunction of glutamatergic systems in neuropsychiatric disorders.

2.6 GABA

Gamma aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the brain. It is present in brain parenchyma at about 15% to 20% of the concentration of glutamate (Govindaraju et al. 2000). GABA contains three methylene groups (Fig. 2), each of which gives rise to a complex signal in 1H-MRS spectra. A GABA multiplet peak at about 3.01 ppm is normally obscured by the creatine singlet at 3.03 ppm. A GABA triplet at about 2.28 ppm is partially overlapped by the glutamate multiplet centered at about 2.34 ppm. A GABA multiplet peak at 1.89 ppm is obscured by the large NAA singlet centered at 2.01 ppm. Because of their extensive overlap with larger signals from other metabolites, none of the three GABA peaks can be reliably distinguished or quantified with conventional brain 1H-MRS acquisitions at 1.5 or 3.0 T field strengths. The GABA resonance at 2.28 may contribute in a small way to the total Glx signal measured with conventional acquisitions. However, specialized pulse sequences including J-resolved and J-difference editing sequences can render some or all of the GABA peaks visible and isolate them from larger overlapping signals, even when used on clinical MRI systems. Perhaps the most commonly used sequence for measuring GABA is the MEGA-PRESS J-difference editing sequence (Mescher et al. 1998). Figure 3 shows the broad GABA peak at about 3.01 ppm after the creatine resonance has been removed by the MEGA-PRESS J-difference editing method.

GABA is synthesized from glutamate by the enzyme glutamic acid decarboxylase (GAD), a reaction that occurs almost exclusively in GABAergic neurons. After it is released during neurotransmission, GABA is taken up by both GABAergic neurons and by astrocytes. Current evidence suggests that neuronal reuptake of GABA predominates and that it occurs primarily in the nerve terminal region (Waagepetersen et al. 2007). After reuptake into neurons, GABA either reenters synaptic vesicles for reuse in neurotransmission, or it is degraded by the mitochondrial enzyme GABA transaminase (GABA-T) and enters the TCA cycle, from which it can be recycled to glutamate and then GABA again. This latter cycle is known as the GABA shunt. The fraction of GABA that is taken up by astrocytes is also metabolized via the GABA shunt, but the resulting glutamate is converted to glutamine and released into the ECF. The glutamine is taken up by either

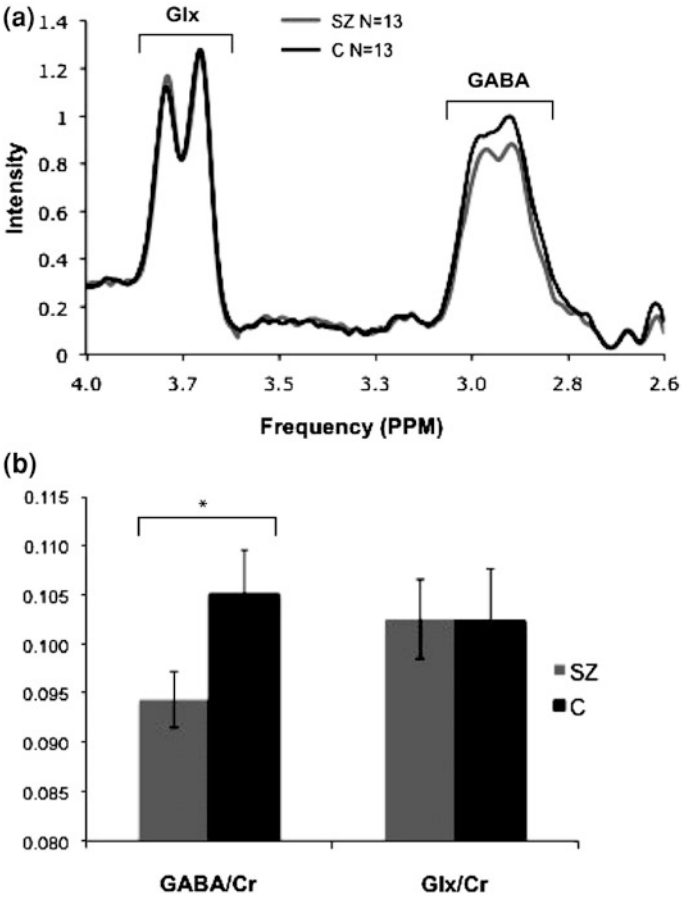


Fig. 3 **a** shows peaks for GABA and Glx from 1H-MRS difference spectra acquired using the MEGA-PRESS pulse sequence for GABA editing (TE = 68 ms) on a 3 Tesla system. The mean difference spectra are shown for 13 schizophrenia patients and 13 healthy comparison subjects. **b** illustrates the finding of significantly lower GABA signal in the patient group ($p<.05$ two-tailed), but no group difference in the Glx signal

glutamatergic or GABAergic neurons, where it either enters energy metabolism or serves as the substrate for neurotransmitter synthesis (Waagepetersen et al. 2007). De novo synthesis of GABA depends on both the anaplerotic production of glutamate by astrocytes, and the conversion of glutamate to GABA by GABAergic neurons (Hertz 2004).

There appear to be at least two distinct pools of GABA in GABAergic neurons, a large cytoplasmic pool and a smaller vesicular pool. Furthermore, two forms of the GABA synthetic enzyme GAD are known, GAD67 and GAD65. GAD67 is widely distributed throughout the cytoplasm and nerve terminals of GABAergic

neurons, and it contributes to the generation of both the cytoplasmic and the vesicular pools of GABA. In contrast, GAD65 is localized to nerve terminals, and it contributes only to the vesicular pool of GABA. Under basal conditions, most GABA are synthesized by GAD67. However, the activity of GAD65 can be upregulated on demand to increase GABA in the vesicular pool (Waagepetersen et al. 2007; Dericioglu et al. 2008).

While it appears that vesicular glutamate may not be detectable by MRS (Kauppinen and Williams 1991), whether or to what extent the vesicular pool of GABA can be detected by MRS is not known. Thus, the 1H-MRS GABA signal arises either mostly or almost entirely from the large cytoplasmic GABA pool in GABAergic neurons under basal conditions. The functional significance of the considerable cytoplasmic store of GABA is not known. It may have metabolic functions or it may act as a reservoir from which to replenish vesicular stores of GABA. However, some evidence suggests that cytoplasmic GABA serves as an important source for "extrasynaptic" GABA release via the neuronal GABA transporter (GAT) from cell membrane regions not associated with synaptic structures or vesicles (Wu et al. 2007; Dericioglu et al. 2008). Extrasynaptic GABA mediates a tonic inhibitory process and plays a key role in regulating both tonic and phasic excitability in GABAergic circuits (Farrant and Nusser 2005; Wu et al. 2007).

Neurophysiological, behavioral, and pharmacological studies indicate that cortical GABA content as measured in human volunteers by 1H-MRS is predictive of the functional status of GABA-mediated processes. It is generally agreed that oscillations in the EEG gamma band (30–90 Hz) depend on the rhythmic activity of local networks of GABAergic interneurons via their synchronizing effects on the output of glutamatergic excitatory neurons (Mann and Mody 2010). Three recent human studies of visual and motor cortices have reported a significant positive correlation between GABA content as measured by 1H-MRS using a MEGA-PRESS sequence and the frequency of evoked activity in the EEG gamma band (Edden et al. 2009; Muthukumaraswamy et al. 2009; Gaetz et al. 2011). Two psychophysical studies have shown that performance on visual tasks mediated by the activity of GABAergic interneurons is significantly correlated with GABA content in the primary visual cortex as measured by 1H-MRS using a MEGA-PRESS sequence (Edden et al. 2009; Yoon et al. 2010a). In addition, several studies have shown that anticonvulsant medications that appear to increase GABAergic tone cause an increase in brain GABA as measured with 1H-MRS (Weber et al. 1999; Petroff et al. 2001). It is worth noting that several studies have found evidence that the 1H-MRS GABA signal varies over the course of the menstrual cycle in women, with GABA signal reduced during the luteal phase (Epperson et al. 2002; Harada et al. 2010). Overall, it appears that 1H-MRS measures of GABA acquired with specialized pulse sequences can provide information about a pool of cortical GABA with a predictive relationship to GABA-mediated responses. It also appears that such measures reflect the functional integrity and capacity of the underlying GABAergic neurons.

2.7 Lactate

Lactate is a three-carbon product of the glycolytic metabolism of glucose (Fig. 2). Its methyl hydrogens give rise to a doublet signal at about 1.32 ppm (Fig. 1b, c). A smaller complex peak from its methine hydrogen arises at about 4.10 ppm but cannot be detected in brain with conventional 1H-MRS methods. Lactate detected at 1.32 ppm in a clinical 1H-MRS acquisition is widely assumed to indicate brain pathology. Indeed, the concentration of lactate in normal brain is rarely greater than 1 mM. The appearance of an obvious lactate signal when no special attempt has been made to optimize its detection strongly suggests that ischemia, tumor, trauma, infection, mitochondrial disease, or other pathological process is present.

Although a high concentration of lactate is a sign of pathology, lactate is a normal and essential component of brain energy metabolism. When measures of brain lactate are of interest in studies of neuropsychiatric disorders, slight modifications to conventional procedures are recommended for its detection with clinical MRI systems. The lactate peak at 1.32 ppm overlaps and is often obscured by the methylene resonances of lipids centered at about 1.30 ppm. Several adjustments to conventional procedures can reduce or eliminate this potentially obscuring lipid signal. Since the relaxation time for the lipid methylene protons is much shorter than for the methyl protons of lactate, much of the lactate signal will be retained while most of the lipid signal will be lost by using a long TE acquisition (such as 144 or 288 ms). Although the inversion of the lactate doublet (due to J-coupling, see Supplement Sect. 4.2.2) at TE = 144 ms can be an aid to the visual identification of the lactate signal, elimination of lipid is more complete and lactate quantification appears to be more reliable when spectra are acquired with TE = 288 ms (Fig. 1b, c) (Roelants-Van Rijn et al. 2001; Maddock and Buonocore 2008). Tissues of the scalp and skull contain high concentrations of lipid. Lipid signal from these and other tissues outside of the voxel of interest can contaminate 1H-MRS data. Use of specialized lipid suppression pulses and attention to optimizing the gradient order can minimize lipid signal originating from outside of the prescribed voxel (Maddock et al. 2006). Because the concentration of lactate in the brain is normally near the low end of the sensitivity range of clinical MRI systems, increasing the signal-to-noise ratio will improve detection of the lactate signal. Thus, 1H-MRS studies of brain lactate often use large voxel sizes and long acquisition times. The use of surface or phased array coils can also improve signal-to-noise ratio from voxels close to the coil (Maddock and Buonocore 2008). Specialized pulse sequences, such as the J-difference editing approach described in Supplement Sect. 4.5.1, can provide even more sensitive and specific measures of brain lactate (Star-Lack et al. 1998).

Although once considered a “dead end” metabolite produced only under anaerobic conditions (e.g. hypoxia), lactate is now recognized as being an essential intermediate in the energy metabolism of organs with high-energy requirements, including muscle, heart, and brain (Brooks 2002; Gladden 2004). In all brain cells,

the first sequence of steps in the generation of ATP from glucose occurs in cytoplasm and proceeds without a requirement for oxygen (glycolysis). The end products of glycolysis are lactate and pyruvate, which are in equilibrium with respect to the reaction catalyzed by lactate dehydrogenase. This equilibrium strongly favors the production of lactate under basal conditions. However, pyruvate is the primary substrate for the oxidative generation of ATP in mitochondria via the TCA cycle and oxidative phosphorylation. The aerobic consumption of pyruvate as part of the mitochondrial TCA cycle promotes the conversion of lactate to pyruvate.

One of the most energy-intensive components of neurotransmission is the clearance of glutamate from the synaptic cleft by astrocytes and the subsequent conversion of glutamate to glutamine for release back into the ECF. This process occurs, in part, in the thread-like filopodia of astrocytes that surround synapses. The filopodia of astrocytes are too narrow to accommodate mitochondria, but they are highly enriched in glycogen granules (a storage form of glucose). Thus, a significant fraction of the ATP required for the astrocytic recycling of glutamate during neural activation is derived from the glycolytic conversion of glucose (and glycogen during strong activation) to lactate (Brown 2004; Pellerin et al. 2007). During neural activation, lactate levels increase and lactate is released into the ECF for uptake into intracellular compartments containing mitochondria, where it can be converted to pyruvate for subsequent metabolism and ATP generation (Hu and Wilson 1997). Although specific details regarding the production and consumption of lactate during neural activity remain to be clarified, it is clear that lactate levels increase during and after neural activation, and that lactate constitutes an important energy source for neuronal oxidative metabolism. It is also clear that astrocytes are the major cell type in the brain for storage of carbohydrate energy as glycogen and that the abundance of lactate transporters in astrocytic and neuronal cell membranes makes lactate a likely vehicle by which carbohydrate energy can be shuttled from cell to cell during times of high-energy demand. 1H-MRS studies in humans using appropriate methods have consistently observed increases in cortical lactate during neural activation (e.g. visual stimulation by a pattern reversal checkerboard) (Prichard et al. 1991; Sappey-Marinier et al. 1992; Maddock et al. 2006; Maddock and Buonocore 2008).

A 1H-MRS finding of substantially elevated brain lactate in the absence of an activation condition is most likely a sign of major pathology. However, small increases in basal lactate may reflect subclinical inflammation, impairment of oxidative metabolism, or increased neural activity. Converging evidence suggests that brain lactate levels increase acutely (over a period of several minutes) in proportion to the degree of glutamatergic activity (Hu and Wilson 1997; Pellerin et al. 2007). Thus, dynamic 1H-MRS studies of the brain lactate response to an experimental activation paradigm can provide insight into the functional state of basic neural and metabolic processes.

2.8 31Phosphorous-MRS

With suitable equipment, clinical scanners can be modified to collect brain MRS data from metabolites containing the 31Phosphorous nucleus (31P-MRS) including phosphocreatine, ATP, phosphomonoesters (mainly phosphorylethanolamine and phosphorylcholine) that are precursors for membrane phospholipid synthesis, phosphodiesters that are breakdown products of membrane phospholipids, and inorganic phosphate. When available, these measurements can provide insight into the status of high-energy phosphates and membrane turnover. The resonance frequency of inorganic phosphate is sensitive to the pH of its microenvironment. Thus, accurate measures of the resonance frequency of inorganic phosphate can be used to estimate the pH of the intracellular compartment in brain tissue (Petroff et al. 1985).

3 MRS Findings in Major Psychiatric Disorders

Since the early 1990s, the non-invasive measurement of brain metabolite concentrations with MRS has provided a unique avenue for extending our understanding of the pathogenesis of neuropsychiatric disorders. There is now a large literature describing the findings of brain MRS studies in the major psychiatric disorders. In this section, we provide an overview of this literature and summarize the most consistent findings in patients with schizophrenia, bipolar disorder, major depression, and anxiety disorders, with an emphasis on findings with potential translational significance.

3.1 Schizophrenia

Schizophrenia is a mental disorder characterized by disordered thinking, perceptual disturbances, and impairment of affect, cognition, and cognitive control. The disorder typically begins in late adolescence or early adulthood and is most often chronic. Of all psychiatric disorders, schizophrenia has been the most extensively studied with MRS methods. Anatomical brain imaging studies and postmortem neuropathological studies of brain tissue provide clear evidence for structural and neuropathological abnormalities in patients with schizophrenia. Recent progress in identifying the neuropathological abnormalities associated with schizophrenia suggests that brain MRS may be a particularly valuable tool for in vivo studies of pathophysiology and treatment effects in this disorder.

The most consistent findings from structural brain imaging studies in schizophrenia include an overall reduction in brain volume, enlarged cerebral ventricles, and regional gray and white matter volume reductions, primarily in medial temporal structures, but also in the lateral temporal lobes, thalamus, and parts of the frontal

lobes (Wright et al. 2000; Ellison-Wright et al. 2008; Jaaro-Peled et al. 2010). Reliable evidence also shows that the reduction in hippocampal volume occurs early in the illness and is also observed in the relatives of patients with schizophrenia, implicating a genetic vulnerability to this phenotypic feature (Jaaro-Peled et al. 2010; Meyer-Lindenberg 2010). Although statistically significant in large samples of patients, these volume reductions are small, and there is extensive overlap between patient and control groups. Several consistent neuropathological findings may account for some of these macroscopic structural observations in schizophrenia. The pyramidal neurons of the frontal cortex, which are the main source of excitatory neurotransmission between cortical regions, are reduced in size and packed more densely without any change in the total number of such neurons (Selemon and Goldman-Rakic 1999). There is similar evidence for reduced size of the pyramidal neurons in the hippocampus. These findings suggest a reduction in neuronal tissue in schizophrenic patients, which would be expected to be associated with a reduced concentration of NAA. Consistent neuropathological abnormalities have also been observed in the GABAergic interneurons of the cerebral cortex in schizophrenia. These observations are discussed in Sect. 3.1.2.

3.1.1 NAA

Steen et al. (2005) conducted an extensive review and meta-analysis of published 1H-MRS data on brain NAA content that spanned over 1,250 patients with schizophrenia and over 1,200 control subjects. They found consistent evidence that NAA is reduced in many brain regions in schizophrenia patients compared to control subjects. In general, the extent of reduction of NAA appeared to be similar in gray matter and white matter. However, they found evidence that the degree of schizophrenia-related NAA reductions varied across brain regions. Specifically, NAA levels did not appear to be reduced in the basal ganglia, occipital cortex, or posterior cingulate cortex. In contrast, NAA levels were consistently and substantially reduced (>5% reduction compared to control subjects) in temporal gray and white matter, hippocampus, frontal gray and white matter, and cerebellum, with the largest reductions (>10%) in temporal white matter and the hippocampus. Smaller, but consistent, reductions were also seen in the anterior cingulate cortex and thalamus. The authors reported no compelling evidence to suggest that NAA is significantly elevated in any brain region in schizophrenia. Although most patients in the studies they reviewed had chronic schizophrenia, over 200 of the patients had been studied while in their first episode of the illness. There was no robust evidence for a difference between first episode and chronic patients. However, in a comparison of 74 first episode and 171 chronic schizophrenia patients in whom frontal cortex NAA levels were measured, the authors noted a trend toward lower NAA in the first-episode patients.

The 1H-MRS studies of NAA compliment the findings of structural imaging and neuropathological studies and offer further evidence of reduced neuronal tissue in schizophrenia, especially in the temporal lobes, frontal lobes, hippocampus, and

cerebellum. The reduction in NAA is present from the onset of clinically overt illness and there is little evidence to suggest that it is attributable to treatment with antipsychotic medications (Steen et al. 2005).

MRS studies showing that reductions in NAA levels are reversible in some disorders (Sect. 2.1) and neuropathological findings of reduced size but not number of pyramidal neurons in schizophrenia leaves open the possibility that reduced NAA in specific brain regions in schizophrenia may represent a neurotrophic change rather than an irreversible loss of viable neurons. If correct, it is conceivable that NAA levels could increase toward normal with effective treatments for schizophrenia. However, longitudinal studies of treatment effects on NAA levels in schizophrenia have yielded mixed results. A few small longitudinal studies of treatment with antipsychotic medications have found increased NAA in selected brain regions, but the studies with larger samples and longer treatment intervals have generally found no effect (Bertolino et al. 2001; Pae et al. 2004; Bustillo et al. 2008, 2010). Only a few small studies have looked at the effect of non-pharmacological treatments on brain NAA in schizophrenia. Premkumar et al. (2010) examined the effects of adding cognitive-behavioral therapy to ongoing treatment with antipsychotic medication in outpatients with schizophrenia. Following 8 months of add-on psychotherapy, they observed a decrease in positive symptoms and an 8% increase in anterior cingulate cortex NAA (the only region they studied). Also in a small sample, Pajonk et al. (2010) observed a 35% increase in hippocampal NAA in schizophrenia patients following three months of aerobic exercise training. No change was seen in a control group of patients who did not participate in exercise training. No randomized, controlled studies have compared the effects of different treatments on brain NAA in schizophrenia, although naturalistic cross-sectional studies suggest NAA levels may be higher in patients taking atypical compared to typical antipsychotic medications (Fannon et al. 2003; Braus et al. 2002; Bustillo et al. 2001). Further studies will be necessary to determine whether NAA levels can be reliably increased by treatment in schizophrenia, and whether such increases are associated with clinically meaningful improvement.

3.1.2 GABA

In a development that has stimulated much theoretical and translational work, postmortem studies on brain tissue have consistently demonstrated a reduction in the GABAergic potential of specific interneurons in many cortical regions, including the frontal cortex and hippocampus in patients with schizophrenia. In particular, the concentration of cortical GABA and the activity of the 67 kDa form of glutamic acid decarboxylase (GAD67, the enzyme responsible for most GABA synthesis in the brain) are reduced in postmortem cortical tissue from patients with schizophrenia (Lisman et al. 2008). Low GABA activity is most consistently observed in the fast-spiking, parvalbumin-positive interneurons. These interneurons are functionally coupled to excitatory pyramidal neurons and

regulate their activity (Lisman et al. 2008). The coordinated activity of these two types of neurons gives rise to EEG activity in the gamma band (30–80 Hz), which appears to be essential for communication and processing of information across cortical regions. Thus, gamma band activity is critically dependent on GABAergic inhibition mediated by the fast-spiking interneurons that are deficient in schizophrenia. Accordingly, gamma band activity is consistently found to be abnormal in patients with schizophrenia (Uhlhaas and Singer 2010).

Several 1H-MRS studies have examined the relationship between cortical GABA and measures of brain function believed to depend on the fast-spiking GABAergic interneurons that are deficient in schizophrenic patients. Muthukumaraswamy et al. (2009) used the MEGA-PRESS method to measure GABA concentration in the visual cortex in normal subjects. They demonstrated a significant positive correlation between resting GABA concentration and the frequency of stimulus-induced visual gamma band EEG oscillations. A second study used the same 1H-MRS method to measure GABA concentration in the visual cortex in normal volunteers who also underwent psychophysical testing on a visual orientation discrimination task. GABAergic inhibition appears to play a key role in visual orientation discrimination. The investigators reported significant positive correlations between oblique orientation discrimination and both visual cortex GABA and the frequency of visual stimulus-induced gamma oscillations in the visual cortex. GABA concentration was also correlated with gamma frequency (Edden et al. 2009). In a psychophysical study of patients with schizophrenia, Yoon et al. (2009) demonstrated a deficiency in visual orientation processing using an orientation-specific surround suppression task. In a subsequent study, Yoon et al. (2010a) measured visual cortex GABA with 1H-MRS using the MEGA-PRESS method and demonstrated significantly lower GABA levels in the schizophrenic patients compared to healthy comparison subjects (Fig. 3). They also found a significant positive correlation between orientation-specific surround suppression and visual cortex GABA levels. These studies suggest that 1H-MRS can be used to measure a pool of cortical GABA that has a direct, functional relationship with GABA-mediated behavioral and physiological responses, at least in the visual cortex, and that these measurements can be used in patient populations to test translational models of schizophrenia. It must be noted that other recent 1H-MRS studies have not observed significantly reduced cortical GABA levels in patients with schizophrenia (Goto et al. 2009; Ongur et al. 2010; Tayoshi et al. 2011). A variety of different MRS acquisition and post-processing procedures were used in these studies, which may account for the differing results. However, only the Yoon et al. study measured GABA in the primary visual cortex and included a parallel behavioral measure to validate the GABA measurements (Yoon et al. 2010a). Although 1H-MRS measures of cortical GABA in schizophrenia appear to have great potential, it is clear that further work is needed to provide more definitive answers to critical translational research questions, such as (1) Does 1H-MRS reliably demonstrate a cortical GABA deficiency in patients with schizophrenia in vivo as has been observed in postmortem brain tissue? (2) If so, do in vivo cortical GABA deficits vary by brain region? (3) Do cortical GABA

deficits predict clinical symptoms or information processing deficits? (4) Do treatment-related changes in cortical GABA predict treatment response in schizophrenia? (5) Can 1H-MRS measures of cortical GABA be used to test GABA-related predictions of the NMDA hypofunction model of schizophrenia? Future studies will clarify the value of 1H-MRS measures of brain GABA in translational studies of schizophrenia.

3.1.3 Glutamate and Glutamine

1H-MRS studies have reported decreased, increased, or no difference in observable brain glutamate or Glx levels in schizophrenia patients compared to healthy comparison subjects (Abbott and Bustillo 2006; Stone 2009; Yoon et al. 2010a). At present, there is no consistent 1H-MRS evidence implicating a specific pattern of abnormal brain glutamate or Glx in schizophrenia. However, models of the neuropathology of schizophrenia suggest that an underlying disturbance of glutamatergic function may be present. Basic studies in animals and 1H-MRS studies in normal volunteers have demonstrated activity-dependent increases in MRS-visible cortical glutamate (Carder and Hendry 1994; Arckens et al. 2000; Qu et al. 2003; Hertz 2004; Mullins et al. 2005; Mangia et al. 2007; Gussew et al. 2010). That is, regional cortical glutamate (or Glx) is observed to increase during neuronal activation. In the NMDA receptor hypofunction model of schizophrenia, NMDA receptor hypofunction leads to both a reduced output from GABAergic interneurons and a downstream hyperglutamatergic state (Lisman et al. 2008). The associated increase in flux through the glutamate/glutamine cycle might be expected to lead to a measurable increase in the levels of these amino acids in the brain. On the other hand, glutamate levels, like NAA levels, may vary with the functional integrity of neurons, most of which are glutamatergic. Neuronal integrity appears to be compromised in many cortical regions in schizophrenia. Impaired functional integrity of glutamatergic neurons could reduce Glx levels in schizophrenia patients. Thus, a combination of factors may predispose to both increased and decreased brain Glx levels in schizophrenia patients. Such counterbalancing effects would make it difficult to detect Glx abnormalities with conventional 1H-MRS approaches.

Glutamate release during neurotransmission leads to astrocytic uptake and conversion of glutamate to glutamine by the enzyme glutamine synthase. NMDA receptor hypofunction appears to increase the activity of glutamine synthase, and thus to increase glutamine levels (Rodrigo and Felipo 2007). Pharmacological blockade of NMDA receptors in animals leads to an increase in cortical glutamine (Kosenko et al. 2003; Rodrigo and Felipo 2007) and in the glutamine/glutamate ratio (Brenner et al. 2005; Iltis et al. 2009). High-field 1H-MRS studies suggest that NMDA receptor blockade has similar effects in the anterior cingulate cortex of human volunteers (Rowland et al. 2005). There have been mixed results from 1H-MRS studies of glutamine measured in patients with schizophrenia. The 1H-MRS signals from glutamate and glutamine partially overlap, and it is difficult to reliably quantify brain glutamine as distinct from glutamate. However, it may be

achievable with higher field scanners, short echo times, and long acquisition times. In this regard, one study using a high-field scanner observed elevated glutamine in the anterior cingulate cortex in treatment naïve patients with schizophrenia (Theberge et al. 2002). However, a second study by the same group found lower glutamine levels in the anterior cingulate cortex in chronic schizophrenia patients (Theberge et al. 2003). Two recent studies (Bustillo et al. 2010; Shirayama et al. 2010) specifically measured the glutamine/glutamate ratio with high-field scanners and both found an elevated glutamine/glutamate ratio in the medial prefrontal cortex or anterior cingulate cortex of the patients with schizophrenia. Both studies also reported a significantly reduced NAA/Cr ratio. In addition, a study of CSF in first episode, drug-naïve patients with schizophrenia also found an increase in the glutamine/glutamate ratio in CSF in the patient group (Hashimoto et al. 2005). The glutamine/glutamate ratio may provide a more useful reflection of the functional status of the glutamine/glutamate cycle in astrocytes and neurons in the context of compromised neuronal integrity in patients with schizophrenia. Similarly, repeated measures, dynamic 1H-MRS studies of activity-dependent increases in glutamate or Glx during an activation paradigm may also offer a useful means of testing hypotheses about NMDA receptor hypofunction and hyperglutamatergic states in the context of compromised neural integrity in patients with schizophrenia.

3.1.4 Other Metabolites

Early 31P-MRS studies suggested that phosphomonoesters were low and phosphodiesters were high in the frontal lobes of patients with schizophrenia, a pattern consistent with increased membrane breakdown in this brain region (Fukuzako 2001). However, more recent studies have not found this to be a consistent finding (Yacubian et al. 2002; Jensen et al. 2006; Smesny et al. 2007). No consistent patterns of abnormalities in brain creatine, choline, or myo-inositol have been observed in schizophrenia (Deicken et al. 2000; Kim et al. 2005; Steen et al. 2005).

3.2 Bipolar Disorder

Bipolar disorder is characterized by episodes of manic and depressed moods interspersed with periods of relatively normal mood. There is strong evidence for a genetic vulnerability to this disorder, which typically follows a relapsing and remitting course in the absence of treatment with lithium or other mood stabilizing medication. High-resolution brain imaging studies demonstrate both global and regional structural abnormalities in bipolar disorder. A recent meta-analysis found evidence for a small but reliable reduction in whole-brain volume (effect size $= -0.15$) and in volume of the frontal cortex (effect size $= -0.42$) in bipolar patients (Arnone et al. 2009). The patient group also showed an increase in the size of the lateral ventricles (effect size $= +0.27$), although lateral ventricle size was

significantly smaller than in patients with schizophrenia across studies directly comparing the two diagnostic groups (Arnone et al. 2009). The bilateral volume of the globus pallidus was found to be significantly larger in bipolar patients across 5 studies, and this effect was associated with the use of mood stabilizer medications (Arnone et al. 2009). A meta-analysis of voxel-based morphometry studies of gray matter observed reduced volume of anterior cingulate and fronto-insular cortex in bipolar disorder (Bora et al. 2010), along with increased basal ganglia volume associated with duration of illness. Mood stabilizers in general, and lithium in particular, have been shown to have neurotrophic effects and to promote neuroplasticity (Manji et al. 2000; Quiroz et al. 2010). The use of lithium by bipolar patients has consistently been associated with increased volume of the anterior cingulate cortex and the hippocampus (Emsell and McDonald 2009). These brain morphometry differences and the neurotrophic effects of mood stabilizing medications should be kept in mind when interpreting the 1H-MRS findings in bipolar disorder.

3.2.1 NAA

There have been many 1H-MRS studies of bipolar patients and, in general, this literature supports the conclusion that NAA levels are reduced in some brain regions. However, variations in MRS acquisition methods, brain regions investigated, metabolite quantification and normalization strategies, sample characteristics, and medication status make it difficult to interpret conflicting findings. Medication status is a particularly important source of variance in studies of NAA, since considerable evidence suggests that lithium and other mood stabilizers may increase brain levels of NAA. We found five published 1H-MRS studies reporting on NAA levels in adult bipolar patients free of recent medication use and matched control subjects. Four of the five studies demonstrated significantly reduced NAA levels in their patient groups. These studies included a total of 53 patients and 65 healthy comparison subjects and observed reduced NAA levels in regions including the hippocampus (2 studies), the dorsolateral prefrontal cortex, and the occipital cortex (Winsberg et al. 2000; Bertolino et al. 2003; Atmaca et al. 2007; Bhagwagar et al. 2007). One study, including 29 patients and 26 healthy comparison subjects, observed no significant difference in NAA levels in composite gray matter and white matter regions obtained from an axial 1H-MRSI slab acquired at the level of the corpus callosum (Dager et al. 2004). Many studies of bipolar patients taking mood stabilizers also show a decrement in NAA levels in frontal and hippocampal regions (Yildiz-Yesiloglu and Ankerst 2006a). In general, these findings are consistent with the meta-analytic evidence for a reduction in global brain and frontal lobe volume in this condition.

The neurotrophic effects of mood stabilizers may include increasing levels of NAA in brain regions where NAA and gray matter volume are reduced in bipolar disorder (Manji et al. 2000). Many cross-sectional studies comparing unmedicated bipolar patients to patients taking lithium have found that NAA levels are higher in the lithium-treated patients (Yildiz-Yesiloglu and Ankerst 2006a). However,

longitudinal studies of the same individuals before and during lithium treatment can provide a more conclusive test of the effects of lithium on regional brain NAA content. One study of 12 adult bipolar patients and 9 healthy volunteers found that 4 weeks of treatment with lithium led to an increase in NAA levels in all regions studied (frontal, temporal, parietal, and occipital lobes) (Moore et al. 2000). However, this effect was not observed in studies of children or adolescents with bipolar disorder (Patel et al. 2008; Dickstein et al. 2009) or in a group of healthy volunteers (Brambilla et al. 2004). There is less consistent evidence for increased NAA with other mood stabilizers (Yildiz-Yesiloglu and Ankerst 2006a).

3.2.2 Glutamate and Glutamine

Elevated gray matter Glx has been consistently observed across a range of brain regions and clinical conditions in patients with bipolar disorder. Yuksel and Ongur recently reviewed the published literature on Glx in bipolar adults through 2009 (Yuksel and Ongur 2010). They found nine 1H-MRS studies that measured Glx in various brain regions, medication conditions, and mood states (depressed, manic, and euthymic) in bipolar patients. Six of the nine studies observed significantly elevated Glx (in cingulate, prefrontal, insular, parietal, occipital, and hippocampal gray matter) in the bipolar patients (Michael et al. 2003b; Dager et al. 2004; Bhagwagar et al. 2007; Frye et al. 2007; Ongur et al. 2008; Senaratne et al. 2009). A seventh study examined Glx in the left dorsolateral PFC in both rapid cycling and non-rapid cycling bipolar II patients. They found elevated Glx in the rapid cycling but not in the non-rapid cycling patients. However, they did not report on the results across all of the bipolar patients (Michael et al. 2009). An eighth study examined only the left amygdala, and found no difference in Glx in the bipolar patients (Michael et al. 2003a). The ninth study found reduced Glx in the right lentiform nucleus in the bipolar patients (Port et al. 2008). Four other studies reported results for glutamate, but not for the combined Glx signal. Two of these reported elevated glutamate in bipolar patients (Colla et al. 2009; Lan et al. 2009). One additional study examined Glx in older adolescents and young adults (mean age = 22) and found elevated Glx in the bipolar patients (Cecil et al. 2002). Considering the variation in technical and quantitative methods used, brain regions examined, and clinical mood state of the patients, these studies provide compelling evidence for a consistent pattern of elevated brain Glx in adult patients with bipolar disorder. Fewer studies have examined Glx in pediatric bipolar patients, and the results are inconsistent (Yildiz-Yesiloglu and Ankerst 2006a; Capizzano et al. 2007). 1H-MRS studies of brain GABA in bipolar patients have produced inconsistent results.

There have been only a few studies examining the effects of medication treatments for bipolar disorder on 1H-MRS measures of Glx. Longitudinal studies in bipolar patients (Friedman et al. 2004), normal volunteers (Shibuya-Tayoshi et al. 2008), and rats (O'Donnell et al. 2003) found evidence for a reduction in brain Glx following lithium treatment. A longitudinal study of lamotrigine

observed no effect on Glx levels in bipolar patients (Frye et al. 2007). A cross-sectional study found no differences in Glx levels attributable to treatment with lithium, anticonvulsants, or benzodiazepines (Ongur et al. 2008). In six cross-sectional 1H-MRS studies, at least 75% of the bipolar patients studied were medication free. Three of these studies observed significantly elevated Glx levels in the bipolar patients compared to the healthy comparison subjects (Michael et al. 2003b; Dager et al. 2004; Bhagwagar et al. 2007). It remains to be determined whether mood stabilizers reduce brain Glx in bipolar patients. However, it appears unlikely that the reliable elevation of brain Glx seen in bipolar disorder is an artifact of medication treatment.

The singular importance of glutamate in neurotransmission, the evidence that some mood stabilizers act, in part, by reducing glutamatergic activity, and the contrasting finding that brain Glx is consistently reduced during episodes of unipolar depression (reviewed below) all support the hypothesis that elevated brain Glx has pathophysiological significance in bipolar disorder. Glutamate and glutamine have important functions in both metabolism and neurotransmission. However, some evidence suggests that 1H-MRS measures a single, integrated pool of cytoplasmic Glx in neurons and glia participating in both metabolic and cell-signaling processes (Hertz 2004). This consideration further supports the possibility that elevated Glx in bipolar disorder may reflect a pathophysiologically significant abnormality. Eastwood and Harrison recently found that bipolar patients have elevated levels of vesicular glutamate transporter 1 (VGluT1) mRNA in the anterior cingulate cortex compared to healthy comparison subjects and schizophrenia patients (Eastwood and Harrison 2010). Their finding reinforces the idea that elevated Glx in bipolar patients reflects an increase in glutamatergic neurotransmission, at least in the anterior cingulate cortex. Assessing the utility of 1H-MRS measures of Glx or glutamate in interrogating pathophysiological models of bipolar disorder or in aiding the diagnostic discrimination between bipolar disorder and other mood disorders will be important objectives of future studies (Yuksel and Ongur 2010).

3.2.3 Choline

There have been consistent demonstrations of elevated choline signal in the basal ganglia of patients with bipolar disorder (Kato et al. 1996; Hamakawa et al. 1998; Dager et al. 2004; Yildiz-Yesiloglu and Ankerst 2006a). Although most evidence suggests that lithium does not change the brain 1H-MRS choline signal (Stork and Renshaw 2005), it is possible that other medications in common use could have such an effect. Thus, studies in unmedicated patients are of particular value. In the only study that reported choline data from the basal ganglia in unmedicated bipolar patients, Dager et al. (2004) found significantly increased choline in the patient group. The 1H-MRS evidence of an increase in mobile choline-containing compounds in the basal ganglia of bipolar patients is consistent with the results of the meta-analysis by Bora et al. (2010) showing that a longer duration of illness is

associated with a larger gray matter volume in the basal ganglia of bipolar patients. Altered metabolism or increased cell density in this region could lead to an increase in the choline signal. Further studies will be necessary to clarify the significance of basal ganglia changes in bipolar disorder. In other brain regions, there is no consistent evidence for an alteration in choline levels in bipolar disorder (Stork and Renshaw 2005; Yildiz-Yesiloglu and Ankerst 2006a).

3.2.4 Myo-Inositol

Lithium can acutely reduce myo-inositol levels by inhibiting the enzyme inositol monophosphatase, which regenerates myo-inositol from inositol monophosphates as part of the phosphoinositol second messenger cycle (Hallcher and Sherman 1980). Recognition of this effect of lithium suggested two related hypotheses: (1) that bipolar disorder may be characterized by elevated levels of myo-inositol; and (2) that depletion of myo-inositol may be an important component of the therapeutic effect of lithium and other mood stabilizers (Berridge 1989). If lithium and other mood stabilizers decrease myo-inositol levels, then the hypothesized elevation of myo-inositol levels may be obscured in studies of medicated patients. However, 1H-MRS studies of sustained lithium administration have not found that it decreases brain myo-inositol (Brambilla et al. 2004; Patel et al. 2006; Silverstone and McGrath 2009) and myo-inositol levels are not consistently lower in untreated than treated bipolar patients (Yildiz-Yesiloglu and Ankerst 2006a; Silverstone and McGrath 2009). This suggests that sustained treatment may not be a significant confound in studies of myo-inositol levels. Generally, neither unmedicated nor medicated bipolar patients show consistent abnormalities of brain myo-inositol levels (Yildiz-Yesiloglu and Ankerst 2006a; Silverstone and McGrath 2009).

3.2.5 Other Metabolites

Two publications have systematically reviewed brain 31P-MRS studies in bipolar patients. From these meta-analyses, the most consistent finding is a decrease in phosphomonoesters (PMEs) in euthymic bipolar patients, which has been observed in four of six studies of the frontal lobe and in one temporal lobe study (Yildiz et al. 2001; Stork and Renshaw 2005). This effect appears to be mood state specific, as frontal lobe PMEs are frequently observed to be higher in currently depressed or manic patients than in currently euthymic bipolar patients. The apparent, state-specific alterations of brain PMEs may reflect an underlying abnormality affecting membrane metabolism in bipolar disorder. 31P-MRS can also be used to measure intracellular pH in the brain. This derives from the effect of pH on the chemical shift of inorganic phosphate, which has a primarily intracellular localization. Five out of five studies (albeit from the same group) have observed lower intracellular pH in euthymic bipolar patients. Most of these studies

examined whole-brain pH, but one study also found lower pH in the basal ganglia region (Stork and Renshaw 2005). There is preliminary evidence that low intracellular pH may be specific to the euthymic state, as pH has been observed to be higher in currently depressed or manic patients than in currently euthymic patients. Both the PME and pH abnormalities may be evidence of mitochondrial dysfunction in bipolar disorder (Stork and Renshaw 2005). The relative normalization of PMEs and pH during periods of active mood disturbance could reflect dysregulatory processes triggered by homeostatic mechanisms attempting to compensate for the mitochondrial deficiency. The 1H-MRS finding that brain lactate is elevated in bipolar patients is also consistent with a mitochondrial deficiency and compensation model (Dager et al. 2004).

3.3 Unipolar Major Depression

Unipolar major depressive disorder is characterized by episodes of sustained depressed mood, loss of motivation, and the associated somatic, emotional, and cognitive symptoms of depression. There is clear evidence for a genetic vulnerability to this condition, and most patients have recurrent episodes of illness. Brain morphometric studies have found no reliable evidence for a global reduction in brain volume in major depression (Konarski et al. 2008). However, there is consistent evidence for a volume reduction in prefrontal regions, especially the orbital frontal cortex, the anterior cingulate cortex, and its rostroventral terminus, the subgenual cingulate cortex, in patients with major depression (Hajek et al. 2008; Konarski et al. 2008; Savitz and Drevets 2009). Volume reduction in the hippocampus also appears to be a consistent pattern in major depression, although this finding may be most marked in older or chronically depressed patients (Konarski et al. 2008; Savitz and Drevets 2009). There is some evidence for volume loss as well as consistently reduced metabolic activity in the dorsolateral prefrontal cortex and for volume loss in the basal ganglia in major depression (Konarski et al. 2008; Savitz and Drevets 2009). Neuropathological studies in postmortem brain tissue from patients with major depression report generally consistent evidence for reduced glial cell number and/or density in frontal and limbic regions, including orbital, anterior cingulate, subgenual and dorsolateral prefrontal cortices, and the amygdala (Hercher et al. 2009; Yuksel and Ongur 2010). Molecular neurobiology studies have found evidence consistent with reduced neuroplasticity in frontal and limbic regions in major depression (Krishnan and Nestler 2008). Together, the findings from structural neuroimaging, neuropathological, and molecular studies suggest that frontal and limbic regions, including the hippocampus and basal ganglia, may be specifically implicated in the pathophysiology of major depression and that impairments in glial functions and neuroplasticity may be involved.

3.3.1 NAA

Reviews and meta-analyses of the 1H-MRS literature on major depression through 2006 found no consistent evidence that NAA was either increased or decreased in adult or pediatric patients with major depression (Yildiz-Yesiloglu and Ankerst 2006b; Capizzano et al. 2007; Kondo et al. 2011). Most of the studies reviewed examined the frontal lobes and most included only medication-free patients. Few studies have examined the medial temporal region, but preliminary evidence suggests it may be characterized by reduced NAA levels in major depression (MacMaster et al. 2008; Reynolds and Reynolds 2011). There is little evidence that antidepressant medications alter NAA in frontal regions (Capizzano et al. 2007). The observations of volume loss in prefrontal regions without a corresponding loss of NAA signal are consistent with the hypothesis that the pathophysiology of major depression involves an impairment of prefrontal glial integrity.

3.3.2 Glutamate and Glutamine

The most frequently replicated brain 1H-MRS finding in major depression is reduced glutamate and Glx in prefrontal and limbic regions when patients are currently in a depressive episode. In their 2010 comprehensive review, Yuksel and Ongur (2010) identified 9 studies that measured Glx levels in prefrontal or limbic regions in currently depressed adult patients with major depression. Although there was substantial variation in the 1H-MRS methods used and the specific brain regions examined, 6 of the 9 studies reported significantly reduced Glx in prefrontal regions, the hippocampus and the amygdala. A similar consistent reduction in prefrontal Glx was recently described by Kondo and colleagues in their review of 1H-MRS studies of major depression in children and adolescents (Kondo et al. 2011). A recent study found that diabetic patients with major depression also showed a significant reduction in basal ganglia Glx compared to non-depressed diabetic control patients and compared to healthy volunteers (Ajilore et al. 2007). Another recent study reported a specific decrease in glutamine in the anterior cingulate cortex of highly anhedonic patients with major depression, but this finding was based on only five patients (Walter et al. 2009).

The reduction in Glx in prefrontal and limbic regions appears to be a state-specific characteristic of major depression. Two studies reviewed by Yuksel and Ongur and an additional more recent study scanned euthymic patients subsequent to the remission of their major depressive episode. Two reported normal Glx levels in prefrontal regions, while one observed elevated Glx in the occipital cortex (Taylor et al. 2009; Yuksel and Ongur 2010). Two additional studies showed a normalization of prefrontal Glx levels following successful treatment with electroconvulsive therapy (Yuksel and Ongur 2010). A more recent study examined 22 depressed patients with varying degrees of response to antidepressant medication and found that Glx levels in the pregenual cingulate cortex, but not in the anterior

insula, demonstrated a significant negative correlation with Hamilton depression rating scores (Horn et al. 2010).

Converging observations support the hypothesis that reduced prefrontal and limbic Glx has pathophysiological importance during active episodes of major depression. Glx levels appear to normalize during clinical remission, and the apparently state-dependent reduction of prefrontal and limbic Glx in unipolar depression contrasts sharply with the state-independent elevation of Glx seen in bipolar disorder. Furthermore, blockade of the NMDA receptor by ketamine leads to a hyperglutamatergic state, and also leads to rapid improvement of symptoms in patients with major depression (Zarate et al. 2006). The apparent anatomical specificity of reduced Glx for frontal and limbic regions in major depression is concordant with the selective volume loss seen in these brain regions with structural MRI studies (Hajek et al. 2008; Konarski et al. 2008; Savitz and Drevets 2009). MRS-visible Glx largely reflects the sum of glutamate and glutamine in neuronal and astrocytic cytoplasm. Brain glutamine participates in no metabolic reactions other than those involving its initial conversion to glutamate, primarily within glutamatergic neurons (Albrecht et al. 2007). Thus, the reduced prefrontal and limbic Glx seen during major depressive episodes suggest a pathological process occurring within glutamatergic neurons or their associated glia. Normal levels of prefrontal NAA combined with MRI evidence for prefrontal volume loss suggest an impairment of glial integrity in major depression. Postmortem studies of brain tissue from patients who suffered from major depression have found consistent evidence for reduced number and/or density of glia in prefrontal and limbic regions. Two of the major functions of astrocytes are the de novo synthesis of glutamate from glucose (via the anaplerotic reaction catalyzed by pyruvate carboxylase) to replenish the glutamate–glutamine pool and the uptake and conversion of neurotransmitter glutamate to glutamine (via glutamine synthase) for recycling glutamate back to neurons (Hertz 2004). A deficit in these astrocyte-specific processes would be expected to compromise glutamatergic activity and lead to a reduction in the pool of glutamate and glutamine. Recent gene expression studies in postmortem brain tissue have found consistent evidence for a decrease in the expression of the astrocyte-specific enzyme glutamine synthase in patients with major depression (Choudary et al. 2005; Klempan et al. 2009; Sequeira et al. 2009). Similarly, expression of the glial excitatory amino acid transporters, EAAT1 and EAAT2, which are responsible for most glial glutamate uptake, has been found to be reduced in patients with major depression (Choudary et al. 2005; Miguel-Hidalgo et al. 2010) and in an animal model of depression (Zink et al. 2010). A reduced capacity of the astrocytic components of the glutamate–glutamine cycle could either cause, or be a trophic consequence of, reduced glutamatergic activity. In either case, the consistent 1H-MRS finding of low prefrontal and limbic Glx along with postmortem evidence for a loss of prefrontal glial integrity and deficits in the molecular mechanisms required for glutamate recycling support the hypothesis that glial dysfunction and dysregulation of glutamatergic function are important factors in the pathophysiology of major depression (Hercher et al. 2009; Valentine and Sanacora 2009; Yuksel and Ongur 2010). Continued MRS studies

of prefrontal and limbic glutamatergic function are likely to further advance understanding of the role of this system in the mechanisms of pathogenesis and treatment response in major depression.

3.3.3 GABA

Although the published 1H-MRS literature on GABA in major depression is not extensive, it suggests that cortical GABA is reduced during episodes of depression and normalized following successful somatic treatment. Two studies have examined occipital cortex GABA levels in drug-free depressed patients, and both observed decreased GABA levels (Sanacora et al. 1999; Sanacora et al. 2004). A subsequent study examined dorsal and ventral regions of the prefrontal cortex in drug-free depressed patients, and found decreased GABA only in the dorsal prefrontal voxel (Hasler et al. 2007). A recent study of occipital and anterior cingulate cortical GABA in treatment-resistant MDD, non-treatment resistant MDD, and control subjects found reduced GABA only in the treatment-resistant patients (Price et al. 2009). One study examined GABA levels only in frontal white matter in drug-free elderly depressed patients (ages 61–91), but found no difference between the patient and control groups (Binesh et al. 2004). Four studies have examined the effect of treatment on cortical GABA in patients with major depression. Two studies found that SSRI's increased occipital GABA (Sanacora et al. 2002; Bhagwagar et al. 2004) and one found that electroconvulsive therapy increased occipital GABA (Sanacora et al. 2003). In contrast, depressed patients showed a trend toward decreased occipital GABA following effective treatment with cognitive-behavioral therapy (Sanacora et al. 2006). In unmedicated, remitted patients, one study noted a normal level of GABA in the prefrontal cortex (Hasler et al. 2005) and one study found significantly reduced GABA in the occipital cortex (Bhagwagar et al. 2007) compared to healthy controls. In general, the evidence suggests that cortical GABA is reduced during episodes of major depression and that effective somatic treatment of depression is associated with a normalization of cortical GABA. This pattern of 1H-MRS findings is congruent with evidence from postmortem studies showing reduced size and density of calbindin-positive, GABAergic interneurons (Rajkowska et al. 2007) and reduced levels of GAD67 (Karolewicz et al. 2010) in prefrontal cortex, as well as reduced density of calbindin-positive, GABAergic interneurons in occipital cortex (Maciag et al. 2010) from patients with major depression. Given the evidence for glial dysfunction in major depression, it is important to note that GABA recycling and metabolism rely on the functional integrity of astrocytes, although to a lesser extent than glutamate recycling, This promising literature suggests that dysfunction of GABAergic systems may have an important role in the pathophysiology of major depression. If this work is substantiated and extended by further research, it may provide a translational rationale for studies of treatments targeting GABAergic systems.

3.3.4 Other Metabolites

Although not quite as consistent a finding as in bipolar disorder, a number of studies have observed elevated choline-containing compounds in the basal ganglia of patients with major depression (Yildiz-Yesiloglu and Ankerst 2006b). In light of the basal ganglia volume loss observed in major depression, choline elevation suggests increased membrane metabolism is occurring in this region. It is unclear to what extent this effect is influenced by medication use. Of three 31P-MRS studies of patients with major depression, two have found evidence for reduced ATP levels in the basal ganglia of unmedicated patients (Moore et al. 1997) and in the frontal lobes of medicated patients (Volz et al. 1998). A third study found no evidence for a group difference in ATP levels in medicated depressed patients and control subjects (Iosifescu et al. 2008). If consistent, low ATP levels would suggest a brain bioenergetic deficit is present in untreated major depression. There is no consistent evidence for alterations in brain creatine or myo-inositol in major depression (Yildiz-Yesiloglu and Ankerst 2006b).

3.4 Anxiety Disorders

The anxiety disorders that have been investigated by MRS experiments include panic disorder, posttraumatic stress disorder (PTSD), obsessive–compulsive disorder (OCD), social phobia, and generalized anxiety disorder. Of these, OCD, PTSD, and panic disorder have been the most extensively studied, and some consistent findings with translational implications have emerged from MRS studies of these disorders. However, none of the anxiety disorders have been studied as extensively with MRS as schizophrenia, bipolar disorder, or major depression. Brain MRI morphometry studies of patients with anxiety disorders have often grouped together patients with different anxiety disorders. Across anxiety disorders, the most consistent morphometric finding has been reduced gray matter volume in the anterior cingulate cortex and dorsomedial prefrontal cortex (Radua et al. 2010; van Tol et al. 2010).

3.4.1 Panic Disorder

Panic disorder is a condition characterized by the repeated occurrence of panic attacks, at least some of which are spontaneous (unprovoked). Panic disorder is often accompanied by agoraphobia—the fear and avoidance of situations that would be difficult to escape from or in which it would be difficult to get help in case of sudden incapacitation. There is strong evidence that the vulnerability to panic disorder is partly genetic, with heritability estimated to be about 48% (Hettema et al. 2001). In addition to the gray matter reduction in medial prefrontal regions seen across anxiety disorders, replicated brain morphometric findings in panic disorder include reduced volume of lateral and medial temporal lobe regions

(Ferrari et al. 2008) and increased gray matter volume of the midbrain and pons (Protopopescu et al. 2006; Uchida et al. 2008). We could find no published studies of postmortem brain tissue from patients with panic disorder. Neurobiological models of panic disorder often propose a role for increased reactivity of amygdala, hypothalamic, midbrain, or brainstem regions in the generation of panic attacks and a role for reduced function of orbital and medial prefrontal regions in the relative inability to regulate the anxiety originating in lower regions (Coplan and Lydiard 1998; Gorman et al. 2000). Patients with panic disorder are unusually sensitive to the panic-inducing effects of agents that increase brain acidity or respiratory drive, including CO_2 inhalation and intravenous sodium lactate infusion (Esquivel et al. 2010). Several models have specifically posited an important role for increased reactivity of acid-sensitive chemoreceptor systems in subcortical and brainstem nuclei in the generation of panic attacks (Klein 1993; Coplan and Lydiard 1998; Maddock 2001; Ziemann et al. 2009; Esquivel et al. 2010).

The most consistently replicated MRS finding in studies of patients with panic disorder has been an elevated brain lactate response to metabolic challenges. Prior to the first MRS studies in panic disorder patients, several investigators had demonstrated exaggerated systemic lactate responses to metabolic challenges in panic disorder (Maddock 2001). Dager and colleagues were the first to use 1H-MRS to examine brain lactate responses in panic disorder. In a series of studies examining the brain lactate response during an intravenous lactate infusion, the panic patients were consistently observed to have significantly greater increases in brain lactate in spite of receiving the same dose of intravenous sodium lactate. This effect was observed in both symptomatic, unmedicated patients (Dager et al. 1997, 1999), and asymptomatic medicated patients (Dager et al. 1997; Layton et al. 2001). While several of these studies examined a single voxel placed in the insular cortex, one study used the PEPSI sequence (discussed in Supplement Sect. 4.3.3) to obtain a 2D multivoxel axial slab of spectral data and concluded that the exaggerated increase in lactate in the panic patients was generalized across all brain regions studied (Dager et al. 1999). Hyperventilation is a metabolic challenge that leads to increases in brain lactate in normal volunteers. Panic patients demonstrate a significantly greater brain lactate response to hyperventilation than healthy comparison subjects, despite similar degrees of hypocapnia in the two groups (Dager et al. 1995). It was initially suggested that these findings of elevated brain lactate may have resulted from ischemic cerebral hypoxia due to excessive vasoconstriction triggered by the metabolic disturbance and anxiety induced by the lactate infusion and hyperventilation procedures. However, more recent studies have demonstrated significantly increased brain lactate responses in the visual cortex during visual stimulation in patients with panic disorder, a paradigm in which hyperemia, rather than ischemic vasoconstriction, is known to occur. Maddock and colleagues demonstrated significantly greater increases in visual cortex lactate during a 10 min period of viewing a flashing checkerboard pattern in a group of symptomatic, unmedicated panic patients compared to matched control subjects (Maddock et al. 2009). The visual stimulation procedure did not provoke more anxiety in the patient group than the control group. A second study showed

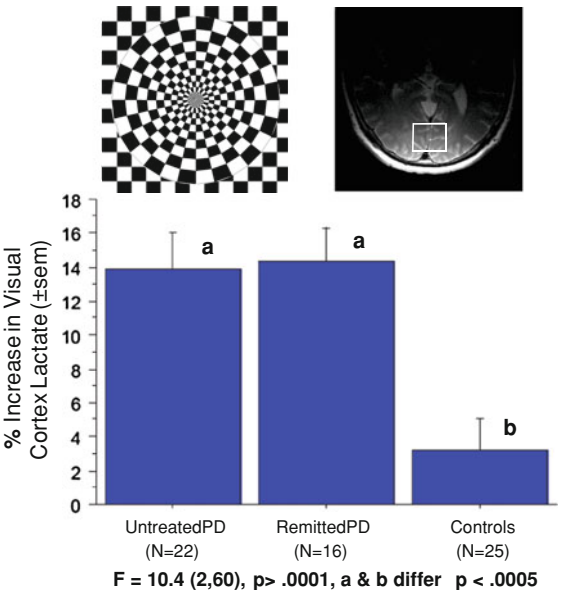


Fig. 4 An 8 Hz pattern reversal checkerboard stimulus was used to stimulate visual cortex lactate production in 22 symptomatic, untreated panic disorder patients, 16 remitted panic disorder patients, and 25 healthy comparison subjects. Percent change in lactate/creatine ratio averaged across 10 min of visual stimulation and 12 min of post-stimulation eyes-closed rest was calculated relative to a pre-stimulation eyes-closed resting baseline. Lactate accumulation was significantly greater in both patient groups compared to control subjects

that remitted panic patients (medicated and unmedicated) demonstrated the same significantly exaggerated visual cortex lactate response to visual stimulation (Maddock and Buonocore 2010). Figure 4 summarizes the visual cortex lactate responses in 22 symptomatic panic patients, 16 remitted panic patients, and 25 matched control subjects. Increased visual cortex lactate accumulation during visual stimulation in panic patients suggests that this metabolic abnormality is evident even during ordinary neural activity. The observation that exaggerated brain lactate responses are seen in both symptomatic and remitted panic patients suggests that it is an enduring or "trait" feature of the disorder and is consistent with metabolic models of the vulnerability to panic disorder.

As discussed in Sect. 2.5, glutamatergic neurotransmission triggers the glycolytic production of lactate from glucose and glycogen, most likely by astrocytes. The lactate is subsequently taken up by neurons for oxidative metabolism. A family of monocarboxylate transporters (MCTs) mediates the co-transport of lactate and hydrogen ions (H+) across glial and neuronal cell membranes. MCT-1 and MCT-4 are expressed in astrocytes, while MCT-2 is the primary form expressed in neurons (Pierre and Pellerin 2005; Bergersen 2007; Hashimoto et al. 2008). MCT-2 has a higher affinity for lactate (Km $\sim$ 0.7 mM) than MCT-1 (Km $\sim$ 4-6 mM) or MCT-4 (Km $\sim$ 32 mM) (reviewed in (Erlichman et al. 2008). When astrocytic production of lactate is stimulated, the relative affinities of these cell-specific subtypes of MCTs favors the rapid movement of lactate and H+ out of astrocytes into the ECF and then more slowly into neurons (Bergersen 2007; Erlichman et al. 2008; Hashimoto et al. 2008). Thus, lactate and H+ accumulate temporarily in the ECF of the synaptic zone, with the magnitude and duration of

the pH change determined by the amount of lactate transported and the buffering characteristics of the ECF. Acid-sensing ion channels (ASICs) respond to ECF pH changes associated with neural activity and are widely distributed in brainstem and hypothalamic regions and in the amygdala (Coryell et al. 2007). ASICs have been demonstrated to mediate fear responses in mice, including the fear response to CO_2 inhalation (Ziemann et al. 2009). Similarly, acid-sensing chemoreceptor systems in the brainstem have been shown to increase their activity in response to increased lactate accumulation (Erlichman et al. 2008). If increased accumulation of lactate during neural activation in panic disorder patients occurs in brain regions mediating fear and arousal responses and is accompanied by a temporary acidification of brain ECF, then the resulting stimulation of acid-sensing chemoreceptor systems might have an important role in triggering "spontaneous" panic attacks, as posited in some models (Klein 1993; Esquivel et al. 2010). In this regard, it is of interest that a recent 31P-MRS study examined the pH-related resonance shift of inorganic phosphate during hyperventilation and found suggestive evidence for altered acid–base regulation in the direction of increased brain acidity in patients with panic disorder (Friedman et al. 2006). 1H-MRS and 31P-MRS are likely to have an important role in future studies testing models of metabolic and acid/base mechanisms in the pathophysiology of panic disorder.

Brain GABA levels have been studied in two samples of unmedicated patients with panic disorder using validated GABA-editing 1H-MRS methods. In the first study, Goddard and colleagues demonstrated significantly lower GABA concentrations in the occipital cortex in panic patients (Goddard et al. 2001). In the second study, Hasler and colleagues found no difference in GABA levels in dorsal prefrontal or ventrolateral prefrontal regions (Hasler et al. 2009). In an extension of their original study and using the same patients, Goddard and colleagues reported that occipital cortex GABA in panic patients did not change following an acute oral dose of clonazepam, while GABA levels decreased significantly in the control group (Goddard et al. 2004). Some pharmacodynamic and PET studies have implicated reduced sensitivity of the GABA-A linked benzodiazepine receptor system in patients with panic disorder (Hasler et al. 2008). However, this abnormality may not involve a reduced concentration of cytoplasmic GABA, as measured by 1H-MRS. Future studies will be needed to establish whether and in which brain regions reduced GABA is a consistent finding in patients with panic disorder.

3.4.2 Post-Traumatic Stress Disorder

Post-traumatic stress disorder (PTSD) is a condition that develops in some individuals following exposure to a traumatic event that threatens a person's life or personal integrity. It is characterized by specific symptom patterns, including intrusive re-experiencing of the event, emotional blunting or avoidance, and generalized hyperarousal. In addition to the bilateral reduction in gray matter volume in medial prefrontal regions observed in common with other anxiety disorders, patients with PTSD also consistently demonstrate reduced volume of the hippocampus compared

to both trauma exposed controls without PTSD and healthy controls (Karl et al. 2006). Based on existing evidence, it appears that antidepressant medication ameliorates the reduction in hippocampal volume in PTSD patients compared to trauma exposed control subjects (Karl et al. 2006). Consistent volume reduction is also seen in the left amygdala in adults and in the corpus callosum in children with PTSD (Karl et al. 2006). Very few postmortem brain studies have been conducted in patients with PTSD, and none have examined hippocampal or amygdala tissue. However, studies showing dysregulation of the hippocampal–hypothalamic–pituitary–adrenal axis and impairments in declarative memory, along with brain volumetric studies, support the basic hypothesis that impairment in hippocampal function has a key role in the pathophysiology of PTSD (Bremner 2006). Functional imaging and lesion studies also support central roles for the amygdala and medial prefrontal cortex in PTSD (Etkin and Wager 2007; Koenigs et al. 2008; Liberzon and Sripada 2008).

In agreement with the results of other neurobiological studies, the most consistent 1H-MRS finding in patients with PTSD has been a reduction in NAA levels in the hippocampus. This effect has been reported as significant in nine of the 10 published 1H-MRS studies that have investigated the hippocampus in patients with PTSD (Schuff et al. 2008; Trzesniak et al. 2008). A recent 1H-MRS study in a mouse model of PTSD found that low NAA in the left dorsal hippocampus prior to electrical footshock trauma predicted the development of persistent PTSD-like symptoms (Siegmund et al. 2009). It is not yet clear whether antidepressant treatment influences hippocampal NAA in PTSD patients. A consistent finding of reduced NAA in the anterior cingulate cortex has also been observed in PTSD patients. This effect has been reported as significant in 4 of the 5 published PTSD studies that have investigated the anterior cingulate cortex (Schuff et al. 2008; Trzesniak et al. 2008). An episode of single, prolonged stress in a rat model of PTSD was recently shown to cause a reduction of glutamate and glutamine in medial prefrontal cortex (Knox et al. 2010). Overall, the 1H-MRS studies of patients with PTSD provide strong support for models of pathogenesis in which dysfunction of the hippocampus and anterior cingulate cortex play central roles. Notable gaps in the current literature include the absence of postmortem tissue studies of the hippocampus, amygdala, or medial prefrontal cortex in PTSD and no 1H-MRS studies of Glx or GABA in any brain regions in PTSD. Because of the unambiguous role of trauma in the pathogenesis of PTSD, it is a psychiatric disorder for which the use of animal models may be particularly fruitful. MRS studies in animals may have an increasingly valuable role in advancing our understanding of PTSD.

3.4.3 Obsessive Compulsive Disorder

Obsessive compulsive disorder (OCD) is a condition characterized by the persistent recurrence of obsessions (intrusive, unwanted thoughts, or images), compulsions (ritualized, repetitive behaviors), or both. The vulnerability to OCD is strongly genetic (Pauls 2010). In addition to bilateral gray matter volume reduction in the medial prefrontal and anterior cingulate cortices, as seen in other anxiety

disorders, patients with OCD also show decreased volume of the orbital frontal cortex and increased volume of the thalami and basal ganglia (lenticular and caudate nuclei) bilaterally (Rotge et al. 2009; Radua et al. 2010). Many of these morphometric findings appear to be independent of the use of antidepressant medications (Radua and Mataix-Cols 2009). Neuroimaging and neurosurgical studies support a general model of involvement of prefrontal cortex–basal ganglia–thalamic–prefrontal cortex circuits in the pathogenesis of OCD (Huey et al. 2008).

Although over 20 1H-MRS studies of pediatric and adult patients with OCD have been published, only a few findings have been consistently replicated. Four studies have demonstrated reduced NAA in the anterior cingulate cortex in adult patients with OCD (Yucel et al. 2007; Trzesniak et al. 2008). One of these studies showed that anterior cingulate NAA normalized after 12 weeks of treatment with citalopram (Jang et al. 2006). However, a recent study reported that NAA levels are increased in this region in OCD (Fan et al. 2010). A relatively large series ($N = 27$) of pediatric OCD patients demonstrated increased choline-containing compounds in the medial thalamus (Smith et al. 2003). A small group of adult SSRI non-responders with OCD showed increased thalamic choline compared to responders (Mohamed et al. 2007). Consistent 1H-MRS abnormalities have not been reported in the basal ganglia in OCD (Trzesniak et al. 2008). Further study will be needed to establish whether abnormalities in MRS-measurable brain metabolites are consistently observed in specific brain regions in OCD patients.

3.5 Summary

This review of the brain MRS literature highlights a number of frequently replicated findings in patients with psychiatric disorders. Some of these consistent findings are convergent with other neurobiological observations. For example, NAA is reduced in many but not all brain regions in patients with schizophrenia, in frontal and hippocampal regions in patients with bipolar disorder, in the hippocampus in patients with PTSD, and in the anterior cingulate cortex in patients with OCD. In each disorder, the reduction in NAA is congruent with evidence for reduced brain volume in similar regions. While irreversible neuronal damage is an important cause of reduced NAA, consistent evidence indicates that reversible reductions in neuronal function can also lead to reduced NAA. Serial 1H-MRS measures of NAA may have value in discerning whether or not specific interventions have remediating effects on an underlying, reversible neuronal dysfunction in psychiatric disorders. Preliminary evidence suggests that this may be the case for the effect of cognitive-behavioral treatment on the anterior cingulate cortex (Premkumar et al. 2010) and exercise on the hippocampus (Pajonk et al. 2010) in schizophrenia, lithium treatment on many brain regions in bipolar disorder (Moore et al. 2000), and SSRI treatment on anterior cingulate cortex in OCD (Jang et al. 2006). However, larger controlled longitudinal studies will be needed to confirm these preliminary findings.

Some of the MRS findings reviewed here provide support for specific models of pathogenesis. Elevated Glx in patients with bipolar disorder and reduced Glx in patients with unipolar major depression accord with models of increased and decreased glutamatergic function, respectively, in those conditions. Reduced phosphomonoesters and intracellular pH in euthymic bipolar patients and elevated dynamic lactate responses in panic disorder patients are consistent with metabolic models of pathogenesis in those conditions. Preliminary findings of an increased glutamine/glutamate ratio and decreased GABA in patients with schizophrenia are consistent with a model of NMDA hypofunction in that disorder. Additional studies are needed to fill in important gaps in this literature. As the sensitivity and specificity of methods continue to improve, MRS studies can be expected to play an important role in the testing of translational models of the pathogenesis of psychiatric disorders.

4 Conclusions

MRS provides a unique, non-invasive method for assessing the metabolic state of the living human brain. Steady growth of the technical capabilities of MRS systems is increasing the range of metabolites that can be measured and the sensitivity and reliability of these measurements. A growing understanding of the pathophysiological significance of abnormalities of the observable metabolite signals, especially with regard to those arising from amino acid neurotransmitter pools, is increasing the value of MRS experiments in neuropsychiatric research. The information gained from MRS studies can be used in conjunction with other non-invasive clinical imaging methods, neuropathological studies, and animal studies to achieve more complete understandings of the natural history of psychiatric illnesses and to test translational models of their pathogenesis. In addition, MRS has the potential to increase understanding of the therapeutic mechanisms of action of effective treatments and to allow clinical monitoring of the neurobiological effects of interventions on brain metabolic markers of psychiatric illnesses.

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fMRI as a Measure of Cognition Related Brain Circuitry in Schizophrenia

Laura A. Libby and J. Daniel Ragland

Abstract Functional magnetic resonance imaging (fMRI) has played a prominent role in the quest to identify the brain systems responsible for cognitive dysfunction in schizophrenia. This chapter describes the evolution of these research efforts, which have alternated between efforts to localize specific cognitive impairments to work trying to understand broader network dysfunction. After a concise summary of localization efforts, the remainder of the chapter describes how different groups of scientists have developed and tested broader network theories. This includes a description of both task-activation and resting state studies, and involves a wide array of analytic techniques. The chapter closes with an understanding of how current default-mode and task-positive network theories grew out of these earlier resting-state and task-activation approaches, and provides some recommendations about future directions.

Keywords Functional neuroimaging · Human brain mapping · Brain physiology · Psychosis · Cognitive neuroscience

Contents

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1 Introduction

From the beginning of functional imaging research on cognition in schizophrenia, scientists have oscillated between functional localization approaches designed to map the pathophysiology of schizophrenia to discrete brain regions and cognitive functions, and functional integration approaches attempting to demonstrate how a broader breakdown in communication between multiple brain regions may explain disordered cognition in schizophrenia. Fortunately, these two worlds often collide—providing us with a more nuanced understanding of how information processing goes awry in the brains of individuals diagnosed with schizophrenia. The goal of this chapter is to describe how studies examining group differences in broader network activity (e.g., functional connectivity) grew out of initial studies designed to functionally segregate distinct areas of task-related activation. In so doing, we will briefly touch upon what has been learned from focal activation studies, including references to recent quantitative meta-analyses. However, these focal activation studies have been extensively reviewed (Ragland et al. 2007; Brown and Thompson 2010; Gur and Gur 2010), and the primary focus of this chapter will be on functional integration approaches—beginning with functional connectivity analyses using regions of interest (ROI) during active task conditions, moving to multivariate approaches aimed at examining resting default mode network (DMN) and activated task positive network (TPN) states, and ending with a discussion of more recent approaches employing multi-modal functional and anatomical imaging techniques.

2 Functional Localization

Functional imaging of individuals with schizophrenia has traced an interesting circle, from resting state, to "activation" studies employing task paradigms, and back again to resting studies. The first functional imaging study to localize pathology in schizophrenia was performed by Ingvar and Franzen (1974) who used ^{133}Xenon blood flow (rCBF) during resting state to demonstrate that, compared to healthy participants, individuals with schizophrenia had relatively lower flows in the frontal cortex (i.e., "hypofrontality"), and higher flows in occipito-temporal regions. However, this resting hypofrontality was not always replicated (Gur et al. 1987a, b), leading to a refinement of the model, stressing that, "hypofrontality appears to be dependent on the behavioral state of the patients during the brain imaging experiment" (Weinberger et al. 1991, p. 276). This refinement emphasized the importance of using the subtraction technique to contrast activity during a task state with activity during a baseline condition designed to constrain what subjects were thinking about, and isolate brain regions responsible for the specific function that the task was designed to measure. The power of this approach had previously been demonstrated by Peterson et al., in a word fluency study (Posner et al. 1988), and was widely embraced by the schizophrenia research community.

Since this transition from initial resting state studies, activation paradigms have measured the impact of schizophrenia on regional brain function during a wide array of cognitive, sensory, and emotional processing tasks, leading to over 800 references in a recent PubMed search. These studies initially struggled with group performance differences that confounded interpretation of physiological differences. This difficulty was largely overcome with the development of event-related designs (Zarahn et al. 1997), which allowed one to follow recommendations (Carter et al. 2008) to restrict analysis to correct only trials. The difficulty of reviewing such a vast literature was somewhat simplified with the development of a quantitative meta-analytic technique termed Activation Likelihood Estimation (Turkeltaub et al. 2002; Laird et al. 2005). ALE measures the probability that spatially smoothed activation foci from individual studies occur across multiple studies, and tests the null hypothesis that the location of these activated foci is equal at every voxel, against an alternative hypothesis that activated foci are spatially distributed. To date, we are aware of six studies that have used ALE to perform meta-analyses of functional imaging studies of schizophrenia.

The first three ALE analyses examined working and episodic memory studies, respectively. In the first analysis, Glahn et al. (2005) examined working memory studies employing the N-Back task, and found predicted reductions in dorsolateral prefrontal cortex (DLPFC) activity in patients during task performance. However, patient reductions were not restricted to the DLPFC, extending to more rostral and ventrolateral/insular portions of the prefrontal cortex (PFC). Moreover, there was evidence of patient "overactivation" in the frontal pole, dorsomedial prefrontal cortex, and anterior cingulate gyrus, leading the authors to conclude that working memory dysfunction in schizophrenia cannot be classified as a simple DLPFC deficit, but should be considered in the context of the larger network of activity engaged by the task. Subsequent analyses of episodic memory encoding and retrieval studies also showed areas of both under- and over-activation in patients across multiple brain regions (see Fig. 1). Reduced activation in patients was observed in multiple areas of the PFC including dorsolateral and ventrolateral prefrontal cortex (VLPFC) (Achim and Lepage 2005; Ragland et al. 2009), and in non-prefrontal regions including the medial temporal lobe (Achim and Lepage 2005), thalamus (Ragland et al. 2009) and posterior cingulate gyrus (Achim and Lepage 2005; Ragland et al. 2009). Both analyses found that patients abnormally increased parahippocampal activation during episodic retrieval, and the study by Ragland et al. (2009) found that group differences in VLPFC activity were not present in studies that provided subjects with encoding instructions—thereby reducing strategic memory demands.

The three remaining ALE studies also found a distributed pattern of group differences. In an examination of a variety of executive function tasks Minzenberg et al. (2009) and Li et al. (2010) found patient reductions in the DLPFC, posterior cingulate gyrus, rostral and dorsal anterior cingulate gyrus and thalamus, and patient increases in pre-supplementary motor area and inferior parietal cortex. A study of facial emotion processing (Li et al. 2010) found patient over-activation in the insula, and patient underactivation in the amygdala, parahippocampal gyrus, fusiform gyrus, superior frontal gyrus, and lentiform nucleus. Finally, analysis of

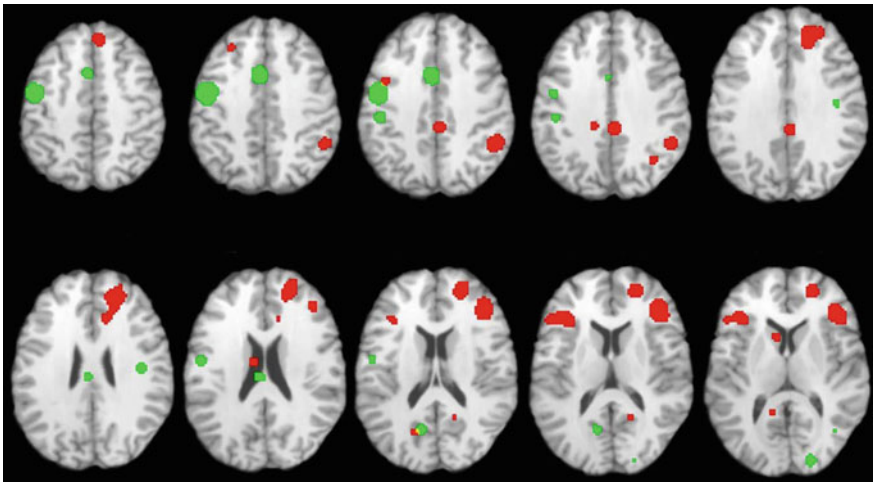

Fig. 1 Illustration of group differences in fMRI activation during memory encoding in healthy participants and patients with schizophrenia based upon an activation likelihood estimation (ALE) meta-analysis of seven previously published studies. Areas of greater activation in healthy participants versus patients are indicated in red, and areas of greater activation in patients versus healthy participants are indicated in green. Image is adapted from original figure published in Ragland et al. (2009)

three studies of time perception (Ortuño et al. 2011), found that patients had reduced activation in middle and superior frontal gyrus, precentral gyrus, lentiform nucleus, thalamus, precuneus, and anterior cingulate, and did not show any areas of atypically increased activation.

As can be appreciated from this review of ALE studies, functional imaging research of schizophrenia using task paradigms and univariate statistical approaches has failed to reveal a "smoking gun" in which dysfunction of a single brain region can fully explain group differences in task performance. This should probably not come as a surprise as the behaviors most commonly impacted by and studied in schizophrenia (e.g., attention, memory, executive control) are multi-factorial—requiring integration of multiple brain regions. Moreover, even if a functional deficit is secondary to impairment of a single brain region, such a focal lesion would likely produce downstream effects on other brain areas, making it difficult to isolate group differences to a single location. There have been two primary responses to the challenges of functional localization. One has been to try to improve localization procedures by translating cognitive neuroscience paradigms designed to identify specific neural mechanisms into clinical studies that can more precisely map brain-behavior relationships. This approach holds great promise and is being developed for clinical trials research to discover new cognitive-enhancing agents, and is being facilitated by the NIH sponsored CNTRICS initiative begun in 2007 (Carter and Barch 2007). The second approach, and the focus of the remainder of this chapter, has been to employ multivariate statistical approaches designed to characterize the impact of schizophrenia on broader network activity.

3 Early Network Theories

One year after Ingvar and Franzen (1974) posited their "hypofrontality" hypothesis, they speculated that their results could be explained by disrupted transmission in a mediothalamic-frontocortical projection system (Franzén and Ingvar 1975) rather than disruption in a single brain region. The notion of disrupted integration of brain activity in schizophrenia was not formally tested until almost 10 years later when Clark et al. (1984) calculated Pearson correlations in positron emission tomography (PET) glucose metabolism between specified ROIs during electrical stimulation (mild shock) versus resting baseline conditions. In a descriptive comparison of patient and control groups, they found that the strong frontal coupling observed in healthy volunteers appeared reduced in the patient sample. Patients also appeared to show reduced anterior–posterior coupling in the left hemisphere and reduced bilateral coupling within anterior brain regions. Group differences in PET regional metabolic correlations were more rigorously tested several years later by Volkow et al. (1988) who employed resting and smooth pursuit eye movement conditions, and tested whether the overall pattern of regional correlations differed between patients and controls using the Kullback procedure. This analysis revealed that patients had a global decrease in the number of significant correlations, which was descriptively characterized as reduced anterior–posterior and cortico-thalamic correlations.

These initial correlational results, combined with clinical speculations that hallucinations and delusions might result from abnormal integration between different brain regions, led Friston and Frith (1995) to propose that schizophrenia may best be characterized as a disconnection syndrome. In this word production study, investigators adapted methodology developed in electrophysiological research to create a measure of functional connectivity defined as, "the temporal correlation between spatially remote neurophysiological events." (Friston and Frith 1995). By using a stepwise regression-like procedure to identify patterns of functional connectivity between all of the voxels in the brain (i.e., eigenimages) the investigators found that the majority of the covariance in the time-series was captured by a negative interaction between prefrontal and superior temporal cortex in healthy controls during the word production task. In contrast, patients showed a positive correlation in fronto-temporal connectivity, leading the authors to conclude that schizophrenia is characterized by a reversal in typical prefrontal and temporal lobe integration. In a subsequent paper, Andreasen et al. (1996) reviewed a number of their previous PET activation studies to argue that this disconnection syndrome is not limited to the frontal and temporal lobe, and extends to a broader failure of integration between prefrontal–thalamic–cerebellar circuitry (termed "cognitive dysmetria"). The disconnection syndrome and cognitive dysmetria hypotheses provided a theoretical foundation for subsequent functional connectivity studies.

4 Task-Related Functional Connectivity

Prior to 2006 when researchers began to investigate resting-state networks, functional connectivity studies of schizophrenia examined group differences in network activity while participants performed various task activation paradigms. Several of these studies used working memory tasks, including the first study by Meyer-Lindenberg et al. (2001) who had healthy participants and patients with schizophrenia perform an N-Back working memory task and a sensorimotor baseline during PET rCBF imaging. As in the previous Friston and Frith (1995) study eigenimages were generated to characterize the variance–covariance matrix of the time-series. These eigenimages were created for a prespecified set of prefrontal, temporal, and parietal ROIs based on task activation results. The pattern that captured the most variance was negative relationships among the temporal cortex, hippocampus, and cerebellum in patients, and a positive relationship between the DLPFC and cingulate gyrus in healthy controls. This group dissociation in functional connectivity remained regardless of task condition, leading the authors to conclude that disruption of fronto-temporal interactions appears to be a trait marker of schizophrenia.

In the late 1990s the field transitioned from PET rCBF to blood oxygen level-dependent (BOLD) fMRI imaging procedures, which provided investigators with better temporal resolution, allowing implementation of new methods to examine functional connectivity. These subsequent fMRI studies examined functional connectivity with a variety of task paradigms. As a test of the disconnection syndrome hypothesis Lawrie et al. (2002) examined correlations between DLPFC and superior temporal gyrus (STG) ROIs in patients and controls while subjects performed a sentence completion task during fMRI. In support of the hypothesis of fronto-temporal disconnection, they found that left fronto-temporal correlations were reduced in schizophrenia, and showed greater reductions for patients with more severe hallucinations. The next study used a somewhat different analytical approach termed psychophysiological interaction analysis (PPI), which examined how correlations between BOLD signal activity in a "seed" region in the right anterior cingulate gyrus (ACC) and activity in the rest of the brain were modulated by task demands (Boksman et al. 2005). This was done for fMRI data collected while participants were performing a word generation task, and revealed that the right ACC showed localized interaction with the left temporal lobe associated with increased verbal fluency in healthy volunteers, that was absent in patients. Using a similar approach with fMRI data obtained during a continuous performance attention task, Honey et al. (2005) found evidence of both increased and decreased ACC connectivity in patients with schizophrenia during a degraded stimulus version of an attentional continuous performance task. When functional connectivity was examined in relation to degraded versus non-degraded stimulus conditions, patient decreases were seen in ACC connectivity to the cerebellum and, patient increases were seen in ACC connectivity to the prefrontal cortex. These disruptions in connectivity with cerebellar and motor areas were viewed in support

of the cognitive dysmetria hypothesis. Finally, this timeseries correlational approach was also used to examine connectivity between STG and parahippocampal gyrus seed regions in the temporal cortex with voxels in the rest of the brain during a word encoding task that manipulated levels-of-processing (Wolf et al. 2007). This analysis of fMRI data revealed that, for deep versus shallow encoding conditions, patients had reduced temporal lobe connectivity to the DLPFC, but increased connectivity to the VLPFC, suggesting that the ventrolateral portion of the PFC may play a compensatory role for patients during episodic encoding.

One of the limitations of previously described functional connectivity analyses is that they do not provide information about the directionality of functional interactions. Therefore, an additional study of the N-Back paradigm (Schlösser et al. 2003) used structural equation modeling to assess "effective connectivity" in patients and controls. Effective connectivity is defined as, "the influence one neural system exerts over another" (Friston et al. 1994), and is examined by constructing anatomical models (i.e., path models) of the directional connections between pre-specified ROIs. Goodness-of-fit statistics are used to select the path models with the smallest residual error. Based upon previous anatomical and functional studies, Schlösser et al. (2003) proposed a unidirectional pathway from parietal cortex to the DLPFC and VLPFC, reciprocal pathways between DLPFC and VLPFC, and a cortico-cerebellar feedback loop involving bi-directional connections among prefrontal cortex, thalamus, and cerebellum. Between-group comparisons of these models revealed that patients receiving typical antipsychotics had reduced prefrontal-cerebellar and cerebellar-thalamic connectivity, and enhanced thalamo-cortical path coefficients. There was also evidence of enhanced interhemispheric connectivity in a group of patients receiving second-generation antipsychotic medications, suggesting that investigators should consider medication effects when performing these studies.

In sum, these functional connectivity studies employing activation tasks and ROI or seed-based correlational approaches have fairly consistently demonstrated that connectivity of the prefrontal cortex and the anterior cingulate gyrus with the temporal cortex appears to be reduced in patients with schizophrenia, consistent with the dissconection syndrome hypothesis. However, these results are not invariant—sometimes showing increased connectivity in patients, and sometimes differing depending upon clinical state, medication status, and the regions that are selected as seeds or as ROIs. As these studies progressed, concerns were also raised about the use of task activation paradigms to examine functional connectivity. When interpreting functional connectivity results, investigators were interested in drawing conclusions about anatomical connections (i.e., areas that fire together, wire together), and one concern about use of activation tasks was that they could create task-related correlations in the time-series that may obscure trait-like differences in anatomical connectivity (Biswal et al. 1995; Lowe et al. 1998; Raichle and Gusnard 2005). A related concern was that group differences in how subjects perform these activation tasks could contribute to group differences in functional connectivity apart from true differences in brain physiology.

These concerns contributed to a directional shift back toward resting-state studies, which eliminate performance demands, and might, ostensibly, provide better trait-like measures of brain physiology.

5 Default Mode Network and Task-Positive Network

Resting-state functional connectivity analysis capitalizes on the finding that the brain appears to be intrinsically organized into functionally correlated networks that are independent of task (Fox et al. 2005; Fransson 2005; Buckner et al. 2008). Early observations in PET imaging found a consistent set of brain areas that showed higher metabolism during resting versus task conditions, particularly in posterior parietal and medial frontal areas (Gusnard and Raichle 2001; Raichle et al. 2001). Around the same time, fMRI studies revealed that BOLD signal in a similar set of brain regions fluctuated spontaneously and coherently at low frequencies (<0.1 Hz) when subjects were resting in the scanner, or simply "doing nothing" (Biswal et al. 1995; Greicius et al. 2003; Fransson 2005). This network comprises medial prefrontal, posterior parietal, and medial temporal regions—all brain areas that have been implicated as dysfunctional in schizophrenia. Moreover, signal fluctuations in this network are anti-correlated with BOLD signal in regions typically engaged by tasks, such as the lateral prefrontal cortex, suggesting a physiological tradeoff between the engagement of "task-negative" and "task-positive" brain states (Fox et al. 2005). This "task-negative" network is thought to represent a baseline brain state from which metabolic resources are diverted during cognitive demand, and has been termed the "default-mode" network (DMN; (Greicius et al. 2003)). The DMN has also been conceptualized as supporting internally directed thought, whereas task-positive networks are thought to support externally oriented cognitive processes (Buckner et al. 2008). Given previously discussed communication breakdowns between brain regions needed for cognitive tasks in schizophrenia, investigators hypothesized that group differences in functional connectivity during activation tasks could be co-occurring with, or driven by abnormal functional connectivity in the DMN. This has led to several fundamental questions about the nature of intrinsic brain networks in schizophrenia.

One question of primary interest has been whether functional connectivity differences between patients with schizophrenia and controls are detectable at rest. Liang et al. (2006) were the first to address this question, using an automated anatomical parcellation procedure to divide the brain into nine regions, and then comparing pairwise regional correlations in resting BOLD fluctuations between patients and controls. They found that resting-state correlations were reduced in patients across brain regions, with the most prominent differences noted in the insula, temporal lobe, striatum, and prefrontal cortex. Additionally, correlations between the cerebellum and all other regions were increased in patients during rest. The results from Liang et al. (2006) established that functional connectivity is

abnormal in patients during rest, and that abnormalities occur across the entire brain, and are not limited to a few regions.

Rather than parcellating the whole brain into anatomical regions, Bluhm et al. (2007) focused their analysis of resting-state functional connectivity in schizophrenia on candidate regions of the DMN. To accomplish this, they began with a seed in the posterior parietal cortex (PPC), a region consistently identified as part of the DMN, and identified correlated voxels across the brain. Then, for each voxel in this network, they compared the degree of connectivity with the PCC between the healthy and patient groups. Functional connectivity between PCC and medial prefrontal cortex, parietal cortex, and cerebellum was reduced in patients. These combined studies suggested widespread functional disintegration within the DMN in schizophrenia patients even at rest. However, it remained unclear whether DMN connectivity was also abnormal in patients during active cognitive tasks, and whether DMN abnormalities were related to dysfunction in brain areas recruited by these tasks.

Thus, a second question was whether group network differences observed during rest were also apparent during task. To address this question, investigators employed a technique called independent component analysis (ICA), which is a data-driven approach to pinpointing temporally consistent, spatially independent brain networks in fMRI data, that occur regardless of task conditions (Calhoun et al. 2009). ICA can be applied equally in resting-state and task-related fMRI, and is particularly useful for identifying the task-negative DMN even during cognitively demanding tasks. The first study to detect DMN abnormalities in schizophrenia patients during task conditions (Garrity et al. 2007) used ICA on fMRI data acquired while subjects performed an auditory oddball detection paradigm. Researchers identified the independent component that most overlapped with DMN brain regions, and then compared the temporal and spatial characteristics of this component between patients and controls. Across both groups, the DMN component showed the greatest activation decrease during presentation of the auditory target stimuli, consistent with the notion that the DMN engagement decreases with greater cognitive demand. Patients had reduced activation compared to controls in the posterior cingulate and precuneus, but increased activation in a different part of the posterior cingulate, the anterior cingulate, and in the superior medial frontal gyrus. It is important to note that these group differences represented differences in regional BOLD signal intensity in those regions, rather than differences in functional connectivity, and reflected topographical differences in the DMN between patients and controls. Similarly, in the temporal domain, patients had relatively less power in low-frequency DMN oscillations, but relatively greater power in higher frequency DMN oscillations, suggesting that DMN abnormalities in patients extended beyond regional differences in network localization. A subsequent study from the same group (Calhoun et al. 2008) established that spatial and temporal DMN differences in patients found during the oddball task were also atypical during rest, suggesting that DMN findings during rest generalize to tasks, and vice versa. However, the spatial layout of both the DMN component and a component consistent with the task-positive network (TPN) were

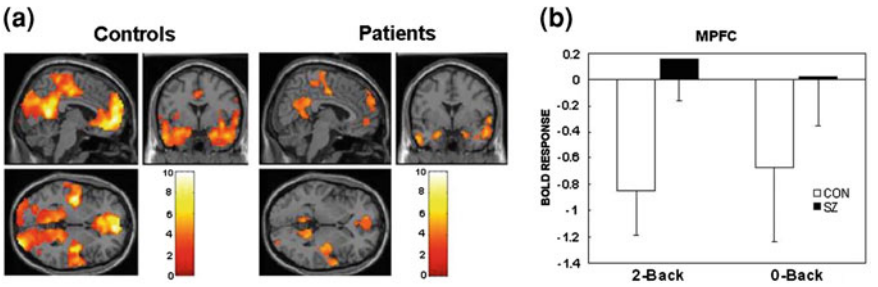

Fig. 2 Task-related suppression of default network regions is lower in patients. **a** Greater activation during rest than task (2-back working memory paradigm) in DMN regions for controls (CON) and patients (SZ). **b** Task-related suppression of MPFC (a DMN region) during 2-back and 0-back conditions. Error bars are 95% confidence intervals. Image is adapted from original Figure published in Whitfield-Gabrieli et al. (2009)

slightly different between activated and resting scans, suggesting that DMN networks were somewhat modulated by task, and that this modulation was not consistent across diagnostic groups.

Several studies have attempted to unravel the relationship between the DMN and the TPN in patients with schizophrenia, with conflicting results. Comparing resting-state networks associated with signal in PCC and DLPFC seed regions, Zhou et al. (2007) found that anti-correlations between the DMN and TPN were greater in patients than in controls. Contrary to earlier findings of reduced connectivity, the study revealed greater within-network functional connectivity in patients. However, this analysis examined only anti-correlated brain regions, and may have missed other important between-group differences. A resting-state study by the Calhoun group (Jafri et al. 2008) compared correlations between seven key independent components and found that, in patients, the DMN independent component was more temporally similar to three of the other non-DMN components including the lateral frontal cortex. This finding suggested that schizophrenia patients might not adequately suppress the DMN when task-related regions come online. Whitefield-Gabrieli et al. (2009) tested this hypothesis by examining interactions between DMN and TPN activity across periods of rest and periods of performance on a working memory task in patients and controls. They found that DMN activity decreased during task, that better task-related DMN suppression was related to better working memory performance in patients, and that this task-related DMN suppression was greater in controls than in patients (see Fig. 2). Additionally, in patients, areas of the DMN were relatively more functionally connected during task, and less anti-correlated with TPN regions during both rest and task conditions. Based on these results, investigators posited a DMN hyperconnectivity/hyperactivity hypothesis, proposing that, in patients, a state of DMN over-synchronization results in failure to divert physiological resources away from the DMN during cognitively demanding tasks.

Whitfield-Gabrieli et al. (2009) also speculated that this hyper-DMN state, in which regions putatively responsible for internally directed thinking are constantly more engaged, may be related to thought disturbance in schizophrenia. They showed that reduced task-related DMN suppression was related to higher scores on a psychopathology index in both the schizophrenia patient group and in healthy individuals. In the Bluhm et al. (2007) study, greater functional connectivity within the DMN during rest was positively associated with positive and negative symptoms in patients. Because of its relationship to both cognitive and clinical variables, it is possible that abnormally high engagement of the DMN is a state-level feature of schizophrenia that can explain both psychopathology and cognitive deficits. However, in these previous studies, patients were receiving antipsychotic medications, which may have contributed to group differences in DMN activity. Olanzapine, an atypical antipsychotic, has been shown to ameliorate clinical symptoms and potentially improve working memory performance. In a double-blind, counterbalanced study examining the effects olanzapine treatment, Sambataro et al. (2010) compared DMN functional connectivity during a working memory task when patients were on and off medication. Olanzapine dose was associated with increased functional connectivity in medial frontal areas of the DMN, but did not affect posterior regions. Although this study revealed that antipsychotic medication can mediate DMN connectivity, and may contribute to variability in functional connectivity differences across schizophrenia studies, DMN connectivity remained aberrant in the un-medicated patient group, suggesting that network-level differences in schizophrenia patients reflect underlying neuropathology regardless of medication effects.

While network connectivity analysis adds another level of complexity to our understanding of brain disruption in schizophrenia, questions remain about exactly what *biological mechanisms* drive the coherence of these functional networks. We now know that coherent signal fluctuations in intrinsic brain networks, particularly the DMN, are highly robust over time and across subjects in healthy populations (Biswal et al. 2010; Van Dijk et al. 2010), suggesting trait-like properties. Moreover, combinations of resting-state fMRI and either anatomical tracer studies in animals (Vincent et al. 2007) or white matter tractography in healthy humans (Greicius et al. 2009) show that intrinsic functional connectivity maps well—but not perfectly—with anatomical connections in the brain. That is, areas that are connected directly or indirectly by white matter tracts show correlated BOLD signal fluctuation during resting-state fMRI. If this functional–anatomical relationship holds in patients, one possible explanation for differences in intrinsic functional connectivity in schizophrenia is that the framework of anatomical connectivity that underlies a functional network is similarly atypical. Zhou et al. (2008) examined both functional and structural connectivity of the anterior hippocampus, a region important for episodic memory often implicated in schizophrenia, and found that both were reduced in patients, suggesting a relationship between functional disintegration and degraded white matter tracts. However, this functional–anatomical relationship may

be less tightly coupled in patients than in controls. Skudlarski et al. (2010) directly compared diffusion tensor-based tractography and resting-state functional connectivity, and found that, while certain brain regions showed either increased or decreased intrinsic functional connectivity in patients versus controls, anatomical connectivity was generally degraded regardless of brain region in the patient sample. Furthermore, while functional networks reflected anatomical connectivity moderately well in controls, functional connectivity between any two brain regions was less predictive of the anatomical connections of those regions in patients. A key assumption of functional connectivity analysis is that network coherence is an indirect measure of anatomical connections. These most recent results challenge whether this assumption can be applied to schizophrenia populations.

6 Conclusions

Has the addition of functional connectivity analysis improved our understanding of pathophysiology in patients with schizophrenia? This research has clearly established that task-related and intrinsic functional connectivity are dysfunctional in patients, but studies do not agree on the exact nature of these disruptions. Task-based and default-mode networks are inversely related in healthy subjects, and this relation appears disrupted by schizophrenia. Patients generally fail to disengage DMN during task conditions, but it is not clear whether this impairment is driven by failure of task-related regions such as the lateral prefrontal cortex to deactivate DMN activity, or by hyperconnectivity within the DMN that makes it more resistant to task-related inhibition (Whitfield-Gabrieli et al. 2009). An added challenge of resting-state versus task activation studies is that resting-state studies do not employ experimental manipulations to test specific hypotheses, thereby reducing the interpretative leverage of the resting-state approach. Regardless of the answer to this question, this current state of affairs suggests caution in interpreting group differences in activation studies that use resting baselines as reference conditions, since these differences can be driven either by the task or by the reference condition. This work has clearly demonstrated that we can no longer think of schizophrenia as an impairment of a single brain region, and that we need to continue to work toward an understanding of group differences at a network level. This progress will likely depend upon the convergence of multiple approaches, including use of carefully controlled cognitive neuroscience tasks to more precisely map brain and behavior interactions, and use of multi-modal imaging techniques (structural and functional) to provide a convergent understanding of the DMN in schizophrenia.

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MRI Studies in Late-Life Mood Disorders

Carmen Andreescu and Howard Aizenstein

Abstract There are well-established patterns of structural brain changes associated with aging. The change in brain volume with age and with the diseases of aging presents a particular challenge for MRI studies in the elderly. Structural MRI is important for studies in normal aging, late-life depression, dementia, Alzheimer disease and other cognitive disorders to examine how age-associated changes in neuroanatomy are associated with specific age-related changes in brain function. Functional MRI has been a major advance for the fields of cognitive and affective neuroscience by allowing investigators to test theories of the underlying neural pathways controlling cognitive and emotional processes. In this chapter, we will review the contribution of MRI studies to late-life mood and anxiety disorders: major depression, bipolar disorder and anxiety disorders in late-life.

Keywords Structural MRI · Age-related brain changes · Functional MRI · Methodological challenges in late-life MRI · Late-life mood disorders

Contents

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Over the last decade there has been a rapid increase in the availability of MR imaging. It is likely that the increase in accessibility, as well as the decrease in scanning costs, will continue to increase the use of MRI. Neuroimaging may offer not only insights into the neurobiology of late-life mental disorders, but may also contribute to the effort of personalizing existing treatments and discover new, more efficacious ones. In this chapter, we will review the contribution of MRI studies to late-life mood and anxiety disorders: major depression, bipolar disorder, and anxiety disorders in late-life.

1 Methodologic Challenges of MRI in Late-Life

1.1 The Influence of Brain Morphometric Changes on fMRI

There are well-established patterns of structural brain changes associated with aging. With increasing age, the brain decreases in overall volume, the cortical gyri become smaller, and the sulci and ventricles become larger. These changes in brain volume vary across individuals and occur even in individuals who are otherwise apparently healthy. The changes have been described in a number of studies (e.g., Raz et al. 1997; Resnick et al. 2000) and seem to vary across the brain with most prominent decrease in volume reported in the frontal cortex.

The change in brain volume with age and with the diseases of aging presents a particular challenge for functional MRI studies in these populations: how should these structural changes be accounted for when comparing the functional signal? In a standard fMRI analysis plan the functional images from all the subjects in a study are lined-up with each other (alignment, cross-registration, warping, normalization). If the brains have significantly different shapes and sizes then the brain alignment may bias the results by contributing more CSF (due to the larger sulci and ventricles) of the more atrophic brains as compared to more gray matter from the less atrophic brains. The standard alignment algorithms vary in their ability to account for the variability in brain structure (Wu et al. 2006). Some investigators have addressed this problem by using a larger smoothing kernel in studies of aging subjects (e.g., 10 mm instead of standard 6 mm or 8 mm full-width half-maximum Gaussian). This approach recognizes that the alignment may be worse in the elderly population, and corrects for it by making the images blurrier. This allows the statistical voxel-wise comparison to find group differences even if there is some discrepancy in the spatial co-localization. An alternative approach that other investigators have used involves avoiding the registration

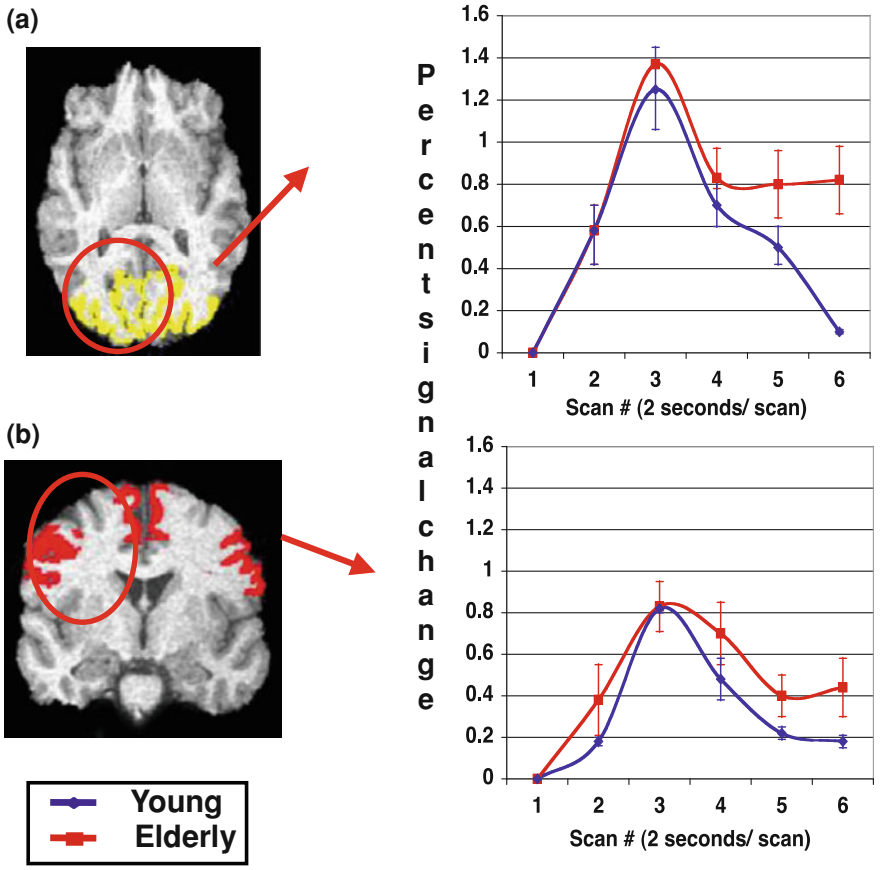

Fig. 1 Time series for **a** visual and **b** motor ROIs. *Blue line* represents mean young percent signal change from baseline; *red line* is elderly percent signal change. Error bars represent ±1 SEM

problems altogether, by focusing on a region-of-interest (ROI)-based analysis (Aizenstein et al. 2011), using ROIs defined in the acquired fMRI space, rather than relying on normalizing the images.

1.2 The BOLD Hemodynamic Response in Healthy Aging

Functional MRI depends on an intact BOLD hemodynamic response function (HRF), i.e., the cascade of neurophysiologic events that leads from neural activation to a change in the measured T2* MR signal. Aging is associated with cerebrovascular changes, so one would expect that it might also alter the BOLD signal. This is of critical importance in interpreting whether the signal identified in an fMRI study of aging reflects changes in neural activity (as is often presumed) or

whether the changes are due to the age-related changes in the coupling of the neural activity to the fMRI signal (i.e., the BOLD HRF). To examine the BOLD HRF in aging we compared healthy college-age subjects and healthy elderly control subjects while they performed a simple visual and motor task (e.g., tapping with their index finger in response to the word TAP in the center of the screen). The resulting fMRI time series (see Fig. 1) show a similar peak for both the young and the elderly subjects in both the visual and motor regions. This suggests that by focusing the analysis on the peak of the HRF the difference in signal observed on fMRI will likely reflect differences in neural activation.

2 MRI Methods for Studying Late-Life Mood Disorders

2.1 Structural Imaging

Structural MRI methods can be used to identify and quantify patterns of changes in volumetric neuroimaging studies. The various structural MRI sequences enable the identification of structural alterations such as (a) volume in gray matter, white matter, and cerebrospinal fluid from high resolution T1-weighted images (Raz et al. 2005, 1998; Rosano et al. 2005) (b) white matter hyperintensities (WMH) from FLAIR images (Gunning-Dixon and Raz 2000; Soderlund et al. 2003) (c) white matter integrity from diffusion weighted imaging (Pfefferbaum et al. 2005; Salat et al. 2005) (d) myelination from magnetization transfer imaging (van Es et al. 2006). Advanced neuroimaging sequences like diffusion spectrum imaging (DSI) and Q-ball imagings are currently being used for studying the white matter tracts (Schmahmann et al. 2007; Fig. 2).

Structural MRI is useful for studying the patterns of neuroanatomical changes in geriatric research. Structural MRI is important for studies in normal aging, late-life depression, dementia, Alzheimer disease, and other cognitive disorders to examine how age-associated changes in neuroanatomy are associated with specific age-related changes in brain function, such as the changes that may be in cognition. Structural MRI allows for identification of both macrostructural and microstructural neuropathologic changes, including atrophy, cerebrovascular changes, demyelination, and changes in membrane integrity.

2.2 BOLD Functional MRI

In the early 1990s a number of investigators showed that not only could MR be used to visualize neuroanatomy and structural pathology but, by tuning the MR contrast appropriately, MR could be used to visualize the dynamic changes in blood oxygenation across the brain; this was the beginning of functional MRI

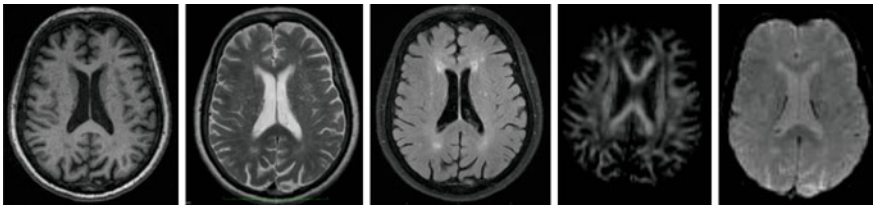

Fig. 2 T1, T2, FLAIR, DTI, and T2* images from 3T scanner

(Schmahmann et al. 2007). Over the subsequent years, a number of studies have shown that this Blood Oxygenation Level Dependent (BOLD) signal could be used to map brain activity on a variety of cognitive and affective tasks.

Functional MRI has been a major advance for the fields of cognitive and affective neuroscience by allowing investigators to test theories of the underlying neural pathways controlling cognitive and emotional processes. This approach is often referred to as 'human brain mapping.' In addition to studying 'normal' human brain function, fMRI can also be used to characterize the functional activation patterns in patient groups. This area of clinical fMRI research has recently led to a number of new insights into the nature of psychopathology and treatment—including the description of a dorsal versus ventral processing imbalance in depression (Phillips et al. 2003), overlap in response patterns with placebo and with medication (Mayberg et al. 2005), and paradoxical nonlinear activation patterns in mild cognitive impairment (Wierenga and Bondi 2007), suggesting a compensatory stage prior to the onset of dementia.

Functional MRI has utilized either resting state paradigms or activation paradigms involving various emotional or cognitive tasks.

2.2.1 Resting State

Over the last decade fMRI has been adapted to examine the connectivity of the Default-Mode Network, an organized functional network of several brain regions active during resting state and inhibited during the performance of active tasks (Raichle et al. 2001). Analysis of resting state activity may enhance the understanding of the biological underpinning of mental illnesses pathophysiology. A primary component of the resting-state network, is the default-mode network, a functionally connected network, which includes as core nodes the posterior cingulate cortex and the medial frontal cortex. Activity of the default-mode network, is believed to reflect self-referential thought, that is suppressed with goal-directed task activity. Activity in the default-mode network is affected in Alzheimer's disease, Major Depressive Disorder (Greicius et al. 2007; Sheline et al. 2009) and anxiety disorders (Zhao et al. 2007; Fig. 3).

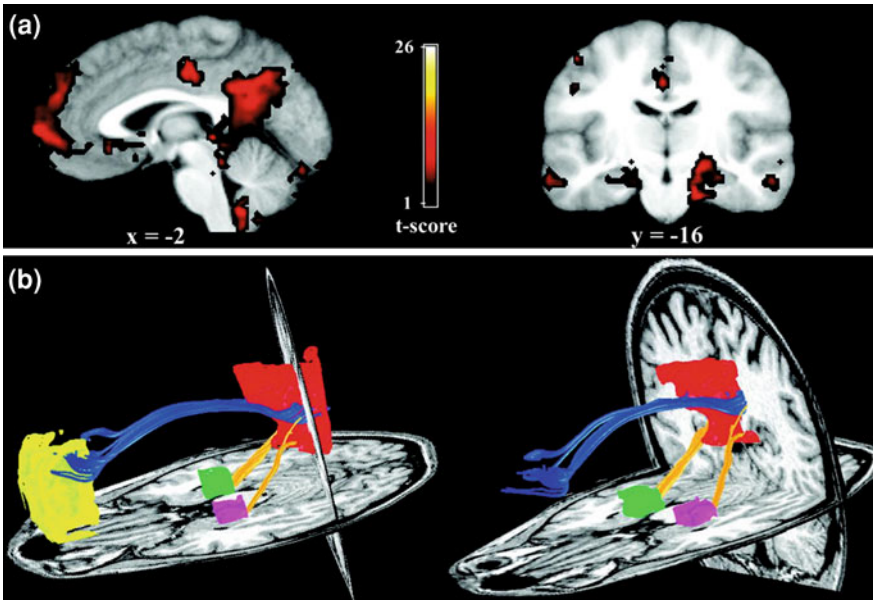

Fig. 3 Functional connectivity reflects structural connectivity in the DMN. **a** Task-free, functional connectivity in the DMN is shown in a group of six subjects. The PCC/RSC and MPFC clusters are best appreciated on the sagittal view. Prominent bilateral MTL clusters are seen on the coronal image (*left side* of image corresponds to *left side* of brain). **b** DTI fiber tractography in a single subject demonstrates the cingulum bundle (*blue tracts*) connecting the PCC/RSC to the MPFC. The *yellow tracts* connect the bilateral MTL to the PCC/RSC. Note that generally the tracts from the MPFC enter the more rostral aspect of the PCC/RSC ROI corresponding to the PCC proper, whereas the tracts from MTL enter the more caudal aspect of the PCC/RSC ROI corresponding to the RSC proper. *Left* and *right* columns show slightly different views of the same tracts to highlight the distinct entry points into the PCC/RSC. There were no tracts connecting the MPFC to the MTL. (from Greicius et al. 2008). For permissions, please e-mail:journals.permissions@oxfordjournals.org

2.2.2 fMRI Studies of Affective Processing in Late-Life Mood Disorders

Several computer-administered paradigms for measuring affect processing are amenable to functional MRI. These include having subjects respond to emotional faces (e.g., Ekman faces, (Ekman and Friesen 1971), images (e.g., the International Affective Picture System, (Lang and Bradley 1997) words, and stories. Studies with these paradigms have identified an affect processing circuit, which includes bilateral medial ventral structures including the amygdala, the ventral striatum, the orbitofrontal cortex, and the pre-and sub-genual anterior cingulate cortex. These ventral regions seem to show increased activation corresponding to the peak of the 'emotional' experience, whether it has positive or negative valence. There has also

been a strong association of these regions with activity in dorsal (described as less affective and more cognitive) structures including the dorsal anterior cingulate cortex and the dorsolateral prefrontal cortex.

Several investigators have integrated these findings into models of affective processing (Mayberg 1997; Phillips et al. 2003; Siegle et al. 2002). The key feature of these models is that the dorsal information processing circuit is more specific for the cognitive elements and regulates the affective activation and processing that occurs in the ventral structures. Thus, for instance, in the fMRI study by Ochsner (2002) the amygdala is activated by negatively valenced emotional stimuli. With cognitive reappraisal of the negative stimuli, the dorsal prefrontal cortex becomes active and the amygdala shows decreased activation, apparently secondary to modulation by the dorsal PFC-mediated reappraisal.

Models of dorsal and ventral cognitive and affective processing have been specifically applied as a framework for studying mid-life depression (Mayberg 1997; Phillips et al. 2003). Both of these models describe mid-life depression as resulting from impaired cognitive and affective processing in these circuits. These models have not yet been applied to LLD. However, the notion of disconnection between the dorsal and ventral circuits would seem to apply even more in late-life as compared to mid-life depression, since in LLD there is more evidence of microstructural changes in the PFC white matter tracts that connect these regions (Alexopoulos et al. 2002; Taylor et al. 2004). Future studies are needed to test this hypothesis.

2.2.3 fMRI Studies of Cognitive Processing in Late-Life Mood Disorders

Several functional neuroimaging studies of late-life mood disorders have been conducted during cognitive activation (e.g., de Asis et al. 2001). Bilateral deficits in dACC and hippocampus were observed during word generation task. These cognitive probes are used for studying the basis of cognitive changes in late-life mood disorders, and for engaging key structures implicated in these disorders (e.g., ACC, dlPFC, hippocampus). Functional imaging performed during controlled cognitive tasks standardizes behavior and therefore decreases variability in brain response.

2.3 Perfusion Functional MRI

A significant limitation of BOLD fMRI is concern that the BOLD hemodynamic response is inherently relative. That is the raw BOLD signal does not provide a reliable estimate of regional blood flow in a region. Rather it is contrast of the BOLD signal on alternating experimental versus control tasks that provide the meaningful signal. In contrast to this limitation, PET imaging with an O-15

radioligand is capable of providing quantitative blood flow measures. In MR imaging, a technique analogous to O-15 PET is also available, and is referred to as Arterial Spin Label (ASL) imaging, or perfusion imaging (Detre et al. 1992; Aguirre et al. 2005). In perfusion MR imaging the MR excitation signal is inverted to provide a 'tagged' signal, which is alternated with an 'untagged' image. Comparing the tagged and untagged provides a quantitative measure the perfusion of the region. Full-brain voxel-wise perfusion images provide a quantitative image of the perfusion across the brain. Investigators have recently used perfusion imaging to demonstrate similar findings as with PET blood flow studies, e.g., decreased parietal-temporal resting perfusion with Alzheimer's disease (Alsop et al. 2010). In addition to providing quantitative resting perfusion, ASL has also recently been used for investigating the blood flow changes associated with tasks (Fernández-Seara et al. 2007). Perfusion fMRI, however, is limited due to slow acquisition time, reduced coverage, and lower SNR compared to BOLD fMRI. The two primary methods for perfusion imaging are referred to as Continuous Arterial Spin Labeling (CASL) and Pulsed Arterial Spin Labeling (PASL). CASL is believed to provide better signal quality, but generally requires special hardware for providing the continuous tagging pulse.

3 MRI Changes in Specific Late-Life Mental Disorders

Throughout its history, DSM architects have struggled with the seemingly fundamental, but complex question of how to define a mental disorder. Current proposals indicate that a spectrum model of mental illness will be embraced in DSM-5, prompting renewed concern and debate about pathologizing normal behavior (Pierre 2010).

Recently the NIMH launched the Research Domain Criteria (RDoC) project to create a framework for research on pathophysiology, especially for genomics and neuroscience. The RDoC project is intended to be the next step in the process of ensuring valid and reliable diagnosis (Insel et al. 2010). Thus, the project intends to classify mental disorders based on dimensions of observable behavior and neurobiological measures, dimensions such as fear and its extinction, response to stress, impulsive behavior, executive function, and working memory. Increasing evidence suggests that abnormality in one dimension frequently occurs in multiple diagnoses of mental disorders. Cutting across traditional diagnostic categories, RDoC will encompass multiple levels of analysis, from genes to neural circuits to behaviors and will be developed for the research community to help break out of diagnostic formulations that may have more reliability than validity.

We will present in this chapter MRI changes in DSM-IVTR disorders, while keeping in mind that an increasing body of the literature focuses on emotion regulation in late-life, executive function and working memory.

3.1 Late-Life Major Depression (LLD)

Depression in the elderly causes significant distress, disability, and loss of life. The importance of considering late-life depression separately from mid-life depression follows from an extensive literature that has identified biological, psychological, and social factors specific for late-life depression. Loss of function and loss of social support are common psychosocial factors in the presentation of depression in the elderly. However, biological factors are also prevalent in LLD. Two key features that distinguish the brain in elderly versus young subjects are cerebrovascular disease and neurodegeneration, and both are known risk factors for depression [reviewed in Lavretsky and Small (2004)]. However, as these processes (cerebrovascular disease and neurodegeneration) exist on a continuum, it is likely that even subsyndromal disease (e.g., cerebrovascular disease without overt strokes and pre-morbid Alzheimer's disease) could also contribute to the depressive syndrome in elderly.

3.1.1 Structural Neuroimaging in Late-Life Depression

The neuroimaging findings in LLD overlap with other diseases of aging, including Alzheimer's disease and cerebrovascular disease. Central and cortical atrophy have been widely reported on both CT [reviewed by Morris and Rapoport (1990)] and MRI (Ballmaier et al. 2004a; Pantel et al. 1997; Rabins et al. 1991). LLD is also associated with reduced frontal lobe volume in general (Kumar et al. 2000), and in particular, the orbitofrontal cortex (Ballmaier et al. 2004b; Lai et al. 2000; Lee et al. 2003) as well as the gyrus rectus and anterior cingulate (Ballmaier et al. 2004b). There also are basal ganglia lesions (Rabins et al. 1991; Steffens et al. 1998; Tupler et al. 2002), especially in the caudate (Krishnan et al. 1992), and the putamen (Steffens et al. 1998; Tupler et al. 2002), that may be worse among late-onset patients. Finally, there is an association between chronic, treatment-resistant depression in groups of mixed ages and right, frontostriatal atrophy (Shah et al. 2002) and reduced volume of the left temporal cortex including the hippocampus (Shah et al. 2002). Recently volumetric studies have identified differences between early versus late-onset LLD (Ballmaier et al. 2004a, b; Andreescu et al. 2011), with the late onset showing less frontal and more temporal and parietal atrophy.

The hippocampus and amygdala appear to be especially sensitive to the effects of major depression. In a study in which the subjects ranged in age from 23 to 86 years of age, both the hippocampus bilaterally and the amygdala core nuclei bilaterally showed reduced volume in depressed subjects relative to controls (Sheline et al. 1999). Reduced hippocampal volume is particularly, but not exclusively, related to later age-of-onset (Steffens et al. 2002), and hippocampal volume was inversely related to conversion to dementia (Steffens et al. 2002). Moreover, the lifetime duration of depression (measured either as years since first

episode or total number of days spent depressed) is very closely associated with hippocampal volume (Bell-McGinty et al. 2002; Sheline et al. 1999).

In addition to studies of regional volume there have also been a number of reports of differences in the MR signal within the white matter of individuals with LLD. Several studies using semi-quantitative ratings (Butters et al. 2004; Greenwald et al. 1998) and semi-automated measures (Taylor et al. 2003) have found increased presence of white matter hyperintensities in periventricular and subcortical regions. Salloway et al. (1996) found the periventricular and subcortical hyperintensities to be most severe in those LLD subjects with late-onset depression. More recently, diffusion tensor imaging has been used to more specifically study the white matter tracts, with results showing decreased fractional anisotropy (a measure of diffusion orientation which is used as a marker of white matter integrity) in prefrontal white matter (Alexopoulos et al. 2002; Taylor et al. 2004). While some studies have found that the disturbances in the white matter are associated with poor treatment response (Alexopoulos et al. 2002, 2008) others have not found this to be the case (Salloway et al. 2002).

3.1.2 Functional Imaging in Late-Life Depression

To date, most of the functional neuroimaging studies reported on LLD have focused on the resting state and have identified changes in baseline (i.e., resting) cerebral activity between patients and controls (reviewed in Table 1). One of the earliest studies (Sackeim et al. 1990) demonstrated global decreased CBF using the xenon inhalation technique. A decrease in global brain metabolism in LLD was also found with PET (Kumar et al. 2000). Baxter et al. (1989) and Bench et al. (1993) using PET have found the decreased blood flow and metabolism in depression, in samples with age ranges extending from mid-life through late-life, to be most prominent in the frontal cortex. Other specific areas with reported decreases in LLD versus controls in PET studies include the medial temporal lobe (Grön et al. 2002) and the caudal ACC (de Asis et al. 2001).

In a recent study exploring resting-state connectivity in the default-mode network in late-life depression, we reported that, compared with non-depressed elderly, depressed subjects pretreatment had decreased connectivity in the subgenual anterior cingulate cortex and increased connectivity in the dorsomedial prefrontal cortex and the orbitofrontal cortex. The abnormal connectivity was significantly correlated with the white matter hyperintensity burden. Remitted elderly depressed subjects had improved functional connectivity compared to pretreatment, although alterations persisted in the anterior cingulate and the prefrontal cortex when remitted elderly depressed subjects were compared with non-depressed elderly. These results provide evidence for altered default-mode network connectivity in late-life depression and emphasizes the role of vascular changes in late-life depression etiopathogenesis (Wu et al. 2011).

Several functional neuroimaging studies of LLD have been conducted during cognitive activation (e.g., de Asis et al. 2001; Grön et al. 2002). The cognitive

Table 1 Review of functional neuroimaging findings in late-life depression

Reference	Imaging modality	Subjects	Primary finding
Sackeim et al. (1990)	SPECT (rCBF using 133Xe inhalation)	30 LLD; 30 EC	Reduced global rCBF
Kumar et al. (1993)	PET (Glucose-15)	8 LLD; 8 EC	Reduced global cerebral metabolism
Lesser et al. (1994)	SPECT (rCBF using 133Xe & Technetium-99 m-HMPAO)	39 LLD; 20 EC	Reduced global rCBF
Smith et al. (1999)	PET (Glucose-15)	6 LLD; 6 EC	Increased activity in right ACC pre-treatment, which decreased with treatment
de Asis et al. (2001)	[^{15}0]H_20 PET (paced word generation)	6 LLD; 5 EC	Decreased activation b/l in dorsal ACC & Hippocampus
Gron et al. (2002)	Block-design FMRI (declarative memory)	12 LLD; 12 EC	Increased vlpfc, decreased hippocampus
Aizenstein et al. (2005)	Event-related BOLD fMRI (sequence learning)	11 LLD; 12 EC	Decreased b/l PFC and increased R striatum
Aizenstein et al. (2006)	Event-related BOLD fMRI (cognitive control task)	14 LLD; 15 EC	Decreased PFC and decreased ACC
Brassen et al. (2008)	Block-design fMRI (emotion reactivity) pre- and post-treatment	13 LLD; 12 EC	Decreased response to negative stimuli in the vmPFC, correlated with symptoms severity and attenuated by symptom improvement
Smith et al. (2009)	PET study	16 LLD; 13 EC	Increased cortical glucose metabolism in brain regions with cerebral atrophy (compensatory response)
Andreescu et al. (2009a)	Event-related BOLD fMRI (cognitive control task)	8 LLD	Sustained activation in the dorsal ACC in subjects with LLD and increased anxiety
Kenny et al. (2010)	Resting-state fMRI	16 LLD; 17 EC	Increased connectivity in the frontal, limbic, parietal, and temporal areas.

Note SPECT single photon emission computerized tomography, *PET* positron emission tomography, *BOLD* blood oxygen level dependent, *PFC* prefrontal cortex, *ACC* anterior cingulate cortex

activation functional imaging studies conducted in LLD have replicated the general patterns of regional activity found during resting studies. In a study using a word generation task, de Asis et al. (2001) found reduced CBF bilaterally in the dorsal anterior cingulate and the hippocampus (as measured compared to controls), and on a verbal declarative memory task, Grön (2002) found decreased left VLPFC and hippocampal activation compared to elderly controls. Recently, on a cognitive control task comparing LLD to elderly controls (Aizenstein et al. 2005); and see Sect. 3 of this chapter) we found decreased BOLD activation in the DLPFC and

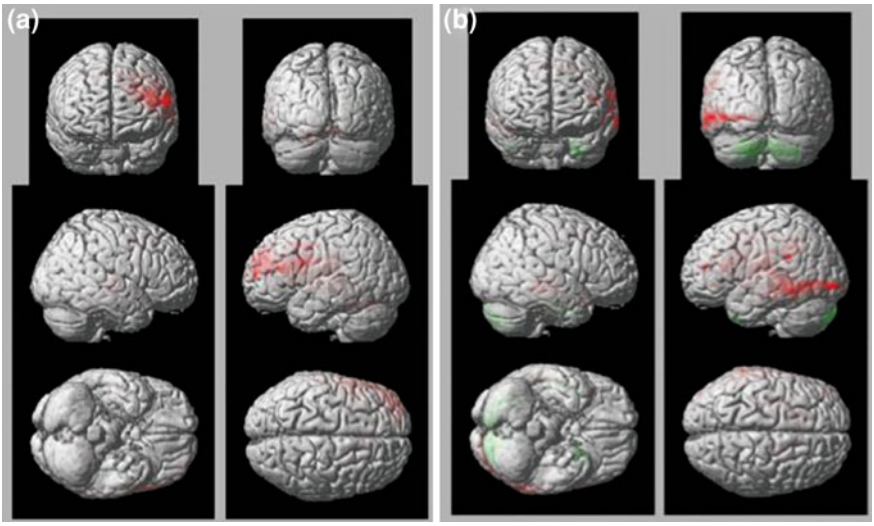

Fig. 4 Elderly non-anxious subjects engage the PFC in suppressing worry (**a**). Elderly GAD subjects (**b**) are not effective in engaging the PFC and maintained an increased activation the posterior areas (temporo-occipital) (**b**)

ACC, and in a sequence learning task comparing LLD to elderly controls we found the depressed elderly to have decreased prefrontal activation and *increased* striatal activation. The increased striatal activation occurred during the trials that violated the predictive sequential pattern, and thus are consistent with reports in mid-life depression of increased negative reward activity in depression.

3.2 Late-Life Bipolar Disorder (LLBD)

Although the prevalence rates of Bipolar Disorder are relatively low among community-dwelling elderly (up to 0.1%), there is significantly higher prevalence (and higher morbidity) in institutional settings, such as personal care homes and nursing homes where prevalence rates may be as high as 10%. Bipolar disorder the elderly is probably heterogenous and its etiopathogenesis is complex.

Bipolar disorder may be divided into two distinct subtypes, the late-onset bipolar (LOB) and the early onset bipolar (EOB) groups. LOB patients tend to have a milder illness in terms of manic severity but they have higher medical and neurological burden. They also have lower familial burden of bipolar illness as compared to EOB patients. There is an increased risk of dementia and stroke in patients with late-life bipolar disorder (Vasudev and Thomas 2010).

Structural and functional neuroimaging data in LLBD is quite scarce. The few studies exploring the neurobiology of late-life bipolar disorder have reported that relative to elderly controls and EOB, late-onset bipolar subjects have increased

Fig. 5 Amygdala (*red*)- sACC (*blue*) functional connectivity during worry induction and worry suppression in elderly controls (*up*) and elderly GAD subjects (*down*)

hyperintense lesions on T2 images around the putamen, as well as in the deep white matter in frontal and parietal regions (Altshuler et al. 1995; Beyer et al. 2004). The authors concluded that their results provide empirical support to the link between vascular risk factors and late-onset BD. It is plausible that, as in the case of MDD, the T2 hyperintensities, which reflect ischemia, area long-term consequence rather than a cause of bipolar illness. One possibility is that people with BD have an excess of atherosclerotic risk factors that lead to microvascular pathology at an even earlier age than MDD. However, using a strict selection of elderly cases with BD and careful case–control matching for clinical and demographic variables, no volumetric differences were found between LLBD subjects in the hippocampus, amygdala, entorhinal, and anterior cingulate cortex, nor a higher degree of WMH, when compared with healthy individuals (Delaloye et al. 2009).

3.3 Late-Life Anxiety disorders

With an estimated community prevalence of 7.3%, late-life generalized anxiety disorder (GAD) is the most common anxiety disorder among the elderly (Wetherell et al. 2001; Wittchen and Hoyer 2001). Late-life GAD is associated with decreased quality of life (de BEURS et al. 1999; Wetherell et al. 2001), cognitive impairment (Mantella et al. 2007; Caudle et al. 2007), increased health

care utilization (de BEURS et al. 1999), and poorer recovery after disabling medical events (de BEURS et al. 1999; Astrom 1996). Recent fMRI studies have reported that, when attempting to regulate their emotional responses, elderly anxious subjects failed to activate prefrontal regions involved in the down-regulation of negative emotions. These results, showing that elderly anxious subjects are not effectively engaging the PFC in suppressing worry, may be clinically relevant for developing personalized therapeutic strategies for the treatment of late-life GAD (Andreescu et al. 2011).

Moreover, time-series analysis of non-anxious subjects showed that amygdala and sACC activate in reverse synchronicity during phases of worry modulation (see Fig. 4). In contrast, elderly GAD subjects displayed same direction activation of the sACC and the amygdala during worry induction. Moreover, time-series cross-correlation analysis showed a decrease correlation between the amygdala and sACC in elderly GAD (Fig. 5).

These results suggest a possible age-related inability of the regulatory regions such as sACC to modulate the worry process in late-life GAD (Andreescu et al. 2009b).

4 Future Directions

MRI has revolutionized clinical neuroscience research and has led to a more sophisticated understanding of the neural substrates of mental disorders (Kumar and Ajilore 2008). There has been tremendous increase in the use of MRI (functional and structural) in studying the brain. These neuroimaging studies have provided deep insight of how the brain works in terms of brain development, function, aging, and other diseases. The use of neuroimaging is important for studying the aging brain as it provides a platform for non-invasive studies of structure and function to increase our understanding of the cognitive aging (Reuter-Lorenz and Lustig 2005) and other age-related changes in the brain.

Applying MRI approaches to the identification of predictors of treatment response may allow us early in the course of therapeutic interventions to identify sub-groups of subjects with difficultly in treating mood disorders. In the future, such subjects may be selected, based on their MRI profile, for more aggressive interventions. Markers of white matter pathology may identify elderly patients for whom the risk of antidepressant treatment may not be balanced by a high probability of treatment response (Kumar and Ajilore 2008; Alexopoulos et al. 2008). In a more personalized medicine era, advances in neuroimaging and genomics could provide a personalized database that may help tailor and guide treatment choices (Kumar and Ajilore 2008)

A potential new treatment is real-time fMRI (de Charms 2008), that has been recently introduced as a method to directly control activation of localized brain regions to affect neurophysiological mechanisms that mediate behavior and cognition (de Charms 2007). Positive results have been reported in modulating pain

perception (de Charms et al. 2005), and more recently in the down-modulation of the sACC (real-time fMRI neurofeedback) (Paul Hamilton et al. HBM 2011). Incorporating real-time fMRI in the future offers the promise of clinical translation in which neuroimaging may also be used for clinical interventional purposes.

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The Role of Diffusion Tensor Imaging in the Study of Cognitive Aging

Owen Carmichael and Samuel Lockhart

Abstract This chapter gives an overview of the role that diffusion tensor MRI (DTI) can play in the study of cognitive decline that is associated with advancing age. A brief overview of biological injury processes that impinge on the aging brain is provided, and their overall effect on the integrity of neural architecture is described. Cognitive decline associated with aging, and white matter connectivity degradation as a biological substrate for that decline, is then described. We then briefly describe the technology of DTI as a means for in vivo, non-invasive interrogation of white matter connectivity, and relate it to FLAIR, a more traditional MRI method for assessing white matter injury. We then survey the existing findings on relationships between aging-associated neuropathological processes and DTI measurements on one hand; and relationships between DTI measurements and late-life cognitive function on the other. We conclude with a summary of current research directions in relation to DTI studies of cognitive aging.

Keywords Diffusion tensor MRI · Cognitive aging · Brain aging · White matter injury · Connectivity

Contents

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1 Scientific Context of DTI in Aging

1.1 Brain Changes Associated With Aging

The aging brain is beset by a variety of deleterious biological processes that develop insidiously over the course of many years, interact with each other, and eventually trigger the neuronal dysfunction and death that underlie late-life cognitive decline (Fig. 1). Beginning in roughly the sixth decade of life, an array of cardiovascular risks promoted by environmental and genetic factors trigger subclinical primary vascular injury through multiple, complex etiological pathways: for example, chronic glucose dysregulation is associated with chronic low-grade inflammation (Miranda et al. 2005); and hypertension and atherosclerotic plaque cause vascular remodeling, i.e. changes to blood vessel structural characteristics that determine the fluid mechanical properties of blood flow (Kiechl and Willeit 1999). The vascular injury processes interact; for example inflammation exacerbates dysfunction of the endothelium by dramatically modulating leukocyte trafficking (Fisher 2008). At approximately the same time, Alzheimer's Disease (AD) risk factors promote the production and aggregation of beta amyloid in extracellular spaces and within blood vessels (Cummings 2004) (i.e., cerebral amyloid angiopathy, CAA). Amyloid exacerbates inflammatory processes, and CAA exacerbates vascular remodeling; vascular remodeling may conversely hinder the clearance of amyloid plaque through vascular spaces (Preston et al. 2003).

Amyloid deposition, inflammation, vascular remodeling, and endothelial dysfunction trigger a complex cascade of secondary injury processes including

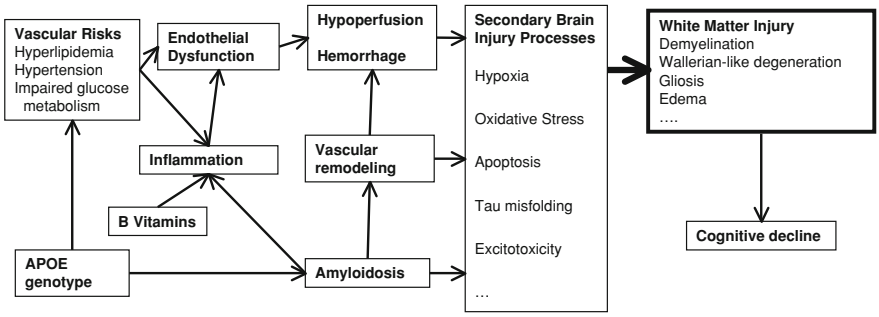

Fig. 1 A partial, simplified view of the complex array of interconnected biological processes that promote white matter injury and subsequent cognitive decline in the aging brain. Because diffusion tensor MRI provides non-invasive measurements of white matter injury, its role in cognitive aging research is to help elucidate the links between biological factors (including vascular, degenerative, nutritional, genetic, and other factors), white matter injury, and cognitive function

apoptosis, hypoxia, oxidative stress, excitotoxicity, and tau phosphorylation. Other factors have the potential to influence these primary injury processes: nutritional factors such as B vitamins can modulate inflammatory processes independent of cardiovascular risks for example (Selhub et al. 2000), and sex hormone levels may modulate injury processes through a number of pathways (Janowsky 2006). The secondary processes cause neuronal injury including demyelination, neurotransmitter dysregulation, and disruption of cell homeostasis. These effects and subsequent neuronal death give rise to the profound loss of cognitive abilities that typify late-life cognitive decline. Importantly, there is a growing sense that the course of late-life brain change is commonly influenced by an admixture of multiple, concurrent, injury processes, rather than a single, isolated pathological process such as AD that is either present or absent (Jagust et al. 2008; Schneider and Bennett 2010; DeCarli 2006).

1.2 Cognitive Changes Associated With Aging

Broad variability across the population in risk factors for aging-related brain injury processes has given rise to broad variability in the time course of late-life brain changes. This heterogeneity in brain change trajectories is presumed to drive the great variability in late-life cognitive trajectories that is one of the hallmark observations of cognitive aging research (Fig. 2). There are robust inter-individual differences in cognitive functioning throughout adulthood and this heterogeneity is amplified by differences in trajectories of cognitive change that emerge as people age. Longitudinal studies of older individuals reveal widely differing rates of cognitive decline, as well as many cases of stable function and even modest

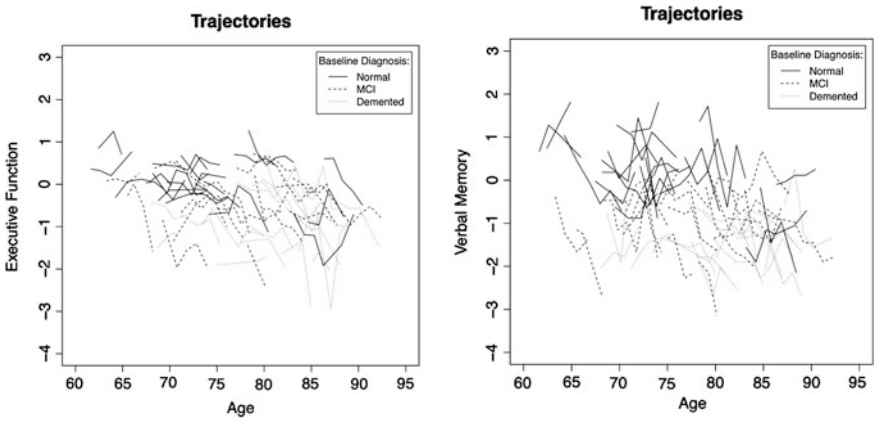

Fig. 2 Population variability in aging-related brain injury processes likely leads to the broad variability in cognitive decline observed in many studies of elderly individuals. Trajectories of psychometrically matched measures of executive function (*left*) and episodic memory (*right*) are plotted for 60 randomly selected individuals from a longitudinal study of aging (Mungas et al. 2010), including 20 individuals clinically diagnosed at baseline with mild cognitive impairment, 20 clinically diagnosed at baseline with dementia, and 20 who were cognitively normal at baseline. On average, the more clinically impaired individuals exhibited lower cognitive function at baseline and greater longitudinal declines; however, there is substantial variability in trajectories and substantial overlap between clinical diagnostic groups. The role of DTI in cognitive aging research is to provide in vivo, non-invasive measurements of white matter injury, from which some of this cognitive heterogeneity is hypothesized to arise. [See Mungas et al. (2010) for more information]

improvement (Albert et al. 1995; Colsher and Wallace 1991; Christensen et al. 1999; Rubin et al. 1998; Schaie 1988; Wilson et al. 2002; Zelinski et al. 1993; Mungas et al. 2010). Variability in longitudinal trajectories translates into the increased variability of function over time that is one of the basic observations of cross-sectional studies of cognitive aging (Christensen et al. 1999). In the context of this broad heterogeneity, the prediction of late-life cognitive decline has emerged as one of the central goals of cognitive aging research.

1.3 Late-Life Cognitive Decline as a Disconnection Syndrome

A convergent body of cognitive neuroscience research supports the notion that a substantial portion of late-life cognitive decline observed in the aforementioned studies is accounted for by disconnection of distributed networks of brain regions that function in concert to give rise to higher-order cognitive abilities (Buckner 2004; O'Sullivan et al. 2001; Seeley et al. 2007). Coordinated activity of distributed sets of brain regions is required for successful memory function, language, attention, and other aspects of higher-order thought. Mapping out the circuitry and

function of these networks, and the ways their architecture relates to domain-specific cognitive function, has been a key goal of cognitive neuroscience research since neuroimaging technologies became widely available.

Visual attention provides a typical example of how crucial distributed brain networks are for successful cognitive functioning, even for tasks that seem relatively rudimentary. It relies on a set of executive control processes critical to successful visual search of the environment, including interaction between top-down, goal-oriented search, and bottom-up, reflexive orienting (Madden 2007). Electrophysiological and neuroimaging research suggests that even such simple behavior relies upon multiple distributed brain regions that interact selectively, synchronously, and successfully as part of a network (Corbetta et al. 2008; Corbetta and Shulman 2002). Specifically, ventral frontal-parietal regions facilitate bottom-up attention, while dorsal frontal-parietal regions contribute to top-down attention (Kastner and Ungerleider 2000; Woldorff et al. 2004; Bisley 2011). Neural activity in frontal and parietal regions is thought to modulate perceptual system activity to enable selective sensory processing for the target (Bar 2003; Hopfinger et al. 2000). Thus, multiple brain regions in distributed networks must be recruited in concert to perform a relatively simple function—attending to visual stimuli—that none could complete in isolation.

Cognitive processes are resolved by neural systems that are both localized and distributed (Mesulam 1990). Indeed, both connectivity and modularity of networks is critical to brain function and cognition (Mesulam 1998; Bullmore and Sporns 2009). Focal, modular brain regions are thought to perform dedicated, specialized processing of particular aspects of cognitive function. Reciprocal connectivity allows for information flow among modules to create larger, distributed cognitive networks. It has been argued that network anatomy and connectivity define the limits of the cognitive tasks that can be performed, and allow humans the flexibility necessary to perform them (Mesulam 1998).

Network modularity and connectivity have been mapped out through non-human primate anatomical and electrophysiological studies, as well as human neuroimaging research (Corbetta and Shulman 2002). In humans, networked activity is important for cognitive functions as diverse as visual attention, working memory (Rissman et al. 2004), episodic memory (Eichenbaum et al. 2007), semantic memory (Hagoort et al. 2008), and cognitive control (O'Reilly et al. 2010). In particular, nodes in prefrontal cortex are thought to be essentially involved in multiple cognitive networks (Mesulam 1998; Miller and Cohen 2001).

The question of how activity is coordinated among diverse brain regions in a connected network is an active area of neuroscience research. Synchronization of neural activity is thought to be required for successful cognitive functioning (Fries et al. 2008). However, in most brain regions, the mechanisms of coordination and integration of information from multiple disparate network modules are unexplained, and discovering these mechanisms is an ongoing challenge.

Because cognitive networks utilize white matter to connect distributed cortical nodes, aging-related changes in network behavior are hypothesized to follow from changes in the integrity of white matter connections, in addition to likely structural

and functional changes to neuronal cell bodies, dendrites, and synapses (Greenwood 2000; Tisserand and Jolles 2003). This "disconnection hypothesis" states that injury to myelin and the axon proper diminish the efficient axonal conduction of electrical signals, thus reducing information processing efficiency in distributed networks, thus leading to diminished cognitive abilities with age (O'Sullivan et al. 2001).

It is within the context of the disconnection hypothesis that DTI serves to provide its most valuable contributions to the study of cognitive aging—as described below, DTI has the potential to assay the integrity of axonal connections between nodes in distributed cognitive networks, and thereby assess relations between network connectivity and age-related cognitive decline.

2 DTI Measurement

2.1 Background

DTI is an MRI method for using magnetic field gradients to measure the directional distribution of water diffusion at each location in the brain. Many reviews have described the technology underlying DTI data acquisition in detail [for example, Bammer et al. (2009), Chanraud et al. (2010), Mukherjee et al. (2008a, b), Beaulieu (2002)]. The simplest way to conceptualize DTI may be as an acquisition of several component images, each of which quantify the degree to which water molecules in the brain appear to be diffusing along a particular direction of travel. Because each such component image only provides diffusion information along a particular direction of travel, several component images, corresponding to several lines of travel, are acquired in rapid sequence and aligned to each other to build a fully 3D representation of the spatial distribution of water diffusion at every location in the brain. If, for a particular location in the white matter, local water diffusion appears to be anisotropic, that is, if water strongly prefers to diffuse along certain well-defined spatial directions and not others, we indirectly infer the presence of highly organized axonal structures: the water is likely to be diffusing within axons along their directions of travel, or within or between myelin sheaths. If, conversely, water is free to diffuse in any and all spatial directions where an axon tract is expected to occur, we can indirectly infer that axonal injury, demyelination, gliosis, or edema may be making this free diffusion possible.

2.2 Preprocessing

Preprocessing must be applied to insure that all of the DTI component images avoid common MRI acquisition artifacts and are in spatial correspondence with each other. Geometric distortions are introduced into each component image as part of the MRI measurement process; these can be corrected at acquisition time

by dynamically modifying parameters of the acquisition sequence, or they can be corrected post-hoc by linearly or nonlinearly aligning the component images to each other or to a group average.

2.3 Diffusion Representations

Once the component images are brought into alignment with each other, each location in the brain is represented by the amount of water diffusion along each of the directions of travel. The key post-processing step in DTI is the extrapolation of this information into a mathematical object that quantifies the amount of water diffusion locally along any and all directions of travel. Traditionally, this spatial distribution of water diffusion has been summarized in terms of a three-by-three matrix that in essence describes three principal, orthogonal directions along which water diffuses, and the amount of diffusion along those directions (Fig. 3, bottom left). Water diffusion along any other direction is interpolated from the three preferred ones. Estimating the three directions and diffusion magnitudes requires only six component images, representing diffusion along orthogonal directions, together with an additional T2-weighted image, and thus this representation has been prominent since the earliest days of DTI. However, the ability of the three orthogonal directions to represent complex spatial distributions of water diffusion is limited; depending on whether water appears to diffuse locally along one, two, or three prominent orthogonal directions, the spatial distribution of water diffusion is required to be represented as pencil-like, pancake-like, or sphere-like, respectively. Now that greater numbers of component images per scan are routinely acquired, the ability to mathematically represent more complex spatial distributions of water diffusion is available. The canonical example is a white matter fiber crossing, at which water diffuses preferentially along two (possibly orthogonal) directions, but water diffusion along all other directions is relatively weak; in a three-orthogonal-direction representation, a pancake-shaped distribution will result in which all directions in between the two preferred ones will appear to have strong water diffusion as well. However, while a number of higher-order diffusion representations have been developed, no single one of them has risen to dominance, and methodologies for manipulating complex diffusion representations are still a highly active area of computational research [see, for example, Frank (2002), Liu et al. (2004), Tournier et al. (2004)].

2.4 DTI Summary Measures of Local White Matter Integrity

Because mathematical representations of DTI data are more complex than those provided for more traditional MRI data, researchers have sought to summarize it into univariate summary measures that capture characteristics of the water

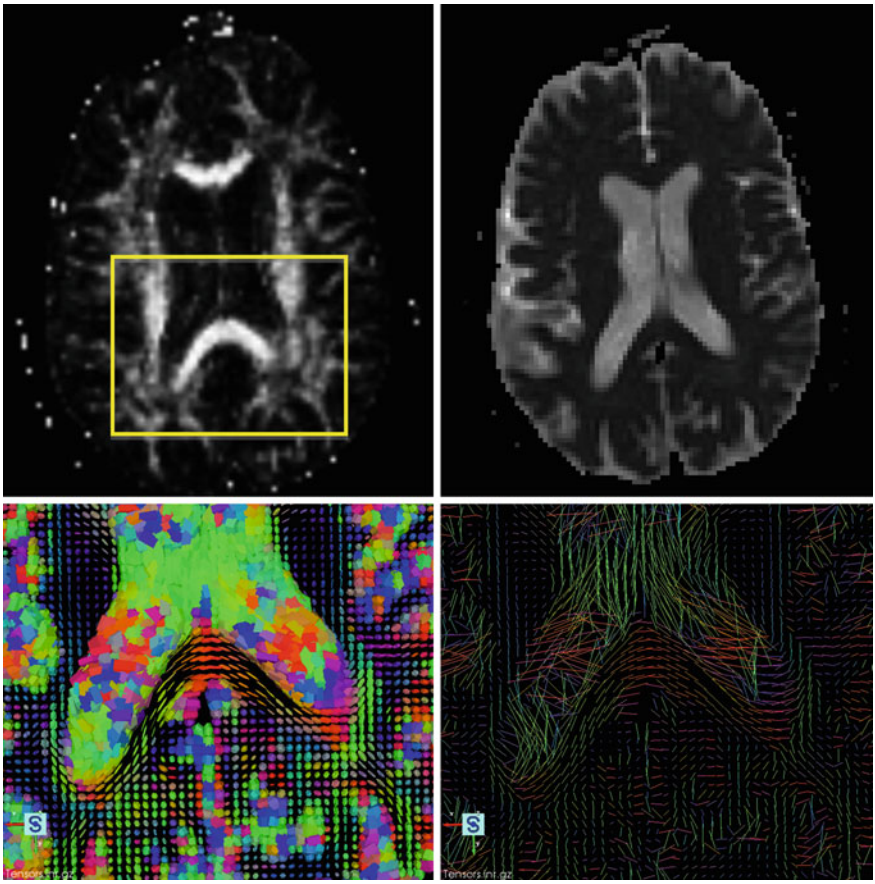

Fig. 3 Univariate summary measures and visual depictions of the diffusion tensor for a typical elderly individual. *Top Images* showing fractional anisotropy (FA, *left*) and mean diffusivity (MD, *right*) for a single slice. For both measures, lesser values are rendered in darker colors. *Bottom* Expanded view of the slice section shown as a *yellow box* in the *upper left* view. Ellipsoids are used to depict the orientations of the three principal orthogonal directions of water diffusion at each location (*left*). An arrow points in the orientation of the first principal diffusion direction at each location (*right*)

diffusion directional distribution. Because the three-orthogonal-direction representation still predominates in neuroscientific studies, the univariate summaries are predominantly calculated from the relative diffusion magnitudes along each of the directions. Fractional anisotropy (FA), for example, calculates the ratio of the magnitude of water diffusion along the most preferred direction to the sum of magnitudes along all three directions, effectively summarizing how preferred the single most preferred diffusion direction is over the others (Fig. 3, top left). This is commonly used as a proxy measure of local white matter microstructural integrity, with clear limitations. Because it is a ratio, FA does not quantify the sheer amount

of water diffusion in an absolute sense; in addition, locations with two or more well-defined directions of strong water diffusion will appear to have low FA. Mean diffusivity (MD), conversely, provides a measure of the total bulk of water diffusion by taking the average of the magnitudes of water diffusion along the three orthogonal directions, and thus does not directly provide a sense of whether that diffusion tends to occur preferentially along one or another direction (Fig. 3, top right). Several minor variants of FA and MD have been presented in the literature, each of which incorporate quantification of the sheer volume of water diffusion and/or the degree to which that diffusion preferentially follows one or another spatial direction. Each of the univariate summaries is limited in its ability to quantify the structural integrity of the underlying white matter, and their inability to disambiguate differing states of white matter organization has largely driven the development of more expressive diffusion representations. From a cognitive aging perspective, the key impact of DTI summary measure limitations has been the inability to establish thresholds on them that differentiate normal, healthy white matter from lesioned tissue. That is, depending on the intrinsic organization of the white matter locally, FA may tend to be relatively low for no other reason than that there are multiple preferred diffusion directions. In other locations where only a single strong diffusion direction is expected, low FA may be a marker of white matter disruption.

Ultimately, to truly disentangle the integrity of white matter from its underlying geometry, higher-order diffusion representations are needed. However, lacking such data, a practical approach to this problem is to use a development sample of healthy young individuals to calculate nominal values of diffusion summaries at every white matter location in a standardized space; white matter integrity in novel subjects is then indexed against these population norms (Lee et al. 2010) (Fig. 4).

2.5 DTI Summary Measures of Connectivity

Univariate DTI summary measures can be useful for characterizing local white matter integrity, and because they are univariate, they can be relatively easy to incorporate into traditional MRI analytic pathways. But DTI additionally provides the capability to integrate multiple water diffusion measurements over a trajectory into summary measures of interregional connectivity, which can then be used to quantify the integrity of structural connections among distributed cognitive networks. Higher integrity of longer-range structural connections is indicated by greater directionality of water diffusion along the trajectory; such trajectories may possess greater myelination, greater axonal integrity, or denser concentration of myelinated axons.

Deterministic tractography, the traditional method for using DTI to assess the integrity of interregional connectivity, traces a set of curved trajectories, or fibers that originate inside of one region of interest, and follow paths along the preferential water diffusion directions implied by the local diffusion representation

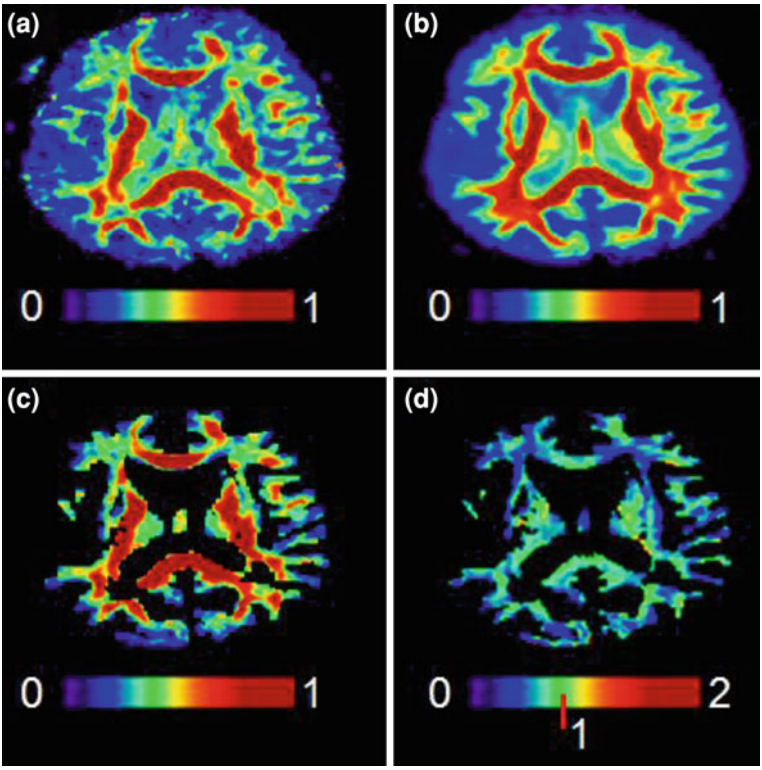

Fig. 4 Indexing FA against normative values is a practical method for converting FA into a white matter integrity measure that is independent of intrinsic local fiber organization. *Top left* Slice of an FA map of a typical elderly individual. *Top right* Co-registered slice of the mean FA map from a population of healthy young individuals, revealing spatial variability in FA values that is due to the intrinsic local geometric organization of fibers. *Bottom left* FA within the white matter pixels of the elderly individual is shown, suggesting a spatially varying pattern of white matter integrity. *Bottom right* Dividing the elderly individual's FA at each location by the corresponding young mean FA reveals a different spatial map of white matter integrity, expressed as percentages of expected young values

(Fig. 3, bottom right). Each fiber path represents a plausible axon tract trajectory. Connectivity between a pair of regions is usually determined by tracing all such curved fibers that originate within any starting voxel in the first region, and isolating only those that terminate in the second region. Complications are added on top of this basic procedure: for example, various mathematical techniques are used to make sure that the traced fiber paths are reasonably straight and smooth, and post-processing steps cull those fibers that are so short and contorted that they probably do not reflect a valid white matter tract. Additionally because traditional DTI generally only provides robust water diffusion data within the white matter, assessing connectivity between a pair of gray matter structures involves the uncertain task of identifying proximal white matter voxels to use as fiber starting

points. In the end, the result is a set of fibers that purport to connect one region to another; the degree of connectivity between the two can be quantified in terms of the number of such fibers.

Probablistic tractography overcomes many of the practical limitations of deterministic tractography at the cost of increased computational burden. The governing principle of probabilistic tractography is to consider, for each starting voxel, all possible fiber trajectories that originate there and terminate somewhere else in the brain. Each such fiber trajectory is assigned a probability representing the degree to which water diffusion directionality along the trajectory are consistent with the hypothesis that a white matter tract follows that path. Interregional connectivity is quantified by summing the probabilities of all such fiber trajectories that originate in one region and end in another. In the abstract, this overcomes the key limitation of deterministic tractography: the requirement that each starting voxel pick exactly one fiber trajectory through the brain. Depending on the configuration of white matter tracts and level of noise in the data, there may be points in the trajectory where multiple directions of travel seems equally plausible, and probabilistic tractography gracefully considers the likelihood of all such putative paths. The practical problem with probabilistic tractography is that there are infinitely many possible fiber trajectories emanating out of each starting voxel, and thus summing fiber probabilities over all possible trajectories is intractable to compute. Therefore, the art of probabilistic tractography involves exploring the space of possible fiber trajectories in a time-efficient way. Even if this space is explored efficiently, however, there is no way to make the jump from one trajectory per starting voxel to multiple without heaping on additional computation.

Lacking high-quality DTI data that allows for robust deterministic tracking, and lacking state-of-the-art computational tools for probabilistic tractography, one common fallback position is to manually trace the paths of interregional white matter tracts using standardized anatomical tracing protocols together with a combination of DTI, anatomical, and functional images, possibly overlaid on top of each other. The univariate summary measures of local white matter integrity may then be summarized over the region to provide an overall summary of interregional connectivity; or, a voxel-based analysis of the local integrity measures can be reduced to focus only on those white matter tract voxels. This effectively converts tractography into a time-consuming manual process, although it provides a clear means for imposing expert neuroanatomical knowledge about tract trajectories onto the DTI analytic process.

2.6 Relation to FLAIR

The application of DTI to white matter characterization in aging has naturally raised the question of whether the information it provides is redundant with or complementary to that provided by several conventional MRI sequences that are useful for white matter characterization (Haller et al. 2009; Wozniak and Lim

2006). The most widespread of these is fluid attenuation inversion recovery (FLAIR), in which the T2 signal from parenchyma is carefully measured while that of cerebrospinal fluid (CSF) signal is suppressed. In white matter, the FLAIR contrast is indirectly determined by the density of lipid protons within myelin, which facilitate the relaxation of water protons bound to its macromolecular matrix (Barkhof and Scheltens 2002). Areas of abnormally high signal in the white matter on FLAIR are a common finding on scans of the elderly brain; such white matter hyperintensities (WMH) are associated with cognitive impairment in these individuals (DeCarli et al. 2005; Nordahl et al. 2006; Nordahl et al. 2005; Yoshita et al. 2006). WMH reflect various pathogenic mechanisms, including cerebral ischemia and degradation of myelin in adjacent fiber tracts (Kim et al. 2008), although lacking additional data, we can only indirectly infer which of these mechanisms contribute to specific WMHs based on WMH size and location (DeCarli et al. 2005; Schmidt et al. 2003).

It appears that as white matter slowly and progressively degrades with advancing age due to various pathological mechanisms, DTI provides fairly sensitive detection of such changes, even when they are quite subtle. White matter on FLAIR, meanwhile, might only become hyperintense when this degeneration reaches a more advanced stage. There are two streams of evidence supporting this notion. First, on co-registered FLAIR and DTI, regions of reduced white matter integrity, as measured by DTI-based FA or MD, extend beyond the boundaries of FLAIR-based WMH, and such abnormal DTI values gradually fall back to normal values with distance from the WMH (Bronge 2002; Firbank et al. 2003; Maillard et al. 2011) (Fig. 5). This suggests that aging-associated white matter damage may involve foci of severely damaged tissue surrounded by more mild damage; FLAIR is only able to identify the former, in the form of WMHs, while DTI can identify both the core and the surround. Second, markers of aging-associated neuropathological processes, including vascular disease and inflammation, may be more strongly associated with DTI than they are with WMH burden; this reinforces the view that DTI may provide a more "continuous" measure of white matter dysfunction while WMHs are only those sites where this dysfunction has passed a threshold of high severity (Fornage et al. 2008; Nitkunan et al. 2008a; O'Sullivan et al. 2004; van Dijk 2005; Wersching et al. 2010).

While DTI appears to have the potential to provide more sensitive measurement of aging-associated white matter dysfunction than FLAIR, its value relative to FLAIR for this purpose is currently not entirely clear for three key reasons. First, the number of studies that have measured both FLAIR and DTI in the same individuals, and directly evaluated what information about prevalent aging-related white matter pathology is shared between the two or unique to one or the other, is still very small. One study has suggested that by investigating relationships between FLAIR signal intensity and DTI measures within WMHs, we can effectively categorize WMHs according to the severity of WMH dysfunction, but the clinical value of such deeper characterization of WMH severity is currently unclear (Zhan et al. 2009). Second, while several serial FLAIR studies have used multiple scans per individual to directly chart the longitudinal course of WMH

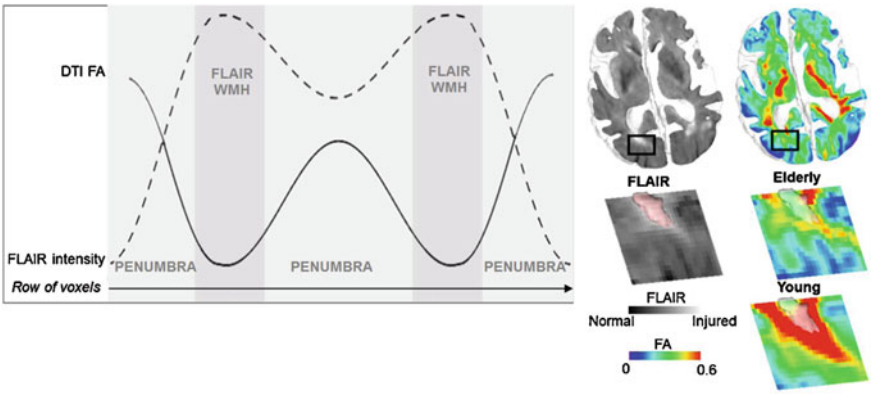

Fig. 5 *Left* White matter hyperintensities (WMHs) from FLAIR MRI may represent only the focal cores of severe white matter injury surrounded by penumbrae of more mildly injured tissue. Fractional anisotropy (FA) from DTI may provide a more graded measure of white matter integrity that captures mild injury to the WMH penumbra. *Top right* Example co-registered slices of FLAIR and FA images of a typical elderly individual. *Middle right* The location of a FLAIR-based WMH is shown overlaid as a *pink/white blob*. Compared to a typical young individual (*lower right*), FA is reduced in a broad zone that extends far beyond the WMH boundary, suggesting a more graded and spatially extended white matter injury process than is captured through WMHs. For more information, see Maillard et al. (2011)

changes in relation to risk factors and cognition, only three analogous serial DTI studies have appeared that report associations between DTI-based measures and ancillary variables of interest (Charlton et al. 2010; Teipel et al. 2010; Sullivan et al. 2010). Third, while FLAIR is a longstanding MRI technology that is relatively easy to acquire, DTI is a recent development, with attendant problems related to data reliability and biases (see Sect. 5.1 below). Thus, it remains to be seen whether FLAIR and DTI will continue to provide complementary information about aging-associated white matter changes as time goes on.

3 Relation Between DTI and Cognitive Aging Risk Factors

3.1 Age

Aging-associated changes to the microstructural properties of white matter in the absence of neurodegenerative pathology were well known from autopsy studies prior to the widespread adoption of DTI (Aboitiz et al. 1996). However, the consensus view at that time was that aging-associated white matter changes detectable in vivo were confined to white matter lesions on T2-weighted MRI; the bulk volume of white matter on conventional T1-weighted sequences, unlike that

of gray matter, did not show strong signals of decline with advancing age [see, e.g., Raz et al. (2005), Raz et al. (1997, 2004)]. However, a set of early studies finding associations between advancing age and regional DTI summary measures established DTI as a useful technology for non invasively detecting such age-related changes in vivo (Moseley 2002; Sullivan and Pfefferbaum 2006). The most consistent findings from these early region-of-interest studies were that FA decreased, and MD increased, with advancing age in cognitively intact elders; and that age-associated white matter microstructural alterations followed an anterior-to-posterior gradient, with prefrontal regions especially vulnerable to such degradation. Specific white matter regions that showed the strongest associations with advancing age included the anterior cingulate gyrus, middle frontal gyrus, genu of the corpus callosum, and centrum semiovale. Later studies largely confirmed the association between DTI measures and advancing age, although the notion that prefrontal white matter is especially vulnerable to aging effects has received mixed support. Furthermore, there has been some variability in the life course of white matter maturation and degradation across the lifespan as reported by DTI studies; they generally agree that white matter integrity follows an inverted U-shape curve over the lifespan, with developmental early-life increases in white matter integrity giving way to aging-associated degradations later in life, but it is not clear when declines in white matter integrity commence in earnest: estimates have ranged from the third to sixth decades of life.

The noteworthy advance of these studies is that they provided a new indicator of brain health that deteriorated with increasing age even among those lacking any evidence of clinically relevant neurodegenerative pathology. In addition because the measurements assayed the integrity of interregional connectivity, they provided a plausible link between brain structure and subtle cognitive declines observed in otherwise-intact individuals. However, in biological terms, the discovery of a connection between DTI and chronological age provided more questions than answers: it suggested that a set of white-matter-damaging biological processes may be acting on the brain over the lifespan, and age can serve as a proxy measure for the combined effects of all such processes, but the roles that specific biological processes—possibly related to AD, CVD, and diverse additional factors—play in promoting such microstructural white matter change remained unclear. Indeed, one of the seminal advances in aging research since the publication of the early DTI studies of aging has been the discovery that a great number of cognitively intact, community-dwelling elderly individuals may in fact be in the process of developing clinically silent neuropathology related to CVD and AD slowly and progressively over the course of years, if not decades. Understanding the effects of such pathologies on white matter microstructure could lead to prevention of age-related FA reductions, and possibly to prevention of the cognitive decline that is associated with advancing age.

3.2 Cerebrovascular Dysfunction

Due to the very high prevalence of cardiovascular disease beginning in midlife, cerebrovascular dysfunction represents a plausible biological substrate driving decline in DTI-based white matter integrity measures in advancing age. DTI measures are sensitive to gliosis, edema, and demyelination, each of which can be associated with either chronic or acute ischemic vascular changes. It is clear that DTI measures are modified in the vicinity of ischemic infarction (Chen et al. 2008). In addition, DTI summary measures suggest reduced white matter integrity in clinical conditions including CADASIL (Holtmannspotter et al. 2005) and ischemic vascular dementia (Assaf et al. 2002; Xu et al. 2010) that are characterized by severe ischemic changes, often in the absence of overt concomitant pathology (Jones et al. 1999). In addition, as noted above, DTI measures may be strongly related to FLAIR-based measures of white matter hyperintensity, which are in turn strongly linked to cerebrovascular dysfunction.

However, while DTI measures appear to indicate reduced white matter integrity in overt cerebrovascular disorders, it is less clear whether DTI can indicate subtle integrity reductions during the many years in which a constellation of vascular risk factors may be promoting progressive, clinically silent cerebrovascular dysfunction, which in turn may be reducing white matter integrity in the absence of clinically relevant behavioral changes. One study reported that, relative to matched controls, FA is reduced among individuals with a history of hypertension, and that FA is further reduced among those with small vessel disease (Nitkunan et al. 2008b). These reductions in FA correlated with a magnetic resonance spectroscopy marker of axonal loss and dysfunction (N-acetylaspartate), and the strength of the correlation increased in a stepwise fashion moving from control, to hypertensive, to small vessel disease groups. A second study noted that individuals with long-standing type 1 diabetes had significantly reduced FA relative to matched controls; moreover, in the diabetic individuals, longer disease duration and poorer glucose regulation was correlated with reduced FA (Kodl et al. 2008). A third study linked adiposity to reduced white matter integrity on DTI (Cazettes et al. 2011); this and another study found associations between decreased white matter integrity and increases in markers of systemic inflammation that commonly accompanies cerebrovascular dysfunction (Wersching et al. 2010). While these studies may have implications for the hypothesis that at least part of the age-associated declines in white matter integrity may be driven by the effects of hypertension, type two diabetes, obesity, and other cardiovascular risk factors even in the absence of an acute cerebrovascular event, a great deal more study is required to further substantiate this view. In particular, the relations between more direct biochemical or imaging-based measures of vascular dysfunction and DTI measures have not been explored to any great depth. Examples of blood markers of vascular injury that would enable such a study include inflammatory cytokines and vascular adhesion molecules; imaging markers of vascular injury, such as perfusion MRI measures of cerebral blood flow, susceptibility weighted MRI measures of microhemorrhage,

and MRI measures of atherosclerotic indices, would also be viable candidates for exploring relationships with DTI.

3.3 Genetics (APOE)

Apolipoprotein E (APOE) genotype is the most strongly validated genetic risk factor for clinical dementia of the Alzheimer type, and studies that assess relationships between DTI measures and APOE genotype typically view the relationships through this lens. From this point of view, cognitively normal elders possessing the 3-4 or 4-4 genotype are at increased risk of developing clinical AD in the future, and therefore may exhibit evidence of early AD pathology that is not so severe as to manifest itself through behavioral changes. As such, cognitively normal elderly carriers of the 3-4 or 4-4 genotype may represent a compelling in vivo model for the earliest AD-associated brain changes, and detecting such brain changes has important implications for the pathophysiology of AD pathology as well as its early detection and prognosis (Reiman et al. 1998; Strittmatter and Roses 1995; Reiman et al. 2009).

Taking a broader view, however, the APOE gene codes for an extracellular cholesterol transport protein that is involved in a wide variety of cellular metabolic pathways (Strittmatter and Bova Hill 2002; Laskowitz and Vitek 2007). Besides the AD cascade, these pathways are implicated in the development of a variety of vascular and neurological injury processes, each of which could contribute to white matter damage evident on DTI. Indeed, possession of one or two e4 alleles may promote inflammation and atherosclerosis (Kontush and Chapman 2006), and may be a risk factor for poorer long-term outcomes in traumatic brain injury (Zhou et al. 2008), hemmorhagic stroke (Martinez-Gonzalez and Sudlow 2006), and possibly multiple sclerosis (Pinholt et al. 2006). With these findings in mind, associations between APOE and DTI measures among cognitively healthy elders should be viewed within the broader context of the role APOE plays in promoting a wide variety of deleterious aging-related biological processes.

Several studies on this topic have reported that possession of at least one e4 allele late in life is independently associated with diminished white matter integrity. The regional specificity of the findings have varied, with reported APOE effects on the left hippocampal gyrus (Honea et al. 2009), across broad regions of the white matter (Heise et al. 2010), on the medial temporal lobe and splenium of the corpus callosum (Persson et al. 2006), and on the parahippocampal gyri (Nierenberg et al. 2005). The possession of both APOE and a family history of clinical AD was reported to be associated with diminished WM integrity in a range of white matter tracts among cognitively normal women (Smith et al. 2010). In addition, APOE may exacerbate the white matter damage that is associated with development of clinical AD (Wang et al. 2010). Only one study to date has examined the relative time course of APOE-related loss of white matter integrity over the life span; APOE was associated with diminished integrity in both young

adult (aged 20–35 years) and older (50–78) groups, and age did not significantly modulate the relationship, suggesting that APOE effects on white matter may in fact assert themselves in early adulthood and remain stable across the remainder of the lifespan (Heise et al. 2010). The consistent pattern of these studies is a detrimental effect of APOE on white matter integrity, possibly due to many biological mechanisms that APOE is linked to.

3.4 Amyloid

Under the amyloid hypothesis of AD, the production and aggregation of amyloid beta is the primary biological event that triggers a variety of secondary injury processes that, acting together, compromise the structure and function of the brain, eventually leading to the profound loss of cognitive function characteristic of clinical AD. Although this pathological cascade has been traditionally viewed as preferentially damaging neuronal cell bodies, dendrites, and synapses, a growing body of DTI studies have suggested that the presence of clinical AD and its clinical precursor, mild cognitive impairment, is associated with diminished white matter integrity as well (see Sect. 4.2). Whether these effects represent secondary results of primary injury to the gray matter, as opposed to primary injury processes acting directly on axons, myelin, and glia, is currently unclear. However, what is clear is that an advanced stage of the AD pathological cascade, characterized by clinically significant cognitive impairment, is associated with diminished white matter integrity as measured by DTI.

The motivation for more specifically studying the relationship between brain amyloid burden and DTI measures is provided by the alarming number of elderly individuals who show no clinically significant cognitive deficit, yet already harbor significant levels of brain amyloid (Aizenstein et al. 2008). These individuals may represent the earliest detectable stage of the AD pathology process: they already show subtle gross anatomical, functional, and neuropsychological deficits (Fagan et al. 2009; Sperling et al. 2009; Pike et al. 2007), and many of them are likely to develop clinical AD in the long term (Morris et al. 2009). However, among cognitively intact elders, the impact of significant brain amyloid on white matter connections is not entirely clear. Studies of white matter changes among clinical AD patients have been limited in addressing this issue because at that later stage of the AD pathology process, white matter deficits may reflect the accumulated effects of amyloid, secondary AD-related injury processes, and possibly injury related to very common concomitant pathology (Cummings 2004; Schneider et al. 2007). Elucidating this issue could clarify the relevance to patients of proposed clinical diagnostic criteria defined by amyloid burden in the absence of significant cognitive impairment (Blennow and Zetterberg 2010).

The scientific rationale for a specific relationship between amyloid and white matter integrity arises primarily from evidence that amyloid beta exacerbates cerebrovascular dysfunction, which in turn promotes white matter disruptions

(Smith and Greenberg 2009; Whitehead et al. 2007). In addition, there is at least one intriguing report that amyloid may more directly impact white matter integrity, by inhibiting the myelin production functionality of mature oligodendrocytes (Horiuchi et al. 2010). However, published data on direct relationships between amyloid burden and DTI measures in asymptomatic elders has been extremely limited. In vivo human data to date has been limited to demonstrations of reduced white matter integrity among individuals clinically diagnosed with intracerebral hemorrhage caused by amyloid deposition in blood vessels (Viswanathan et al. 2008; Salat et al. 2006). Meanwhile, one DTI study of transgenic mice that produce amyloid describes progressive degradation of white matter integrity over the lifespan coinciding with the approximate time course of amyloid plaque accumulation (Sun et al. 2005) [See, however, Harms et al. (2006)].

4 Relation Between DTI and Cognition in the Elderly

4.1 Across the Span of Cognitive Trajectory

Studies relating DTI measures to cognitive function have been driven by diverse hypotheses about the effects of white matter disruption on cognitive aging. Many have emphasized frontal-mediated cognitive domains, especially executive control, following suggestions that frontal white matter is preferentially vulnerable to aging-related disruption (Malloy et al. 2007). Others have focused on the hypothesis that impaired network connectivity leads to declines in processing speed in addition to executive control (Madden et al. 2009a). Finally, the importance of aging-associated white matter disconnection may be that it impairs compensatory recruitment that is required to maintain task performance in the face of gradual and progressive degradation of gray matter during normal cognitive aging (Sullivan and Pfefferbaum 2006; Cabeza 2002). DTI remains one of the most useful tools for testing such disconnection hypotheses in vivo.

The earliest reports of relations between DTI and cognition in cognitively intact elders focused on univariate white matter integrity measures along the interhemispheric tracts. The integrity of the splenium of the corpus callosum and parietal pericallosal regions correlated with reaction time (RT) for alternating, but not unimanual, finger tapping, (Sullivan et al. 2001), and MD in anterior white matter tracts, which allow communication between frontal lobes, was correlated with reduced executive control and attentional set-shifting independent of age (O'Sullivan et al. 2001). Associations between white matter integrity and performance on a visual target detection task may vary with age: in younger adults, splenium FA best predicted reaction time, while in older adults, FA in the anterior limb of the internal capsule was the best predictor (Madden et al. 2004). Meanwhile, reduced working memory performance in older individuals may be associated with reduced FA in frontal white matter and increased MD in frontal

and posterior white matter, independent of age (Charlton et al. 2006). Together, these results suggest that DTI may be assaying the integrity of connections within distributed cognitive networks that are recruited for efficient information transfer in frontal-mediated cognitive tasks.

More recent tractography-based results are largely consistent with a pattern of age-related frontal white matter integrity declines predicting executive control deficits. Poorer performance on the Stroop Test, generally considered a measure of executive control, was associated with lower FA in premotor callosal fibers and post-central callosal fibers (Sullivan et al. 2006), and FA in the genu and right superior longitudinal fasciculus was reported to mediate the age-related variability in performance on a task-switching paradigm (Madden et al. 2009b). These and many other results imply that reductions in integrity of specific tracts are differentially correlated with declines in varied components of executive control.

There are limited reports in normal older adults of relations between DTI measures and episodic memory deficits. One fiber tracking study of older and younger adults found that while FA in anterior white matter tracts including the genu and uncinate fasciculus was associated with executive control in agreement with the studies described above, while FA in posterior tracts including the cingulum bundle, inferior longitudinal fasciculus, and splenium, was related to memory performance (Davis et al. 2009).

4.2 Between Clinical Diagnostic Groups

Progressive neurodegenerative diseases that underlie clinical dementia syndromes are increasingly being thought of from the point of view of cognitive network disconnection (Seeley et al. 2009). Comparisons of DTI-based network connectivity measures between healthy individuals and those diagnosed with clinical dementia may illuminate the relations between network degeneration and the spatial patterning of disease, providing guidance for network-based disease monitoring and treatment (Palop et al. 2006).

Most DTI studies on clinical dementia syndromes to date have focused on AD and MCI. Early investigations generally indicated increased MD and reduced FA in MCI and AD, particularly in long white matter tracts innervating association cortex (Moseley et al. 2002). In many of these studies, the degree of white matter disruption was associated with the degree of memory dysfunction or scores on the Mini Mental State Examination [MMSE, (Folstein et al. 1975)]. Most of these reports describe white matter deterioration in posterior regions. For example, in one report (Rose et al. 2000), poorer MMSE score among AD patients was correlated with decreased diffusion anisotropy in the splenium of the corpus callosum. Among amnestic MCI patients and healthy age-matched controls, the same group reported that reduced FA in posterior white matter regions was associated with reduced cognitive performance, particularly in memory (Rose et al. 2006; Medina and Gaviria 2008).

One study reported that white matter alterations in temporal, frontal, and parietal regions were more pronounced as diagnostic status declined from normal cognition, to MCI, to clinical AD (Huang et al. 2007). Across all subjects, lower temporal white matter FA and higher diffusivity correlated with poorer episodic memory performance, lower frontal white matter FA correlated with poorer executive control, and higher parietal white matter RD correlated with poorer visuospatial function. These findings support the hypothesis that reduced connectivity may underlie decline of cognitive networks in dementia. Additionally, vascular-related changes to white matter integrity that are coexistent with MCI and AD may contribute to dysfunction of neural networks, particular those connecting prefrontal cortex to its targets. Such disconnection, combined with known hippocampal injury, may contribute to cognitive declines observed in these conditions (Mayda et al. 2009).

Few studies have examined the differential effects of degenerative processes, such as AD, and vascular injury processes on brain structure, including white matter connectivity. In one such study of cognitively normal, MCI, and AD participants (Lee et al. 2009), clinical diagnosis was used as an indicator of neurodegenerative disease burden, and a vascular risk score indicated overall cerebrovascular disease burden. These were independently associated with FA in the corpus callosum as well as subcortical white matter, with spatially independent patterns of microstructural damage across diagnostic groups. Neurodegeneration primarily affected FA in more organized white matter tracts, while vascular factors contributed to FA reductions in less organized white matter, which may partially explain functional differences. Further evidence (Lee et al. 2010) confirmed that vascular and degenerative processes make differential, region-specific contributions to white matter impairment as measured by DTI, across the spectrum of cognitive ability, and further suggested that differential patterns of structural injury, and thus functional decline, may be contingent on connectivity changes.

Relatively few studies have investigated relations between DTI and cognition in patients with frontotemporal dementia (FTD). In one study comparing FTD and AD with healthy aging, FTD patients demonstrated reduced FA in frontal and temporal white matter tracts, while AD patients demonstrated reduced FA in parietal, temporal and frontal tracts (Zhang et al. 2009). In direct comparison with AD, FTD patients demonstrated greater reductions overall, particularly in frontal FA; however, due to difficulty of matching subjects based on MMSE performance, the FTD cohort may have been more demented. Such characteristic signatures of white matter impairment may allow for improved differential diagnostic power.

Most studies of FTD subtypes which have distinctive cognitive sequelae, such as the behavioral variant of FTD (bvFTD), semantic dementia (SD), and Progressive non-fluent aphasia (PNFA), have only explored associations between DTI measures and diagnostic status and not specific cognitive tasks. In one recent study, bvFTD patients exhibited decreased FA and increased diffusivity measures in white matter connecting frontal with temporal and parietal lobes, while SD patients showed similar diffusion in tracts such as inferior longitudinal fasciculus and uncinate fasciculus, and PNFA patients showed these tract abnormalities in the

superior longitudinal fasciculus (Whitwell et al. 2010). Clearly, further investigations of DTI-cognition relations in FTD subtypes are needed.

5 Future Directions

5.1 Advances in DTI Methodology

Methods for acquiring high-resolution, anatomically accurate water diffusion MRI data are developing rapidly, and there are several pressing challenges that need to be addressed by methodologists to advance the utility of DTI as a tool to study the cognitive aging process.

The most pressing methodological challenge may be the development of acquisition and post-processing techniques that provide reliable white matter integrity measurements across scan sessions and scanner operating equipment. The few published reports on DTI reliability suggest that white matter integrity and connectivity measurements do vary substantially simply by acquiring DTI data on a different device, or on a different day (Pfefferbaum et al. 2003; Takao et al. 2011; Vollmar et al. 2010). It is currently unclear whether the variability stems from differences in scan prescription, measurement noise, brittleness of post-processing pipelines, or some combination of each. Interscanner variability has made large-scale, multi-site DTI studies difficult to justify, and has made reported DTI findings difficult to replicate. Intraindividual variability in DTI measurements have dampened enthusiasm for the use of DTI as a tool for measurement of longitudinal change, which is critical both for clarifying the biological course of aging-associated white matter changes and for assessment of DTI-based endpoints in clinical trials. These issues represent the key barrier to fully delineating the utility and limitations of DTI as it relates to the study of cognitive aging.

Fortunately, there are tentative signs pointing to the possibility of reliable, serial DTI data collection across multiple scanners in the future. Considerable attention is being paid to the development of compelling physical phantoms that emulate the macroscopic configuration and water diffusion properties of white matter tracts [for example, Klein et al. (2008)]. Such phantoms can play a pivotal role in evaluating the validity of DTI measurement methods and for harmonizing DTI data across scanners. In addition, the Alzheimer's Disease Neuroimaging Initiative (ADNI), whose unprecedented investment in cross-scanner standardization has dramatically boosted the viability of large-scale, multi-site collection of quantitative T1-weighted elderly brain MRI, has recently made a similar investment in DTI (Jack et al. 2010). Finally, there is at least one report that sophisticated post-processing methods may be able to play a major role in ameliorating differences in DTI data across scanners and scan sessions, and the continued development of such methods is a major focus of the computational neuroimaging community (Vollmar et al. 2010).

The mathematics of representing and manipulating DTI data is another major methodological direction. How best to represent the complicated spatial statistics

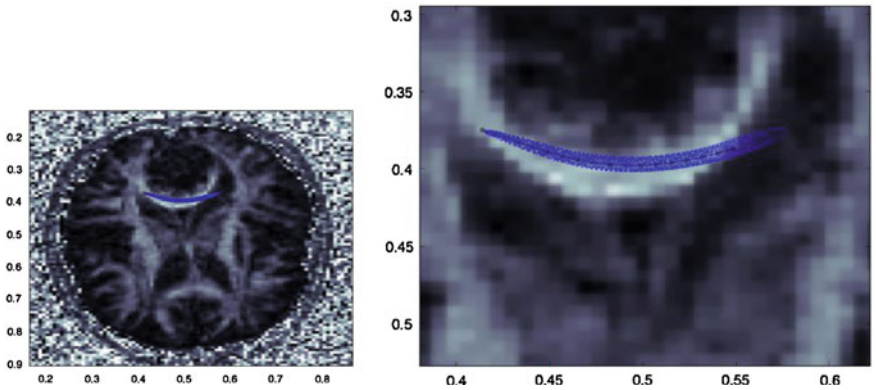

Fig. 6 New mathematical methods for estimating uncertainty in DTI-based white matter connectivity measurements could help to make DTI a more robust and reliable measurement technology. *Left* A slice from an FA map of a typical elderly individual. *Right* In an expanded view of the splenium of the corpus callosum, one white matter fiber tracing is shown as a thin *black curve*. The *blue* envelope around that curve represents uncertainty in the fiber trajectory: due to limited spatial resolution and noise in the acquired DTI data, the true fiber trajectory may actually lie anywhere within the envelope. Such uncertainty information may allow users to quantify the effects of DTI data quality on connectivity measurements, account for data quality in downstream analyses, and identify corrupted scans for which uncertainty is especially high [see Koltchinskii et al. (2007) for more information]

of water diffusion in a way that is relatively stable across scan sessions, computationally feasible, and accurate, is an ongoing research problem among computational scientists. In addition, the complexity of fiber tract trajectories in brain, together with limitations in MRI acquisition technology, make uncertainty in the spatial statistics of water diffusion prominent, and several empirical and analytic methods have been developed for quantifying how confident we should be in our estimated white matter integrity and connectivity measures (Chang et al. 2007; Zhu et al. 2008; Behrens et al. 2003; Koltchinskii et al. 2007; Sakhanenko 2008) (Fig. 6). However, while this uncertainty information can help practitioners make the critical distinction between tract trajectories that are fairly certain, and those that may be artifactual, such uncertainty information is currently very rarely leveraged in real analyses. The incorporation of this uncertainty information throughout data analytic pathways is expected to boost the viability of DTI in cognitive aging as time goes on.

5.2 Needs in Relation to DTI and Risk Factors

As a non-invasive, broadly tolerated technology, DTI seems to be uniquely placed to allow us to observe how white matter connectivity evolves with advancing age. Studies that combine serial DTI measurement with concurrent measurement of

neuropathological processes presumably represent the most viable path toward understanding the relative time course of late-life white matter changes and how that time course is modulated by a complex array of inter-connected biological events. Yet, to our knowledge, there may be as few as three published studies related to cognitive aging that involve multiple DTI scans per human subject (Charlton et al. 2010; Teipel et al. 2010; Sullivan et al. 2010). As noted above methodological issues related to DTI measurement reliability have made serial DTI studies difficult, but their importance for decoding the biology of cognitive aging is clear, and they will remain a future direction of study. In addition, studies that isolate biomarkers of individual neuropathological processes, including AD, CVD, inflammation, and others, and relate those to DTI measurements will be important for our understanding of the differential contributions that each of these processes make in determining the biological substrate on which age-related cognitive decline plays itself out.

5.3 Needs in Relation to DTI and Cognitive Networks

Incorporating measures of functional connectivity using functional magnetic resonance imaging will allow researchers to further investigate the extent to which loss of nodal and network function both depends on and causes changes in white matter connectivity (Madden et al. 2010; Madden et al. 2007). In addition, incorporation of additional modalities such as EEG, MEG, and intracranial recording will allow enhanced understanding of the relationships between brain structure and the spatial and temporal properties of cognitive networks [e.g., Westlye et al. (2009)].

An obstacle may exist in the relatively primitive psychological ontology currently in use in a majority of cognitive neuroscience research laboratories, and the manner in which we relate highly debatable cognitive processes to measured brain structure and function (Bunzl et al. 2010). The problem in localizing functions to discrete nodes is that processing may not be modular at all, in that there may not be a one-to-one mapping between a brain region and a specific cognitive function. Certainly, a better comprehension is required regarding localization as it relates to our understanding of fundamental categories of cognition.

6 Conclusion

Diffusion tensor MRI provides a non-invasive, in vivo technology for measuring the integrity of brain connectivity that is required for higher-order cognitive function but declines with advancing age. Its white matter injury measurements have the potential to provide an anatomical substrate that links a variety of aging-related pathophysiological mechanisms to the cognitive decline that is common in

the elderly. While the past decade of studies generally support the view that reductions in DTI-based white matter integrity measures are associated with advancing age and cognitive decline, a precise understanding of the biological mechanisms that lead to white matter integrity reductions and subsequent cognitive losses in the elderly is still being developed. As methods for DTI acquisition and postprocessing continue to develop and enable large-scale, longitudinal research studies, pressing questions about relations between risk and protective factors, white matter integrity, and clinically relevant cognitive outcomes will be addressed. These studies in turn will clarify the utility of DTI as a marker of aging-associated cognitive decline for clinical diagnosis and clinical trials.

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Structural, Functional and Spectroscopic MRI Studies of Methamphetamine Addiction

Ruth Salo and Catherine Fassbender

Abstract This chapter reviews selected neuroimaging findings related to long-term amphetamine and methamphetamine (MA) use. An overview of structural and functional (fMRI) MR studies, Diffusion Tensor Imaging (DTI), Magnetic Resonance Spectroscopy (MRS) and Positron Emission Tomography (PET) studies conducted in long-term MA abusers is presented. The focus of this chapter is to present the relevant studies as tools to understand brain changes following drug abstinence and recovery from addiction. The behavioral relevance of these neuroimaging studies is discussed as they relate to clinical symptoms and treatment. Within each imaging section this chapter includes a discussion of the relevant imaging studies as they relate to patterns of drug use (i.e., duration of MA use, cumulative lifetime dose and time MA abstinent) as well as an overview of studies that link the imaging findings to cognitive measures. In our conclusion we discuss some of the future directions of neuroimaging as it relates to the pathophysiology of addiction.

Keywords Methamphetamine · Amphetamine · Imaging · Structural · Functional · Addiction

Contents

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Acronyms

5-HT	Serotonin
AC	Anterior commissure
ACC	Anterior cingulate cortex
ADC	Apparent diffusion coefficient
BDI	Beck Depression Inventory
BOLD	Blood-oxygen-level dependence
BPRS	Brief Psychiatric Rating Scale
BSI	Brief Symptom Inventory
C	Coherence index
CC	Corpus callosum
Cho	Choline
Cr	Creatine
CSF	Cerebral spinal fluid
DAT	Dopamine transporter
DD	Delay discounting
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
fMRI	Functional magnetic resonance imaging
Glu	Glutamate
GM	Gray matter
GLX	Glutamate + Glutamine
HIV	Human immunodeficiency virus
MA	Methamphetamine
mI	Myo-inositol
MRI	Magnetic resonance imaging

MRS	Magnetic resonance spectroscopy
NAA	N-acetyl aspartate
NAc	Nucleus accumbens
NMR	Nuclear magnetic resonance
OFC	Orbital frontal cortex
PC	Posterior commissure
PD	Parkinson's disease
PFC	Prefrontal cortex
PCr	Phosphocreatine
PET	Positron emission tomography
PVC	Primary visual cortex
ROI	Region of interest
RT	Reaction time
SCID	Structured clinical interview for DSM-IV
SCL-90	Symptom checklist-90
SERT	Serotonin transporter
VBM	Voxel-based morphometry
VMAT	Vesicular monoamine transporter
WCST	Wisconsin card sort test
WM	White matter
WURS	Wender Utah Rating Scale

1 Introduction

Unlike most organs of the body, the brain has been less amenable to in vivo investigation until recent times. Researchers now have the capability to examine the fine structure and physiology of an intact brain in vivo with different imaging modalities. These imaging techniques have greatly increased our understanding of healthy brain function as well as that of neuropsychiatric disorders, including addiction. In this chapter we will review selected neuroimaging findings related to long-term amphetamine and methamphetamine (MA) use. We will present an overview of structural and functional (fMRI) MR studies, Diffusion Tensor Imaging (DTI), Magnetic Resonance Spectroscopy (MRS) and Positron Emission Tomography (PET) studies that have been conducted in long-term MA abusers. We will also discuss the relevant studies as tools to understand brain changes following drug abstinence and recovery from addiction. Imaging techniques have great potential to elucidate the interaction between neuronal changes, drug use patterns and clinical symptomatology. Furthermore, correlations between brain structure and function with measures of cognitive function have been observed, suggesting that altered neuronal function within select regions may contribute to impairments in behavioral regulation linked to addiction (Chung et al. 2007;

Salo et al. 2007; Sung et al. 2007; Kim et al. 2009a). Group differences in brain structure and function are by themselves important findings, but meaningful associations with drug use patterns and behavior makes them even more clinically relevant. To that end within each imaging section we have included a discussion of the relevant imaging studies as they relate to clinical patterns of drug use (i.e., duration of MA use, cumulative lifetime dose and time MA abstinent) as well as an overview of studies that link the imaging findings to cognitive measures. We have included the majority of imaging studies that were referenced on PubMed in this chapter that focused primarily on MA and to a lesser degree amphetamine. We have not included those studies that involved samples of polydrug abusers, prenatal MA exposure or those that examined the comorbidity of HIV + MA or the comorbidity of MA use with other psychiatric disorders such as schizophrenia and bipolar. The reason for this selective approach is that we felt that the inclusion of other disorders could mask the underlying neural changes induced by MA alone and could weaken the interpretation of the imaging results as it relates to MA. Finally, in our conclusion we will discuss some of the future directions of MRI and PET as they relate to the pathophysiology of substance use disorders and their treatment.

2 Magnetic Resonance Imaging Techniques

MRI has evolved considerably over the past nearly three decades. Currently with higher field magnets and advanced pulse sequence designs one is able to obtain high-resolution structural images of the brain with sub millimeter resolution. Such improved instrumentation allows for contrast between tissue types allowing one to separate/segment brain tissue into gray matter (GM), white matter (WM) and cerebral spinal fluid (CSF). We are thus able to measure tissue volumes for the various brain structures in normal and clinical populations.

Magnetic resonance imaging (MRI) is based upon the phenomenon of nuclear magnetic resonance (NMR). NMR is the physical property by which nuclei of certain elements—such as hydrogen—when placed in a strong magnetic field and exposed to radiowaves of a particular frequency, resonate or emit energy of the same frequency that can be detected as an NMR signal. This NMR signal can be localized in space by applying magnetic field gradients in three directions; subsequently, the localized signals can be converted into an image. The basic MRI requires a strong primary magnetic field (B0) that is parallel with the long axis of the tube-shaped device within which a subject lies. This large primary magnet generates these magnetic fields that align the hydrogen molecules (protons) within that field. The magnetic field gradient added to the main field allows one to select a slice for imaging. The radiofrequency pulses applied perpendicularly to the main field at the same time as the magnetic field gradient perturb the aligned hydrogen molecules of the proscribed slice. These perturbed hydrogen molecules then precess about the axis of the primary magnetic field (B0) and come back to the initial alignment. The pattern of decay of the signal

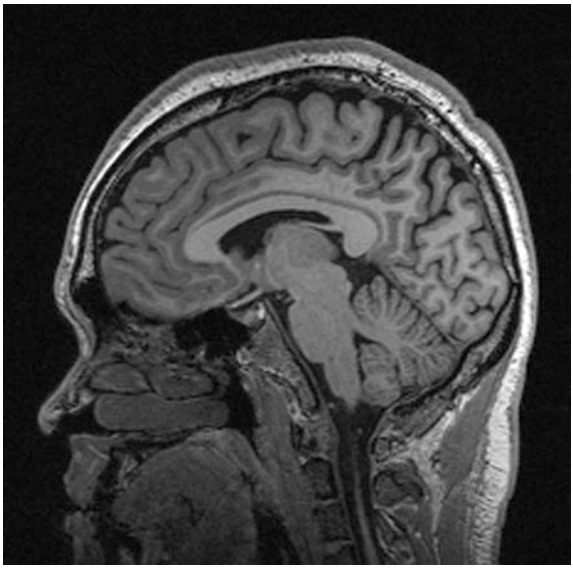

Fig. 1 T1 weighted MR image acquired in the sagittal plane

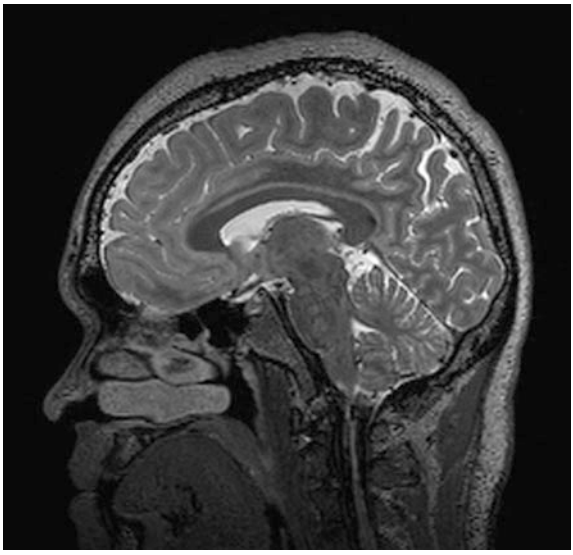

Fig. 2 T2 weighted MR image acquired in the sagittal plane

as the precessing protons come back into initial alignment gives information about the concentration of the signal source, the hydrogen atoms in water being imaged, but also reflects the chemical environment in which the signal source is found. Two characteristic relaxation times, T1 and T2, characterize the chemical environment (Figs. 1, 2).

2.1 Structural MR Imaging Studies of Methamphetamine (MA) Abuse

Evidence that MA induces neuronal damage comes for the most part from the animal literature. Surprisingly relatively few structural brain studies have been conducted in human MA abusers and some of those have been post-mortem examinations whereas others have employed in vivo imaging. Exposure to high doses of MA and related stimulants has been shown to cause long-term changes in the dopaminergic (Ricaurte et al. 1980; Wagner et al. 1980a, b; Ricaurte et al. 1982, 1984) as well as in the serotonergic system (O'Hearn et al. 1988; Axt and Molliver 1991; Zhou and Bledsoe 1996) in humans and non-human primates. Damage has been seen in frontostriatal regions subserving selective attention such as the striatum, frontal cortex and amygdala (Ricaurte et al. 1982; Villemagne et al. 1998) as well as regions subserving memory such as hippocampus and amygdala (Melega et al. 1996). Consistent with the animal work, an early post-mortem study of the brains of MA abusers revealed reduced dopamine transporter (DAT) levels in the nucleus accumbens (NAc), caudate and putamen (Wilson et al. 1996). This study will be discussed in greater detail later in the chapter. There is less evidence of structural changes in posterior brain regions such as visual cortex following MA exposure (Molliver et al. 1990).

Those structural imaging studies that have been published in human amphetamine/MA abusers have revealed patterns of damage consistent with the animal work (Bartzokis et al. 2000; Thompson et al. 2004). One of the first structural MR studies of amphetamine abusers was conducted on a low-field (1.5 T) machine and compared regional brain volumes in nine amphetamine-dependent males, 10 cocaine-dependent males and 16 non-substance abusing male controls (Bartzokis et al. 2000). Both stimulant abusing groups exhibited smaller temporal lobe volumes compared to the controls, localized primarily to GM with no differences observed in the frontal lobes. Although the two stimulant abusing groups did not differ from each other on regional brain volumes, the cocaine abusers exhibited a unique profile in that they exhibited an age-related decline whereas the amphetamine abusers did not. As shall be seen in subsequent structural MR studies of MA abuse, WM hypertrophy was also observed in conjunction with GM decline, and was more evident in the amphetamine abusers than in the cocaine abusing group. A more recent high-resolution structural MRI study later reported evidence of structural abnormalities in chronic MA abusers (Thompson et al. 2004). Cortical maps obtained at 3 T revealed severe GM deficits in the cingulate, hippocampus and paralimbic cortices. On average, MA abusers had 7.8% smaller hippocampal volumes than control subjects and reductions of 11.3% within the cingulate and paralimbic cortices. In conjunction with the GM reductions, WM hypertrophy was also observed in the MA abusers. These results suggest that MA abuse may target hippocampal regions as well as the cingulate-limbic cortex.

In addition to the structural abnormalities reviewed above in the temporal and paralimbic cortices, one study examined the effects of MA on the corpus callosum

(CC) of 27 MA abusers compared to controls (Oh et al. 2005). In this study, automated shape analysis techniques were employed to detect whether group differences might exist within subregions of the CC. Although no group differences in overall CC volume were observed, differential patterns were observed in the curvature and the width of distinct CC sub regions between the two groups. In the boundary model-based shape analysis, decreased width was observed in the posterior midbody and isthmus of the MA abusers, while increased curvature was observed in the genu of the MA abusers. CC abnormalities will be discussed further in the DTI section within this chapter. Although the studies reviewed above have described a consistent pattern of GM reduction following long-term MA abuse, they did not report volumetric changes within striatal regions. However, one study did report evidence of increased striatal volume in a large group of 50 MA abusers who had been drug abstinent more than two years (Chang et al. 2005). The authors suggested that possible mechanisms for the striatal enlargement may include glial activation and inflammatory changes associated with MA-induced injury which in turn reflects a compensatory response to the neurotoxic effects of MA.

A pair of studies used voxel-based morphometry (VBM) to examine volume differences in gray and WM segmented tissue in MA abusers (Schwartz et al. 2010; Kim et al. 2006). The first of the two published studies obtained imaging data from 29 MA abusers and 20 non-substance abusing controls in order to detect group differences as well as to investigate the relationship of drug sobriety on brain volumes discussed below (Kim et al. 2006). GM density decreases were observed in the mid-prefrontal gyrus with no differences observed in WM. The authors also reported volume reductions in the left medial frontal gyrus, right mid-frontal gyrus and right precentral gyrus of the MA abusers; however, when statistical corrections for multiple comparisons were applied these region group differences were no longer statistically significant. A subsequent study also used VBM to examine structural changes in 61 MA-dependent individuals in the early stages of abstinence (Schwartz et al. 2010). In this study lower cortical GM density was observed in the MA abusers within the bilateral insular regions and the left MFG? The authors also observed a density increase within regions of the cerebellum.

2.1.1 Volumetric Findings and Drug Use Patterns

The ability to link changes in brain volume following MA abuse and patterns of drug use (e.g., duration of MA use and length of MA sobriety) is an important endeavor as it provides insight to which brain regions are more susceptible to the neurotoxic effects of MA. At the same time, such approaches offer a valuable opportunity to detect whether or not select brain regions may be resilient following extended periods of MA sobriety. Although the body of structural imaging studies in MA abusers is relatively small to date, some, but not all, have linked observed changes to drug use patterns. One such study of 50 MA abusers reported that those subjects with smaller striatal regions reported the greatest amount of lifetime MA use (Chang et al. 2005). No results were reported in relation length

of drug abstinence. In another study that employed VBM techniques relationships with drug abstinence were examined and reported. In this study lower GM density within the right midfrontal cortex was observed in the MA abusers with short-term abstinence (<6 months) compared to the MA abusers who had remained drug abstinent for longer periods of time (>6 months) (Kim et al. 2006). In fact those MA abusers with long-term MA abstinence had GM volumes that approached those of controls suggesting possibly some degree of normalization following extended periods of drug sobriety.

The relationship between drug abstinence and structural abnormalities was also examined in a recent VBM study (Schwartz et al. 2010). This study of 61 abstinent MA abusers (age range 20–63) revealed that increased time of MA abstinence correlated significantly with increased GM density in the bilateral amygdala, striatum, left fusiform gyrus and right cerebellar tonsil. In addition, a negative correlation was observed between time MA abstinent and cortical density within the right midfrontal gyrus. No correlations were reported for duration of use or cumulative dosage.

2.1.2 Volumetric Findings and Cognitive Function

Several of the studies reviewed above analyzed the relationship between brain volume and behavioral measures. In one of the first studies to correlate changes in brain volume with cognition, reductions in hippocampal volumes were found to correlate with performance on a test of word-recall, such that those MA abusers with the lowest test scores had the most pronounced reductions within the hippocampus (Thompson et al. 2004). Findings of reductions in striatal volume have also been related to measures of cognition. In a study of 50 MA abusers those with smaller striatal volume were more impaired on tasks of both verbal fluency and speeded motor function (Chang et al. 2005). These findings are consistent with neurological studies that have implicated these same regions. Findings from the VBM study reviewed above also revealed neural-behavioral correlations. In this study, those MA abusers who scored the lowest on the Wisconsin Card Sort Test (WCST), a task that measures multiple executive functions had the lowest GM density within the right mid frontal cortex (Kim et al. 2006). This correlation was the strongest in those MA abusers with short-term abstinence. As with many of the studies within this review, correlational findings were observed only in the MA abusing group and not in the controls. This lack of observed correlations in the controls may be due in part to small sample sizes.

A recent VBM study examined the relationship between impulsive behavior and structural abnormalities (Schwartz et al. 2010). This study administered a delay discounting (DD) task to 61 MA-dependent individuals in order to measure the tendency of the MA abusers to select immediate over delayed rewards. Group differences were observed between MA abusers and controls as manifested by increased impulsivity in the MA abusers. In addition to the behavioral differences, significant positive correlations were also observed between impulsivity and GM density within bilateral putamen, left ventral striatum and the posterior cingulate cortex. Negative correlations were also observed in frontal region. To the best of

our knowledge these are the only published findings that have linked measures of cognition to changes in brain volume within MA abusers.

2.1.3 Summary

In a recent review of the MRI literature in MA abuse, Berman et al. examined a group of peer-reviewed MRI studies published between 1988 and August 2000 (Berman et al. 2008). In this comprehensive review the majority of studies reported regional brain volume reductions in the MA abusers with a preferential involvement of medial temporal lobe structures (i.e., hippocampus), and cingulate cortices. In conjunction with GM deficits there was also a consistent pattern of WM hypertrophy. Although the majority of studies reported volume reductions (Schwartz et al. 2010; Thompson et al. 2004), there was moderate evidence for enlargement within striatal regions (Chang et al. 2005). In summary, although there are relatively few structural imaging studies in human MA abusers, those that have been published suggest profound volumetric abnormalities in cingulate, temporal, hippocampal and limbic regions. These findings of structural abnormalities were accompanied by correlations with measures of cognition including verbal fluency, impulsivity, memory and tasks of executive function. Furthermore, the findings of volumetric abnormalities in the MA abusers were also found to correlate with drug use patterns (i.e., duration of drug use, time abstinent).

3 Magnetic Resonance Spectroscopy (MRS)

Magnetic resonance spectroscopy (MRS) is a valuable tool to examine the presence of neuronal damage or deterioration in addiction. MRS is based on the same principles of NMR and produces patterns of signals, or spectra, which allow visualization of neurochemicals in vivo. The individual peaks in the spectrum represent information about the chemical makeup of the tissue under examination, with time change of these spectra following the changes in concentrations of biological compounds. MRS allows for the visualization of diverse group of markers of cellular integrity and function, including those of living neurons N-acetylaspartate (NAA), high-energy metabolic products creatine (Cr), cell membrane synthesis or degradation choline (Cho), glia myonositol (mI), glutamate + Glutamine (GLX) as well as glutamate (Glu).

Although structural MRI employs hydrogen protons almost exclusively, MRS uses a number of elements including hydrogen (proton), phosphorus-31, fluorine-15, lithium-7, sodium-23 and carbon-13. Compared with MRI, MRS requires that a relatively large volume be sampled in order to obtain a sufficient signal. MRS has a volume resolution of about 1 cubic centimeter from (H^1) or proton spectroscopy of metabolites, whereas 6 cubic centimeters or more are necessary for phosphorus spectroscopy. Despite these limitations, MRS has made significant contributions to the imaging of biological function, including the field of addiction.

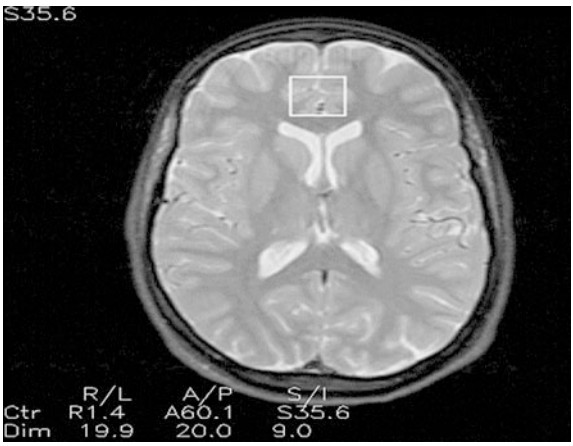

Fig. 3 Oblique axial proton density magnetic resonance image showing the typical location (*white box*) of the voxel sampling within the anterior cingulate cortex

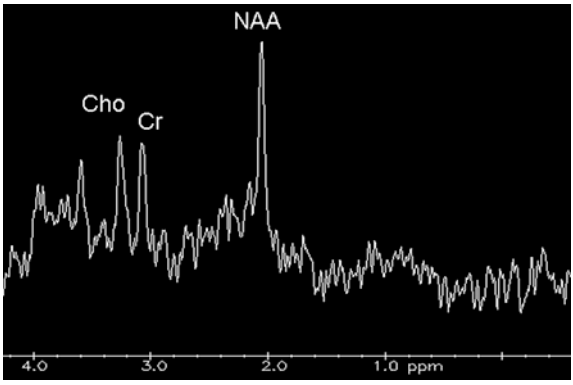

Fig. 4 Representative MRS spectra acquired at 1.5 T

Two MRS techniques are available in proton spectroscopy; single-voxel proton MRS in which a preselected region of interest (ROI) is sampled and MRSI proton magnetic resonance spectroscopy imaging in which several brain regions can be sampled simultaneously and multiple spectra obtained. Although single-voxel techniques may yield greater signal to noise ratios, MRSI possesses the ability to better define neurochemical differences between brain regions. MRS has made significant contributions to the understanding of neurochemical function to date and has great potential to contribute to the further understanding of neurochemical differences associated with addiction (Figs. 3, 4).

3.1 MRS Imaging Studies of Methamphetamine (MA) Abuse

The use of MRS to study neurochemical changes resulting from long-term MA abuse has yielded a number of important findings. Several early proton MRS studies reported evidence of frontostriatal neuronal damage in MA abusers

(Ernst et al. 2000; Taylor et al. 2000; Nordahl et al. 2002). One of the first MRS studies conducted in MA abusers found evidence of abnormally low NAA in the basal ganglia as well as in lateral frontal WM (Ernst et al. 2000). Ernst et al. interpreted the pattern of low NAA accompanied by high amounts of mI and Cho, as consistent with neuronal damage; compromised neurons with glial proliferation. These findings are consistent with the published findings from the animal literature, in which damage was also reported in frontostriatal regions (Ricaurte et al. 1982; Villemagne et al. 1998). Nordahl et al. also found evidence consistent with damage in medial frontal regions and reported reductions in NAA/Cr in the primarily GM region of the rostral Anterior Cingulate Cortex (ACC) of MA abusers compared to controls (Nordahl et al. 2002). No differences were observed in the medial Primary Visual Cortex (PVC). They interpreted the lack of PVC differences to reflect the fact that the PVC receives relatively little DA innervation and thus is less vulnerable to damage following MA abuse. Taylor et al. also reported reduced NAA/Cr within the ACC but not in the caudate nucleus (Taylor et al. 2000). In a later study of 48 abstinent MA abusers by the same group, no group differences were observed in neurometabolites between the substance abusers and controls, but longer duration of lifetime MA abuse was significantly correlated with both increases in frontal GM Cho and elevated mI levels within the basal ganglia, both of which are markers of damage (Taylor et al. 2007).

In another study that focused on striatal regions, Sekine et al. measured NAA, Cr + phosphocreatine (PCr), and Cho levels bilaterally in the basal ganglia of 13 abstinent MA abusers and 11 controls (Sekine et al. 2002). Significantly reduced Cr + PCr/Cho ratios were observed in the basal ganglia of the MA abusers but NAA/Cho ratios in the same region did not significantly differ between groups. These findings suggest that protracted use of MA may cause changes in some, but not all, metabolite alterations in the basal ganglia.

Sung et al. examined alterations in neurochemicals within both WM and GM of 30 abstinent MA abusers. In this study, conducted on a 3 T magnet, increases in mI and decreases in NAA were observed within frontal WM of the MA abusers (Sung et al. 2007). Further analyses examined the relationship of these metabolite values with patterns of drug use and cumulative drug dosage which will be discussed in a later section.

One of the neural mechanisms by which MA is thought to exert damage on the human brain is through neurotoxicity mediated by excess glutamate (Ohmori et al. 1996; Davidson et al. 2001; Quinton and Yamamoto 2006). Despite several studies conducted in animals, direct evidence of the effects of Glu on the human brain is limited. One reason for the lack of studies in this area may lie in the technical challenges in obtaining reliable measures of Glu. At lower fields (1.5 T) the ability to separate the combined glutamate signal (glutamate + Glutamine or GLX) is difficult. Thus the studies reviewed below include those that have measured GLX at lower fields (Ernst and Chang 2008) and more recent studies conducted at 3 T in which the ability to measure uncontaminated Glu is now possible (Hurd et al. 2004; Sailasuta et al. 2010a, b).

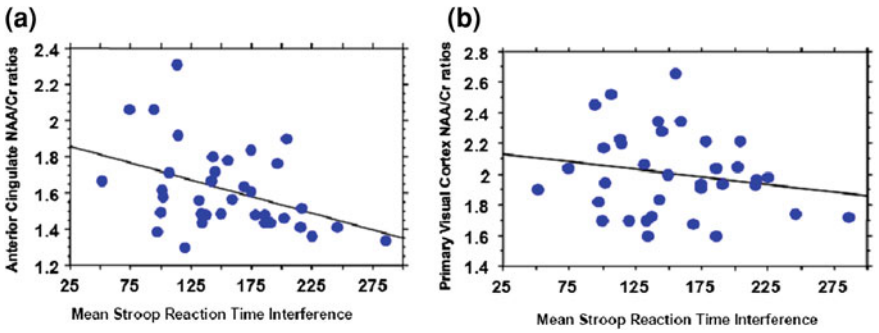

Fig. 5 Correlation between NAA/Cr metabolite levels in the anterior cingulate cortex (**a**), primary visual cortex (**b**) and mean stroop reaction time interference in 36 methamphetamine abusers. (Reprinted from Salo et al. 2007)

Ernst et all examined GLX in a group of 25 abstinent MA abusers and observed lower GLX levels in frontal GM but not in the basal ganglia compared to non-substance abusing controls (Ernst and Chang 2008). In one of the first studies to measure changes in Glu in 18 abstinent MA users, Sailasuta et al. (2010a, b). reported both increases in Glu and NAA reductions in frontal WM. Although globally Glu levels correlated with NAA levels, suggesting a possible role of Glu in mediating MA neurotoxicity, such a correlation was not observed in the frontal WM (Sailasuta et al. 2010a, b).

3.1.1 Summary

Collectively these MRS studies suggest that long-term MA use causes changes in neurochemicals that are more pronounced in frontostriatal regions, with less evidence in posterior brain regions. Most of the studies reviewed thus far have focused on NAA, Cho and MI. With increasing field strengths and technological advances scientists are now able to measure a broader range of metabolites including Glu (Hurd et al. 2004) (Fig. 5).

3.2 MRS Findings and Drug Use Patterns

MRS has proven to be a powerful imaging tool to investigate the relationship between neural changes following MA abuse and patterns of drug use (e.g., duration of MA use and length of MA sobriety). One of the first MRS studies to examine the effects of MA abstinence on neurochemicals reported that in a sample of 24 MA abusing subjects, those MA abusers who had been abstinent for 12 months and longer had lower Cho/NAA levels within the ACC compared to those MA abusers who had been MA abstinent for six months and less (Nordahl et al. 2005). This study

was followed up by an expanded dataset of 47 MA abusers in which increases (i.e., normalization) in NAA/Cr were also observed within the same rostral ACC regions (Salo et al. 2011a). The relative Cho and NAA normalization across periods of abstinence suggests that, following cessation of MA abuse, adaptive changes occur, that may contribute to some degree of normalization of neuronal structure and function in the ACC. These NAA changes may underlie improvements in cognition that have also been observed across periods of drug abstinence (Salo et al. 2009b).

Sung et al. also reported changes in neurometabolites related to patterns of MA use (Sung et al. 2007). Sung et al. reported a marginal difference in frontal NAA levels between those MA abusers with greater lifetime MA exposure (i.e., >100 g) and those with lower lifetime MA exposure. NAA reductions in those MA abusers with the longer lifetime MA exposure were greater in frontal WM than in GM. Unlike the previous MRS studies reviewed above, Sekine et al. focused on a bilateral sampling of the basal ganglia (i.e., primarily putamen) in a sample of 13 MA abusers in order to examine neurochemical differences in MA abusers (Sekine et al. 2002). In this study there was a significant correlation between the Cr + Pcr values and the duration of MA use, driven mostly by the left basal ganglia. In a later study of 48 abstinent MA abusers by the same group, no group differences were observed in neurometabolites between the substance abusers and controls, but longer duration of lifetime MA abuse was significantly correlated with both increases in frontal GM Cho and elevated mI levels within the basal ganglia (Taylor et al. 2007), both of which are markers of damage.

Changes in glutamatergic function have also been observed across periods of drug abstinence (Ernst and Chang 2008; Sailasuta et al. 2010a, b). Ernst et al. reported a varied time course of GLX levels in MA abusers across periods of abstinence. In a sample of 12 MA abusers, those who had maintained drug abstinence across periods of 12 months showed evidence of normalization in the GLX signal followed by subsequent increases (Ernst and Chang 2008). The authors suggested that these findings may reflect that the glutamate system is down regulated early in abstinence and then normalizes over time. In one of the first studies to measure changes in Glu in 18 abstinent MA users, Sailasuta et al. also reported significant reductions in Glu levels within frontal WM across periods of MA abstinence (Sailasuta et al. 2010b). No correlations were observed between time MA abstinent and posterior gray matter.

3.2.1 Summary

Many of the MRS studies reviewed above have detected significant correlations between MA dosage, years use and length of MA abstinences. The findings reveal that those MA abusers who have used the powerful stimulant for a longer period of time and at larger dosages exhibit the greatest neurometabolite abnormalities, consistent with damage. Of even greater importance is the important finding that some degree of neurochemical normalization may occur across extended periods of MA abstinence and that not all the neurometabolite changes observed following

long-term MA use are permanent. The increases in NAA observed across periods of drug abstinence may reflect a partial restoration of mitochondrial function as NAA is synthesized in the mitochondria (Moffett et al. 2007). If this is true then this is of particular relevance as mitochondrial dysfunction is thought to be one of the key mechanisms by which MA produces its neurotoxic effects on brain function (Davidson et al. 2001; Quinton and Yamamoto 2006). The findings of Cho normalization may represent an adaptive response to overt damage. a process that may include reactive gliosis, increased membrane turnover and axonal sprouting. At longer remission periods less membrane synthesis and turnover may occur potentially explaining the normalized relative choline values (Nordahl et al. 2005). The findings of GLX and Glu normalization may represent yet another set of adaptive changes by the human brain (Ernst and Chang 2008; Sailasuta et al. 2010b). Possible explanations for reductions in GLX/Glu following MA abuse might be: (1) the loss of glutamatergic neurons; (2) decreased Glu synthesis or (3) reduced Glu uptake within the glial cells (Yamamoto and Bankson 2005). While yet another potential mechanism for reductions in GLX/Glu might be a result of MA-induced oxidative stress (Tata and Yamamoto 2007). Thus normalization of the GLX/Glu signal across extended periods of sobriety may reflect a reversal of the neurotoxic processes described above. While collectively all of the findings above paint an encouraging picture of positive brain recovery associated with drug sobriety, the findings do suggest that protracted periods of drug sobriety (i.e., >12 months) may be needed for neurochemical function to normalize.

3.3 MRS Findings and Cognitive Function

Although there are many MRS studies that have reported group differences in MRS metabolites between MA abusers and non-substance abusing controls, only a small subset of these studies have linked these MRS data to cognitive measures (Salo et al. 2007). Of particular interest is the relationship between NAA and measures of cognition. NAA is believed to be present almost exclusively in neurons and their dendritic and axonal processes (Tsai and Coyle 1995). As NAA is synthesized within the mitochondria and decreases in NAA correlate with reductions in ATP, NAA can be regarded as a marker of neuronal viability that is related to the energy metabolism of the neuron (Tsai and Coyle 1995; Grachev et al. 2001; Ohrmann et al. 2004; Moffett et al. 2007). Salo et al. examined the relationship between a measure of attentional control (i.e., Stroop Task) and metabolites within the ACC and PVC. In this study of 34 abstinent MA abusers, an inverse correlation was observed between ACC-NAA/Cr and performance on the Stroop color-word selective attention task. Specifically, those MA abusers with the worst performance on the attention task had the lowest NAA/Cr levels within the ACC. No significant correlations were observed in the PVC. In a follow-up study that employed a task of spatial attention, significant correlations were observed between both ACC and PVC NAA/Cr levels in the MA abusers

(Salo et al. 2011b). These findings are of particular interest as the two groups (MA abusers and controls) did not differ in performance on the task of spatial attention. The strong correlation between spatial attention and NAA/Cr levels within the PVC in the MA-dependent individuals suggests that preserved neuronal integrity within the PVC of stimulant abusers may modulate cognitive mechanisms that process implicit spatial information. In summary, although there are only a pair of MRS studies that have correlated metabolite values with cognitive performance in MA abusers, the published findings are consistent with other MRS studies of cognition that have published in healthy control subjects (Jung et al. 1999; Grachev et al. 2001).

3.4 MRS Findings and Clinical Symptoms

There are few MRS studies that have attempted to link changes in neurometabolites with the psychiatric symptoms associated with long-term MA abuse. Given the impact of MA-induced psychiatric symptomatology on emergency rooms and health care services, the identification of the neural correlates of MA-induced psychosis and MA-related symptoms is of paramount importance. In the Sekine et al. study discussed above, reductions in Cr + PCr/Cho ratio observed in the bilateral basal ganglia correlated significantly with both duration of MA use and with the severity of residual psychiatric symptoms (Sekine et al. 2002). In this study a comprehensive approach was used to evaluate psychiatric symptoms in the MA abusers including: (1) self report; (2) family report; (3) medical record review; and (4) administration of the Brief Psychiatric Rating Scale (BPRS). Specifically, the authors found a negative correlation between Cr + PCr/Cho ratios in the basal ganglia and scores on the positive symptoms subscale of the BPRS which measures delusions and hallucinations. In other words, those MA abusers who exhibited the highest scores on the BPRS subscale were those individuals with the lowest Cr + PCr/Cho ratios in the basal ganglia. The authors interpreted this correlation to reflect that residual psychiatric symptoms may be in part attributable to the metabolite alterations in the basal ganglia.

4 Diffusion Tensor Imaging (DTI)

Diffusion tensor imaging (DTI) has emerged as a powerful non-invasive imaging tool that gives a measure of the pattern of connectivity between neural structures by examining the restricted flow patterns of water molecules in axonal pathways and white matter substrates. When water molecules are flowing randomly in CSF or cortical GM, the environment is said to be ‘isotropic’. In contrast, when the environment is constrained by WM cell membranes (i.e., organelles and axonal bundles) the diffusion is restricted such that flow is greater along the axon than

perpendicular to it. This diffusion or flow pattern is called anisotropy. DTI techniques are necessary to measure the directional flow of water molecules in anisotropic environments by combining a number of diffusion weighed images along with a baseline or reference image to characterize the flow of water molecules in three-dimensional space. Different measures can be obtained from the DTI images such as Fractional Anisotropy (FA) and Coherence indices (C). FA is computed on a voxel to voxel basis and reflects the degree or fraction of the total anisotropic tensor. FA values vary across regions and tissue types. Regions such as the CC where fibers are organized in parallel have high FA values approaching 1, whereas FA values in the ventricles CSF are closer to 0 reflecting a random or isotropic diffusion pattern. C represents the coherence between voxels in the WM. DTI techniques are extremely valuable when examining axonal integrity and connectivity of brain structures and have important applications to the study of MA abuse. Although volumetric measurements of WM macrostructure can be obtained through traditional MR techniques, currently DTI is the only in vivo tool capable of measuring WM microstructure within the human brain. To date only a handful of DTI studies have been conducted in MA abusers and the results will be reviewed below.

4.1 DTI Imaging Studies of Methamphetamine (MA) Abuse

Studies of MA exposure using animal models have suggested that chronic exposure may destroy axonal arbors of dopamine neurons with sparing of the cell bodies themselves (Ricaurte et al. 1980, 1982). Compared to the rich body of published animal studies on WM damage following MA exposure, only a small number of studies in humans have been published (Tobias et al. 2010; Thompson et al. 2004; Oh et al. 2005; Bae et al. 2006; Alicata et al. 2009; Kim et al. 2009a; Salo et al. 2009a). Although some studies have examined WM macrostructure (Oh et al. 2005; Bae et al. 2006), others have studied WM microstucture using DTI techniques (Tobias et al.; Chung et al. 2007; Kim et al. 2009a; Salo et al. 2009a). DTI has emerged as a promising imaging technique to examine WM abnormalities in both clinical (Lim et al. 2002; Lim and Helpern 2002; Pfefferbaum and Sullivan 2005; Pfefferbaum et al. 2006) and non-clinical populations (Lim and Helpern 2002; Sullivan and Pfefferbaum 2003; Madden et al. 2004; Pfefferbaum et al. 2005; Barkovich et al. 2006). Information derived from the DTI measures can then be correlated with cognitive measures in order to predict the relationship between changes in WM microstructure and behavior (Pfefferbaum et al. 2000; O'Sullivan et al. 2001; Sullivan et al. 2001; Kubicki et al. 2002; Madden et al. 2004; Kubicki et al. 2005; Schulte et al. 2005).

As long-term MA use can cause significant damage to WM, the emergence of MA imaging studies that have used DTI has been of paramount importance to the addiction literature. Findings of reduced FA may occur as a result of demyelination, as well as from axonal damage (Song et al. 2002). Morphological studies of

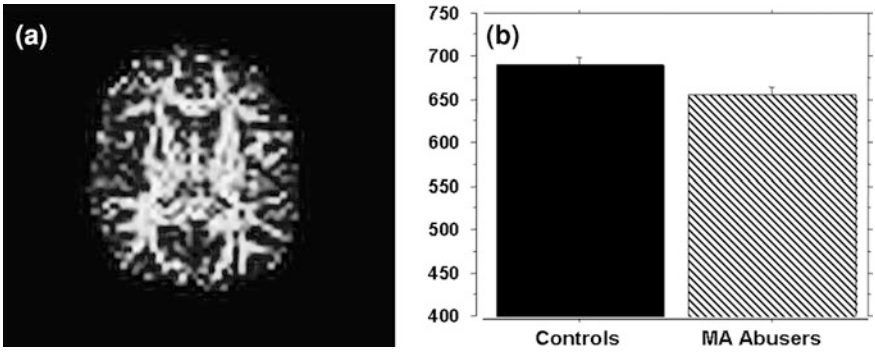

Fig. 6 Group differences in fractional anisotropy (FA) between methamphetamine (MA) abusers and controls (**b**). FA image (**a**). Salo et al. unpublished figures

animals who have been exposed to MA report findings of abnormally low presynaptic DA & 5-HT axonal markers which are related to overt destruction of DA and 5-HT axon terminals as well as the axon proper, typically sparing the nerve cell bodies (Ricaurte et al. 1980, 1982). While the exact mechanism of MA damage to axonal fibers is unknown, there is abundant evidence from animal studies that MA exposure causes both acute disintegration and more chronic fragmentation in the WM fibers of frontal, parietal and temporal cortices, as well as in the hippocampus and cingulum bundle (Zhou and Bledsoe 1996).

One of the first studies to apply DTI to the study of MA abuse reported lower FA within frontal regions of 32 MA abusers (Chung et al. 2007). These changes in WM microstructure were observed bilaterally at the level of the AC-PC plane but not in the posterior regions of the parietal and occipital cortices. Furthermore, extended analyses of gender differences revealed that these WM group differences were accounted for by the male MA abusers. A pair of recent DTI studies focused on the examination of the CC in long-term MA abusers. One study examined WM microstructure in 37 abstinent MA abusers and found differences in FA values within the genu of the CC but not in the splenium (Salo et al. 2009a). Another study also reported abnormal changes in the genu of the CC and further reported that the tensor values observed suggest that altered myelination may be one probable source of the abnormal FA values in the MA group (Kim et al. 2009a) (Fig. 6).

A later DTI study of 30 abstinent MA abusers examined both WM diffusion as well as fiber orientation within the CC, frontal WM, basal ganglia and the cerebellum (Alicata et al. 2009). In contrast to the other DTI studies reviewed above, no differences in FA values were observed in the CC but reductions in FA values were observed in the frontal WM of the MA abusers. In addition to the FA group differences, increased diffusivity was also observed in the striatum of the MA group (caudate and putamen). The authors suggest that this pattern of increased diffusivity within the striatum may reflect a neuroinflammatory response consistent with other imaging studies that have reported increased striatal volume following

MA abuse (Chang et al. 2007). The most recent DTI study of MA abusers examined WM changes in 23 MA abusers and 18 matched controls (Tobias et al.). This study revealed modest (3–12%) FA reductions in several regions, including right prefrontal WM above the AC–PC plane, bilateral midcaudal superior corona radiata, the genu of the CC, and the right perforant path adjacent to the hippocampus. The hippocampal findings were of interest due to previously published findings of structural abnormalities in the hippocampal region (Thompson et al. 2004). Furthermore, in many of these studies the degree of alteration to WM microstructure appears to be related to the duration of MA use and clinical symptoms which will be discussed in greater detail below.

4.2 DTI Findings and Drug Use Patterns

A subset of the DTI studies reviewed above correlated WM indices with drug use patterns (Alicata et al. 2009; Salo et al. 2009a). Although the study by Alicata et al. failed to detect a significant correlation between FA and drug use patterns, they did observe a positive correlation between cumulative drug usage (daily and lifetime) and ADC indices, a measure of diffusivity, within the putamen of MA abusers. Specifically, those MA abusers with the highest ADC values reported using the highest dosages of MA on both a daily basis and across lifetime. No correlations were reported between periods of drug abstinence and WM. In the Salo et al. study correlations were examined between DTI indices, years MA use and time abstinent. No significant correlations were observed (Salo et al. 2009a).

4.3 DTI Findings and Cognitive Function

A number of DTI studies have extended the imaging findings to examine relationships with behavior and cognition. Such an approach is critical to better understand how neural changes following long-term MA abuse can alter the way addicted individuals make decisions and in turn how these neural changes may promote the maladaptive behavior that sustains compulsive drug seeking behavior. Several of the DTI studies reviewed above collected concurrent data from a range of cognitive tasks and related the behavioral performance to the patterns of WM microstructure observed in their study. The first study to examine the relationship of FA values with cognitive performance administered the WCST, a task of frontal executive function (Chung et al. 2007). In this study lower FA values within frontal regions were found to correlate with worse performance on the WCST. A subsequent study also observed a negative correlation between WCST total error and FA in the genu region of the CC (Kim et al. 2009a) in a small group of abstinent MA abusers. There were no significant correlations between WCST total error and tensor measures, including FA in the splenium region of the CC.

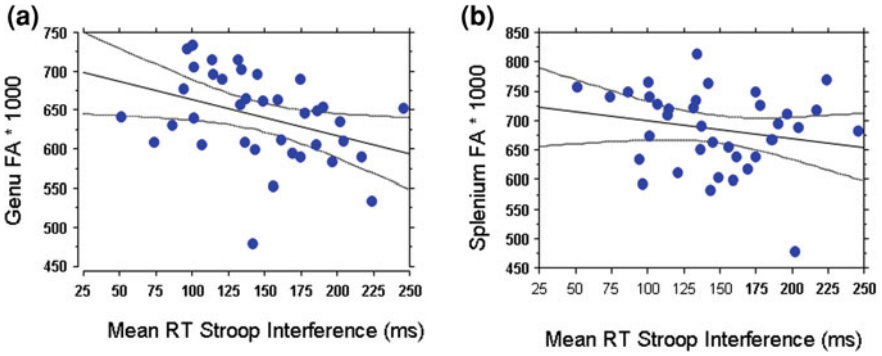

Fig. 7 Correlational scatterplots between millisecond (ms) reaction time (RT) stroop interference and fractional anisotropy (FA) 1,000 values in the (**a**) genu and (**b**) splenium of 37 methamphetamine (MA) abusers. (Reprinted from Salo et al. 2009a)

A later study administered a task of selective attention (Stroop Color Word Task) and found that even after correcting for multiple comparisons, FA within the genu correlated significantly with task performance in the MA abusers but not in controls. Since both the WCST and the Stroop task are known to engage the frontal cortex, this is strong evidence that the WM changes observed across both studies on medial and lateral frontal WM have functional correlates with behavior and cognition. A pair of recent DTI studies of cocaine abusers reported similar findings in that they observed significant correlations between FA in anterior WM and measures of impulsivity (Moeller et al. 2005; Lim et al. 2008) and suggest that the findings are applicable to a broader population of stimulant abusers. Furthermore, the observed correlations with WM connectivity in the MA abusers suggest that neural connections that are vital to top-down cognitive processes such as decision making are disrupted following long-term MA use (Fig. 7).

One possible reason for this correlation with anterior structures is the connectivity pattern of the genu itself. The fibers that cross through the genu connect right and left regions of the dorsolateral prefrontal cortex (PFC), an area with strong interconnections to the ACC (Pandya and Seltzer 1986). Both the ACC and the dorsolateral PFC are thought to mediate top-down cognitive control. Another possibility for the correlation with the genu may be that axons within the genu are usually thinner in diameter and less myelinated than those in the splenium and thus may be more susceptible to damage following long-term drug abuse (LaMantia and Rakic 1990; Aboitiz et al. 1996). In contrast, the fibers that cross through the splenium connect regions within visual association areas (Rockland and Pandya 1986), regions that are certainly involved in the execution of any visual selection task, but may not be recruited heavily by demanding tasks of behavioral control, such as the WCST and the Stroop task.

4.4 DTI Findings and Clinical Symptoms

Although there have been several DTI studies that examined the relationship between WM changes and cognition, few have correlated WM microstructural changes with the clinical and psychiatric symptoms associated with long-term MA abuse. The DTI study by Tobias et al. made an important contribution to the addiction literature by examining the relationship between clinical symptoms and WM microstructure (Tobias et al. 2010). Among the 23 MA abusers studied, the left midcaudal superior corona radiata FA values correlated positively with measures of depression, as well as a subset of general psychiatric positive symptoms (i.e., depression, paranoia). The results seem somewhat counterintuitive in that increased FA values, which generally mean healthy WM, would correlate with increased symptoms of clinical depression and psychotic symptomatology. The authors suggest that subjects with intact WM (i.e., higher FA) may be more aware of their illness and thus endorse symptoms to a greater degree.

4.4.1 Summary

Collectively the rather small body of DTI studies reviewed above points to a consistent picture of WM damage within frontal regions of the brain with relative sparing of posterior regions. Furthermore, in many of these studies the degree of alteration to WM microstructure appears to be related to the changes in executive cognitive function, duration of MA use and clinical symptoms.

5 Functional MRI

Although structural MRI has been a useful tool in pinpointing gross anatomical changes associated with a variety of clinical disorders and typical development, fMRI has allowed researchers to map differing cognitive constructs to specific brain regions in awake, performing humans. fMRI is a relatively new technology, which researchers began to use in the late 90 s to illuminate the neural correlates underlying different psychological constructs of the brain. Its potential to move the field of psychology and neuroscience forward soon became apparent. Not only does fMRI have excellent spatial resolution but it provides researchers a window into the brain as subjects actively perform cognitive tasks. As no harmful effects of fMRI have thus far been revealed, it is an ideal technique for repeated use on the same participant (e.g., in longitudinal studies or within-subject experimental designs). The use of fMRI technology has revealed impairments in a wide range of cognitive domains linked to addiction in general and specifically MA dependence.

Neuronal firing in the brain requires energy and the brain's response to this energy expenditure is to increase local blood flow to that anatomical region. The ratio of oxyhemoglobin to deoxyhemoglobin changes in that region of the brain

and fMRI utilizes the blood-oxygen-level dependence (BOLD) signal, the MRI contrast of blood deoxyhemoglobin, as an indirect measure of neuronal firing in the brain. Therefore the BOLD signal associated with certain cognitive activities can be measured and localized with very good spatial resolution to certain brain regions, as described above for structural MRI. In studies of brain activation, changes in the oxygenation of the hemoglobin are associated with external stimuli or cognitive challenge. It appears that hemoglobin changes between diamagnetic and paramagnetic states are a result of a relative change in oxygen extraction during sensory or cognitive challenge.

The hemodynamic response measured by fMRI is a relatively slow signal, taking about 4–5 s to peak and about 15–20 s in total to rise and fall. This can vary across brain regions and according to particular constructs under investigation. One consideration, which sets fMRI aside from other techniques such as PET, is the fact that there is no implicit baseline. This makes paradigm design a particularly important consideration for fMRI research. Early fMRI studies utilized a block design whereby many events of interest were presented in succession to create a large change in the BOLD signal, which was contrasted against a baseline such as rest, or were measured above a baseline of similar task activity minus the event of interest.

More recent techniques allow for event-related analyses, which allow changes in BOLD signal associated with discrete events of interest to be isolated from on-going task-related activity. However the relatively poor temporal resolution of the hemodynamic response functions remains a limiting factor with regard to separating psychological constructs, which are temporally proximal. At the current time there have been relatively few fMRI studies on MA dependence from a small number of laboratories. Some research groups have focused on cognitive control processes, others on emotion regulation and other on the interplay between systems responding to reward and those involved in control processes.

5.1 fMRI Studies of Methamphetamine (MA) Abuse

5.1.1 Decision Making and Cognitive Control

The earliest fMRI studies of MA dependence come from Paulus and colleagues (Paulus et al. 2003, 2005). This group used fMRI techniques to investigate the neural correlates of decision making in MA-dependent individuals. Understanding decision making in addiction is crucial to understanding the mechanisms by which individuals appear to make poor choices, which can lead to long-term substance abuse and dependence. The same two-choice prediction task has been used throughout these studies to investigate the neural correlates of decision making in MA addiction (Paulus et al. 2002, 2003, 2005, 2008). This task assesses the capacity to make decisions under situations of uncertain outcomes with only 50% of the responses to be reinforced with a correct response outcome. The behavioral

data from these studies consistently report that MA users are more influenced by more immediate or recent success or failure. In contrast, the controls may be more inclined to tailor their response strategy to ongoing outcomes subsequent to choices. The authors conclude that there is a strong stimulus–response association in MA users that is consistent with a dominance of habit-based learning (Paulus et al. 2003, 2005).

The imaging data from these studies showed that functional activity associated with the prediction task was greater in controls in right inferior frontal gyrus (BA 44) and left medial frontal gyrus (BA46) compared to the MA abusers (Paulus et al. 2002). In general, although controls tended to activate left MFG (BA 9/10), bilateral medial frontal gyrus (BA 10) and right OFC (BA 11) to the prediction task over the control condition, the MA users did not display any change in activity in BA 9/10. In fact, subjects with MA dependence displayed a decrease in activity in bilateral dorsolateral PFC and right orbitofrontal (OFC) in the active compared to control condition. Thus, the MA users displayed a lack of task-specific activity for this paradigm. Lastly, less activity in left insula (BA 13) and more activity in right midfrontal gyrus (BA 9) predicted more instances of decisions that were directly influenced by immediately preceding failures in MA users (Paulus et al. 2002).

In a follow-up to their 2002 study, Paulus and colleagues examined functional activity during a similar paradigm in 14 male MA abusers compared with 14 controls (Paulus et al. 2003). MA-dependent subjects were abstinent for an average of 25 days (range 6–46 days). Controls displayed success-related patterns of neural activation in OFC, dorsolateral PFC and parietal cortex. In contrast, independent of whether or not they were successful, MA users displayed less task-related activity in OFC (BA 10), dorsolateral PFC (BA 9), ACC (BA 32) and parietal cortex (BA 7). MA-dependent subjects also displayed less task-related activity in bilateral parietal cortex (BA 7/19), left post-central gyrus (BA 5) and left superior temporal gyrus (BA 39). Both MA-dependent and control participants displayed more task-related activation in the right insula (BA 13), right inferior frontal (BA 44, 45), right middle frontal gyrus (BA 9) at low error rates and least activity when the outcome was most unpredictable (i.e., 50% error rate). However, MA dependent subjects displayed different patterns of neural activity to different error rates compared to controls in a number of regions including in the left insula (BA 13), inferior frontal gyrus (BA 46), middle frontal gyrus (BA 10), precuenus (BA 7) and inferior parietal lobe (BA 40). The authors concluded that although controls activate regions more when they are successful, MA users activate more when the situation is unpredictable.

In a more recent fMRI study, the same group investigated whether functional activation during their two-choice prediction paradigm in users in early remission (3 to 4 weeks abstinent) would predict relapse in 40 MA users (Paulus et al. 2005). Participants underwent fMRI during early remission and were contacted approximately 1 year later for a follow-up interview (median interval to follow-up interview 370 days). Investigators established that 18 of the 40 participants had relapsed and 22 had not. Imaging results revealed that those Individuals who had not relapsed displayed more activity in the right hemisphere in the insula (BA 13), inferior parietal lobe (BA 40) middle temporal gyrus (BA 22), middle occipital

gyrus (BA 19), dorsolateral PFC (BA 6), posterior cingulate (BA 23), inferior frontal gyrus (BA 45), in the left caudate/putamen as well as the cingulate gyrus (BA 32) compared to those participants who had relapsed. Furthermore, activation in the right insula, right posterior cingulate and right middle temporal gyrus best differentiated between those individuals who had relapsed and those who had not. Cross validation techniques were correctly able to predict 19 out of the 22 individuals who did not relapse and predicted 17 out of 18 who relapsed (94% sensitivity; 86% specificity). Time to relapse was best predicted by activity in right midfrontal gyrus, right midtemporal gyrus and right posterior cingulate. This study is the first to date that has successfully used functional imaging data to predict risk for relapse in MA abusers. This study is of paramount importance, as it suggests that there may be cognitive and neural correlates which may identify individuals at greater risk for relapse and may be applicable to a broad range of addicted individuals.

One last study from the Paulus group examined response inhibition and the use of cues to prepare for an impending inhibitory event in 19 recently abstinent MA-dependent individuals (25–50 days) compared to 19 controls (Leland et al. 2008). The authors used a GO/NOGO paradigm with four different stimulus classes, one representing the NOGO event and three representing GO events. One class of the GO stimuli acted as a fairly accurate (87%) predictor of a NOGO stimulus while another class never preceded a NOGO. In the MA abusers the false alarm rate was lower following a cue compared to an uncued NOGO, which was not the case in controls. The authors suggested that the controls did not overtly benefit from the cue because they were already performing well on the task whereas the MA subjects may have an impaired inhibitory system. The imaging data revealed that the MA group had greater cue-related activity in the ventral and dorsal ACC compared to controls. Furthermore, more activity in the ventral ACC was associated with fewer false alarms to following cues.

fMRI techniques have also been employed to examine behavioral regulation and cognitive control processes linked to addiction and MA dependence. Salo et al. utilized a modified Stroop color-word selective attention paradigm to examine how MA abusers adjusted both their reaction time (RT) and accuracy to an experimental event based on previous events or trials (Kerns et al. 2004; Salo et al. 2009c). In this single trial version of the Stroop task trial-to-trial adjustments were measured by comparing RT to conflict trials (i.e., trials in which the word and font color were incongruent) preceded by conflict trials and RT to conflict trials preceded by non-conflict trials (i.e., trials in which the word and font color matched). While controls tended to speed their RT following a conflict trial (consistent with the literature on conflict processing), the MA abusers did not (Kerns et al. 2004). In fact, the MA abusers exhibited no trial-to-trial adjustment and performed as if the previous trial event had not entered their awareness. In other words the MA abusers did not utilize a previous exposure to an event to modify their behavior. This difference in trial-to-trial RT adjustments was found in the absence of mean error rates or general RT differences between groups. Of great importance are the imaging results linked to the trial-to-trial behavioral adjustments. While controls

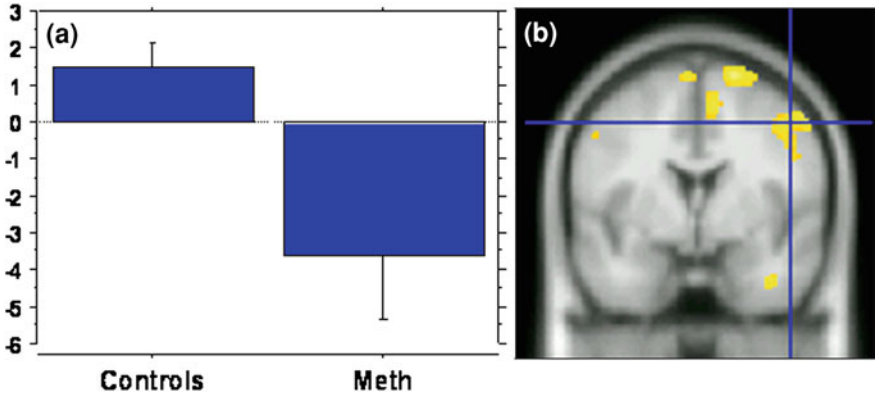

Fig. 8 **b** Dorsolateral prefrontal cortex (DLPFC) region showing increased activity related to trial to trial adjustments in control subjects relative to methamphetamine (MA) abusers. **a** Graph of the average coefficients for the trial-to-trial adjustment statistical contrast in the two groups, averaged within group for the voxels of the DLPFC activation shown. (Reprinted from Salo et al. 2009c)

displayed increased activity in right PFC following conflict trials (consistent with engagement of top-down control) the MA abusers failed to engage the same region (Salo et al. 2009c). These findings have since been replicated in a larger group of MA abusers and controls (Fig. 8).

5.1.2 Emotion Regulation

A number of studies have examined social and emotional processes in MA-dependent subjects. Kim et al. examined empathy processing in 19 abstinent MA users compared to 19 controls (Kim et al. 2011). The authors used cartoons to depict two categories of stories, empathy and physical causality. The empathy condition required subjects to empathize with the character's situation and imagine their emotional state whereas the physical condition required subjects to imagine physical events alone. MA users scored worse than controls on both the empathy and physical conditions. The imaging results revealed that the MA users displayed hypoactivity in OFC, temporal poles and hippocampus, with hyperactivity observed in the dorsolateral PFC. This pattern of activity did not correlate with task performance. The authors concluded that the dorsolateral PFC may have been recruited in MA subjects more than controls due to both compromised OFC functioning, as well as impairments in empathetic processing. Medial frontal cortex, including OFC, has been implicated in processing emotional expressions, and thus may be critical to the experience of empathy.

Payer et al. used fMRI and a facial affect-matching task to evaluate emotion regulation in 12 MA-dependent subjects in very early stages of drug abstinence

(5–16 days) (Payer et al. 2008). Subjects were required to match either emotional facial expressions (active condition) or irregular shapes (control condition). Both MA-dependent subjects and controls activated a similar network of regions to emotional facial expressions including amygdala, ventrolateral and dorsolateral PFC, precentral frontal cortex, parietal cortex and occipital regions. Although no group differences were observed in task performance, the MA group displayed right-hemisphere hypoactivity within ventrolateral PFC, temperoparietal junction, inferior temporal gyrus, middle temporal and fusiform gyri as well as in the left cuneus. In contrast, the dorsal ACC, which has been referred to as the cognitive region of the ACC, was more active in the MA group. As noted above Leland et al. also observed ACC hyperactivity in their GO/NOGO paradigm (Leland et al. 2008), although Paulus et al. found hypoactivity in cingulate suggesting that patterns of activity in this region may be task-dependent (Paulus et al. 2003). ROI analysis focusing on the amygdala revealed that although there was a trend toward amygdala hypoactivity in the MA abusers compared to the controls, it failed to reach significance.

Recently, Payer et al. conducted a second fMRI study using a paradigm examining facial affect in 25 MA users compared to 23 controls (Payer et al. 2011). The authors hypothesized that the MA abusers would be impaired in emotion regulation. Although all participants displayed similar patterns of activation in regions including bilateral amygdala and inferior frontal gyrus during face processing, group differences were observed in bilateral ventral inferior frontal gyrus during visual processing of facial affect. During affect labeling all subjects engaged dorsal inferior frontal gyrus but the MA group displayed hypoactivity within the amygdala. The authors carried out an analysis to investigate whether any potential differences in GM volume were influencing the between-group differences in brain activity. Although VBM analysis revealed lower GM volumes in the MA group, this did not appear to significantly influence group differences in functional activity. The authors also carried out ROI analyses focusing on the amygdala to further investigate any potential group differences and failed to find any significant functional or structural differences. And finally, Payer et al. tested functional connectivity between inferior frontal gyrus and amygdala in both groups (Payer et al. 2011). As predicted there was an increase in connectivity between these regions during affect labeling, particularly in the right hemisphere; however, no between-group differences were observed. The authors concluded that, contrary to their hypothesis, MA users were successful in emotional regulation. They suggested that hypoactivity in ventral inferior frontal gyrus may reflect limited emotional insight, which may ultimately lead to heightened aggression in the MA abusing group.

5.1.3 Reward Processing

Other studies have investigated temporal discounting in individuals with MA dependence. Temporal discounting refers to the tendency for distant rewards to be less preferred over immediate rewards. Temporal discounting is usually investigated with the delay discounting (DD) paradigm, which presents individuals with

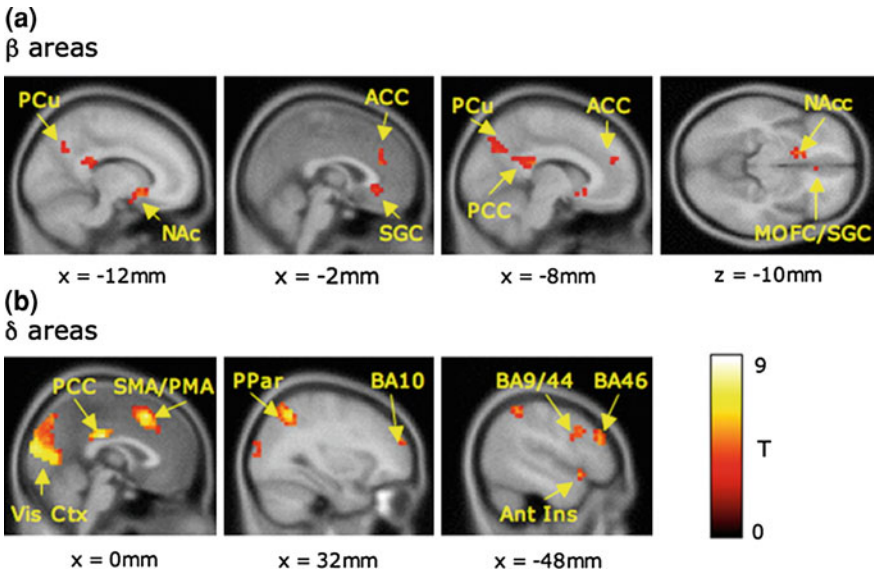

Fig. 9 **a** Brain areas that are preferentially activated by choices involving a reward available at a 0 min delay. **b** Brain areas that are activated by all intertemporal choices. (Reprinted from McClure et al. 2007)

choices between immediate small rewards and delayed larger rewards. An individual's reward-related impulsivity can then be measured and compared to their peers. It has been hypothesized that there are two separate neural systems that regulate this type of decision making process; the cognitive system, including dorsolateral PFC and parietal cortex, which is involved in cognitive control and deliberative processes and the limbic system, that responds more to immediate rewards and can be described as being the "impatient" system (McClure et al. 2004a, b). These two systems are an integral part of everyday decision making processes and are thought to be dysfunctional in addicted individuals (Fig. 9).

Monterosso et al. examined brain activity during a DD paradigm in 12 non treatment-seeking MA abusers and 17 control participants (Monterosso et al. 2007). Control subjects were predominantly smokers due to the high rate of smokers in the MA group. The authors administered the Wender Utah Rating Scale (WURS) to exclude those subjects suspected to have childhood Attention-Deficit/Hyperactivity disorder. Behavioral data revealed that MA-dependent subjects were more impulsive in their reward choice than controls. Areas of activity during the DD task included ventrolateral and dorsolateral PFC, parietal lobe and ACC. Although there were no regions that were significantly more active in the MA compared to the control group, control subjects activated the left dorsolateral PFC and right inferior parietal sulcus to a greater degree than MA-dependent subjects during difficult decisions. Furthermore, although activity in the control group was minimal in the aforementioned regions during easy decisions and

increased substantially during difficult decisions, activity in the MA group did not differ between easy and difficult decisions. Greater activity in the left ventrolateral PFC in all participants was related to a decrease in impulsivity in choosing between small immediate and large delayed rewards.

Hoffman et al. also compared activity during a DD task in 19 MA-dependent subjects compared to 17 drug-free controls (Hoffman et al. 2008). As previous studies in MA abusers have shown an increase in sensitivity to immediate rewards over larger delayed rewards (Monterosso et al. 2007), Hoffman et al. hypothesized impairments in the cognitive circuit and increased activity in the affective circuit thought to mediate selection of small immediate versus large delayed rewards (Hoffman et al. 2008). Contrasting difficult versus easy choices on the DD task resulted in 11 clusters of activity including right insula, dorsal ACC, medial superior frontal gyrus, posterior cingulate, ventrolateral and dorsolateral PFC and regions of parietal cortex. The data revealed that MA users made more impulsive decisions than controls and displayed hypoactivity in bilateral precuneus, right caudate, ACC and dorsolateral PFC compared to controls. Neither Monterosso nor Hoffman located any significant activity in OFC or ventral striatum during their delay discounting paradigms. This may have been due to susceptibility artifacts, which make imaging of these regions difficult. Monterosso and colleagues have suggested that the utilization of hypothetical rewards were not sufficient to recruit limbic areas (Monterosso et al. 2007).

5.2 fMRI Findings and Drug Use Patterns

Relatively few fMRI studies have linked the imaging findings to patterns of drug use. One important study used the patterns of brain activation observed in the MA abusers to predict patterns of relapse (Paulus et al. 2005). In this seminal imaging study, Paulus and colleagues found that higher levels of activity in left midfrontal gyrus and less in left superior temporal gyrus (BA 38) predicted longer period of abstinence. Longer duration of MA abuse correlated with more activity in OFC. Paulus et al. also found that more activity in left middle frontal gyrus (BA 6) and less activity in left superior temporal gyrus (BA 38) correlated with longer periods of sobriety in MA-dependent subjects. Furthermore, subjects with a larger decrease on orbitofrontal activity had a longer history of MA abuse (Paulus et al. 2002). In their 2003 study, Paulus et al. found that duration of MA use predicted differences between groups in left midfrontal gyrus (BA9), ACC (BA32) and left precuneus. Duration of sobriety was associated with more activity in left medial frontal gyrus (8) (Paulus et al. 2003).

5.3 fMRI findings and Cognitive Function

As the strength of fMRI lies in part in its ability to measure cognitive processes online during scan acquisition, it is somewhat redundant to discuss the cognitive findings in any detail here as they have already been discussed above.

5.3.1 fMRI Findings and Clinical Symptoms

Payer and colleagues examined the relationship between scores on the Symptom Checklist 90 (SCL-90-R; Derogatis et al. 1973), the Brief Symptom Inventory (BSI; Derogatis and Melisaratos 1983) both of which assess self-reports of psychological symptoms experienced in the past 7 (SCL) or 30 (BSI) days (Payer et al. 2008). The authors found that higher dorsal ACC activity related to higher scores on the Hostility and Interpersonal Sensitivity subscales of both the SCL-90 and the BSI. The authors also found a non-significant trend ($p = 0.09$) towards high self-reports of interpersonal sensitivity being associated with lower activity in right ventrolateral PFC; however the authors pointed out that the combination of a relatively small sample size in addition to a non-significant trend suggest that the results should be interpreted with caution (Payer et al. 2008). In a later study this group also found that a reduction in amygdala activity during affect labeling was associated with increases in perpetrated aggression in controls and participants with MA dependence (Payer et al. 2011). The authors suggest that these findings may suggest impairment in socio-emotional regulation, perhaps related to socially maladaptive behaviors exhibited.

5.3.2 Summary

In summary the body of fMRI studies conducted in MA abusers have painted a picture of disrupted behavioral regulation and abnormal decision making processes that are linked to differential recruitment of frontal brain regions (Paulus et al. 2002, 2003, 2005; Leland et al. 2008; Salo et al. 2009c). Furthermore additional studies have identified changes in BOLD activity among MA abusers while performing tasks that measure reward processing and delay of gratification (Monterosso et al. 2007; Hoffman et al. 2008). Finally there has emerged a recent fMRI literature that has examined the neural substrates of emotion regulation in MA abusers (Payer et al. 2008, 2011). Thus the body of fMRI studies reviewed here is very consistent with the MRS findings and suggests that MA targets the dopaminergic rich frontostriatal regions to a greater degree compared to posterior cortices.

6 Positron Emission Tomography

Positron Emission Tomography (PET) has evolved significantly over the past quarter of a century. PET provides physiological information about the tissue being sampled. via the detection of paired 511-KeV photons that are emitted by radioactive decay. By detecting the simultaneous arrival of these photons at crystal sensors, the electronics determine the line path (chord) on which the isotope existed at the time that the radioactive decay occurred. The PET study requires (1)

a PET camera with sensors that pick up (simultaneous) pairs of photons emanating from tissue being sampled, (2) a compound labeled with a positron emitter, and (3) a compartmental model characterizing relevant physiological processes that the radiolabeled compound undergoes. Positron emitters release a positron when a proton is converted to a neutron. This released positron then annihilates an electron resulting in the emission of two gamma ray photons at a 180°eccentricity to each other. Numerous elements have positron-emitting isotopes including several commonly found in biological molecules—for example fluorine-18 [F-18], carbon-11 [C-11], oxygen-15 [O-15], and nitrogen-13 [N-13]. The acquired imaging data as well as the compartmental model are then utilized to obtain the physiological rates of the biological processes being sampled. The application of PET to the study of addictive disorders includes the imaging of neuroreceptors, neurotransmitter kinetics, transporters, blood flow and measurement of regional glucose metabolism

6.1 PET Imaging Studies of Methamphetamine (MA) Abuse

PET can be used to examine dopaminergic functioning including dopamine receptor binding and dopamine transporter (DAT) functioning), serotonergic functioning and glucose metabolism in individuals who abuse MA. Animal studies suggest that MA produces profound damage to dopamine (Preston et al. 1985) and serotonin cells (Ricaurte et al. 1980, 1994). Studies in mice have shown that given acutely, MA increases dopamine levels in the synaptic cleft, mainly through the inhibition of the DAT (Giros et al. 1996). However, it may be ill advised to over-extend the results from animal studies to humans as MA-induced neuronal damage has been shown to vary across species (Kita et al. 2003).

One of the earliest PET studies of human MA abusers was a postmortem study of 11 control subjects and 12 individuals with a history of MA abuse. This study revealed a reduction in DAT density following chronic MA abuse (Wilson et al. 1996). Following studies attempted to replicate the finding of a reduction in DAT density in living human subjects with MA dependence. McCann and colleagues found a reduction in DAT density in the caudate (23% less) and the putamen (25% less) of MA abusers compared to control subjects (McCann et al. 1998). However the participants with a history of MA abuse in this study were poly-drug users, also having heavily used methcathinone in addition to MA. Following this study, a number of others also found striatal reductions in DAT in MA abusers (Volkow et al. 2001b, 2001d; Moszczynska et al. 2004; McCann et al. 2008). More widespread decreases in DAT density and binding potential have been found in PFC, caudate/putamen and nucleus accumbens (NAc) in other studies (Iyo et al. 1993; Sekine et al. 2001)

Using a combined imaging approach that applied MRS, SPECT and PET Iyo et al. examined metabolic and neurochemical changes in 11 MA abusers and nine non-substance abusing controls (Iyo et al. 2004). No significant differences were

observed between the MA abusers and controls in the density of striatal dopamine D2 receptors, however the density of DAT in the NAc and caudate/putamen in the MA group was significantly less compared with the controls. Furthermore, this reduction was significantly correlated with the length of MA use and severity of psychotic symptoms which will be discussed in greater detail below. Thus, findings in DAT reduction in MA abusers are relatively consistent, despite differing methodologies and subject characteristics across the different studies.

A significant body of research has reported severe dopamine depletion in the brains of chronic MA abusers. One study of autopsied brains of twenty chronic MA users detected severe dopamine reductions in the caudate of the MA abusers with six of the subjects having 70% dopamine depletion in the caudate compared to healthy controls (Moszczynska et al. 2004). The MA abusers also had significant dopamine reduction in the putamen. The authors compared MA brains to brains of Parkinson's (PD) patients. PD patients tended to have a marked decrease in dopamine in the putamen rather than the caudate (i.e., the opposite pattern from the MA abusers). Furthermore dopamine depletion in the MA abusers was comparable to PD reduction in the caudate but not in the putamen. The authors suggested that this may explain the range of cognitive impairments observed in MA abusers but with a lack of PD symptoms. PET can also assess the distribution of MA throughout the body and brain. A very recent study measured the distribution of MA within various organs of the human body, including the brain and concluded that MA was distributed widely through most organs in the body; with the highest levels of uptake occurring in the lungs, liver and brain with clearance being the slowest from the brain, liver and stomach (over 75 min) (Volkow et al. 2010).

Striatal reductions in dopamine D2 receptors have also been found in MA abusers (Sonsalla et al. 1986; Iyo et al. 1993; Volkow et al. 2001a). An early PET study used the radiotracer 11C-N-methylspiperone to assess the role of dopamine D2 receptors in the striatum of six male MA abusers and ten age- and sex-matched control subjects. Although striatal D2 binding did not differ between groups, the ratio of binding availability in the striatum was related to the psychotic symptoms exhibited in the MA group. These findings will be discussed in greater detail below. D2 receptor availability was also associated with metabolic rate in the OFC (Volkow et al. 2001a), a region associated with impulse control (Sellitto et al. 2010; Damasio 1996). This correlation between D2 receptor availability and OFC metabolism is of interest as it has been reported that short-term exposure to stimulants can disrupt OFC functioning and negatively effect behaviors thought to be subserved by this region (Olausson et al. 2007). The association between D2 receptor availability and OFC metabolic rate was interpreted as potentially contributing to the compulsive nature of drug taking in addicted individuals (Volkow et al. 2001a). However changes in dopamine D2 receptor density were not observed in the striatum of MA abusers who were at least 2 months clean (Volkow et al. 2001b).

The vesicular monoamine transporter (VMAT2) has been identified as a marker of nigrostriatal dopamine neuron integrity in humans (in vivo (Frey et al. 1997; Lee et al. 2000; Boileau et al. 2008) and postmortem (Wilson et al. 1996)) as well

as in animals (Vander Borght et al. 1995; Strome et al. 2006; Sossi et al. 2007). PET studies have utilized (+)[^{11}C]dihydrotetrabenzanine (DTBZ), a VMAT2 PET radioligand, as a marker of dopamine integrity. The use of VMAT2 as a marker of dopamine integrity in PET studies has yielded conflicting results. Wilson et al. found normal VMAT2 levels in the brains of their MA-abusing subjects in their postmortem study despite a finding of reductions in DAT in this group (Wilson et al. 1996). However, it should be noted that this post-mortem brain study included a limited sample size and it is difficult to translate postmortem data to the in vivo scenario. In contrast, a recent study discovered a small, 10% decrease in VMAT2 binding in the striatum of MA abusers who were, on average, three years abstinent (Johanson et al. 2006). An average of three years is a relatively long period of abstinence thus it is possible that there was recovery of dopaminergic function during this time period.

Members of Wilson's group published an additional study measuring dopamine integrity in subjects with MA dependence, this time in vivo (Boileau et al. 2008). In this study, the authors used active MA users as they wished to investigate whether greater decreases in VMAT2 binding would occur compared to the Johanson et al. study mentioned above. The results from their study, however, suggested that VMAT2 might not, in fact, be a reliable marker of dopamine neuronal integrity in PET studies. Contrary to their hypothesis, Boileau et al. found an *increase* in VMAT2 binding in the putamen and head of the caudate in comparison to controls (Boileau et al. 2008). This was particularly pronounced in subjects who had presented with a positive drug screen on the day of the scan and reported having used from 1.5 to 3 days previously. The effect of an increase in VMAT2 in MA abusing participants decreased with time abstinent; in fact VMAT2 binding failed to differ significantly from controls following a period of 7 to 21 days abstinent and subjects who were abstinent for over 30 days were only slightly elevated (9% in the caudate and 4% in the putamen) in comparison to controls. These authors also used an additional control group, which consisted of PD patients. As expected, these PD patients displayed lower VMAT2 in these brain regions when compared to control subjects. The authors suggested that VMAT2 (at low radiotracer concentration as present in PET studies) might be influenced by changes in vesicular dopamine levels (de la Fuente-Fernandez et al. 2009). They argued that administration of MA, which is a powerful dopaminergic drug, would decrease vesicular dopamine and that this would be reflected by increased VMAT2 binding. Thus failure to find a difference in striatal (+)[^{11}C]DTBZ binding between MA abusers and controls may not necessarily indicate preservation of striatal dopamine innervations. In a follow-up to their 2008 study, Boileau and colleagues investigated whether acute MA administration would cause increased VMAT2 binding in the striatum in healthy non-substance using controls (Boileau et al. 2010). Boileau et al. scanned nine subjects pre, 2 h post and in five subjects 24 h post administration of a low dose of MA. The authors failed to find increased striatal VMAT2 binding which they suggested may be due to the low dose administered to their non drug-using cohort. They suggested that a low dose of MA may have stimulated a compensatory increase in

dopamine synthesis which might have replenished vesicles. Thus, the PET literature on MA abuse in humans using VMAT2 binding dopamine neuronal integrity is, as yet, unresolved.

To the best of our knowledge, only two studies have utilized PET to study the effect of long-term MA use on the serotonergic system in humans (Iyo et al. 1993; Sekine et al. 2006). Iyo et al. scanned six male MA abusers, all who had previously experienced MA-induced psychosis and ten male controls (Iyo et al. 1993). In addition, as discussed above D2 receptor binding was also measured in the striatum of the same subjects. The authors did not report group differences in receptor binding in striatal and frontal cortices, however the ratio of binding availability in the striatum was significantly decreased compared to that in the frontal cortex of the MA abusers. The authors conclude that this reduction may represent an imbalance in the activity of the D2 and serotonin receptors. Furthermore as all ten MA abusers reported a history of MA-induced psychosis, this imbalance may be related to the susceptibility to MA psychosis. A later study of long-term MA abuse on the serotonergic system compared 12 currently abstinent MA abusers (mean period of abstinence 1.6 years) to healthy controls. The authors described their subjects as being recreational users with no history of particularly heavy MA use. They used [11C]McN-5652, which has a binding specificity to pre-synaptic serotonin transporter (SERT) to examine changes in the serotonergic system of MA abusers. The authors found decreased SERT density in all ten regions of interest studied, namely, midbrain, thalamus, caudate nucleus, putamen, amygdala, ACC, dorsolateral PFC, OFC and cerebellar cortex.

A number of studies have measured glucose metabolism in MA abusers. Volkow and colleagues used [18F] fluorodeoxyglucose to examine brain metabolism in fifteen MA abusers (2 weeks to almost 3 years abstinent) compared to 21 controls (Volkow et al. 2001c). They found a 14% increase in whole brain metabolism in MA abusers, in particular in parietal cortex (20% increase). Following normalization for whole brain metabolism, the MA abusers displayed decreased metabolism in the thalamus, striatum (larger in the caudate [12%] than the putamen [6%]) and the increase in parietal cortex remained significant. The authors interpreted the increase in parietal metabolism to be related to inflammation and gliosis brought about by MA-induced brain damage. The authors cite animal studies that find gliosis in response to MA administration as support for their theory (Sheng et al. 1994; Escubedo et al. 1998).

London and colleagues measured glucose metabolism using [18F] PET in 17 female MA-abusers who were in early abstinence (4–7 days) compared to 18 female controls (London et al. 2004). Reduced metabolism within the ACC and insula was reported along with concomitant increases in the OFC, mid and posterior cingulate, amygdala, ventral striatum and cerebellum (London et al. 2004, 2005) These are the same brain regions in which reduced DAT density has been observed following long-term MA exposure (Volkow et al. 2001d; Sekine et al. 2003). Another study of glucose metabolism imaged male MA abusers in early abstinence (i.e., 21 days) in a resting state PET study (Kim et al. 2009b). They also found evidence of left inferior frontal hypometabolism in MA abusers compared to controls, close to Brodmann area 9 in dorsolateral PFC.

6.2 PET Findings and Drug Use Patterns

Measures of dopaminergic integrity (DAT binding and dopamine receptors), as well as metabolism ascertained through PET imaging have been correlated with patterns and severity of drug use (i.e., number of months using drug, months abstinent and general quantities of drug consumed). Sekine and colleagues found that DAT reductions in caudate/putamen and in NAc were associated with length of MA use, but not with duration of abstinence (Sekine et al. 2001). Volkow et al. also found a correlation between DAT density and years of MA use in the caudate but not the putamen (trend significant; $p = 0.08$) but again no correlation with abstinence duration nor with MA dosage. A pair of recent PET studies also failed to find any correlation between DAT binding potential and duration of abstinence (Johanson et al. 2006; McCann et al. 2008). In addition to PET studies of DAT binding and dopamine receptor density, investigations were also carried to determine whether a relationship between brain metabolism and drug patterns existed. Iyo et al. found that NAc metabolism correlated negatively with period of MA use but not with months abstinent (Iyo et al. 2004). Kim et al. found that cerebral glucose metabolism in left inferior frontal WM correlated negatively with total cumulative dose of MA (Kim et al. 2009b). In one study which examined serotonergic functioning in MA abusers, Sekine and colleagues found that decreased SERT density in the midbrain, thalamus, caudate, putamen and OFC correlated with longer of MA use; however, there was no correlation with time abstinent (from 6 months to 5 years) (Sekine et al. 2006).

Thus, in the studies reviewed thus far, there is some evidence for a decrease in DAT binding potential with increased time abusing MA, but remaining MA abstinent does not appear to have a major impact on DAT recovery. However there is one important longitudinal PET study of MA abusers that contradicts this conclusion (Volkow et al. 2001b). In this study the authors examined DAT reduction in the striatum of 5 MA abusers who were tested following a short abstinence period (less than six month abstinent) and then again following protracted abstinence (12–17 months). The authors found a substantial increase in DAT following a protracted abstinence period of one year and longer (Fig. 10).

A follow-up PET study of these participants that measured glucose metabolism revealed no differences in global metabolism between short versus long-term MA abstinence. Furthermore regional analyses revealed no differences in absolute metabolic measures within the striatum, thalamus, and occipital cortex. Relative changes were also assessed by using the regional values normalized by the global measures. The relative metabolic measures of the MA abusers were significantly lower after both short- and long-term abstinence in the striatum. In contrast, after extended periods of abstinence, relative thalamic activity did not differ from that of the control subjects (Wang et al. 2004). The authors suggest that the sustained reduction in metabolic activity in the striatum could underlie some of the long-lasting deficits reported in abstinent MA abusers. In a direct comparison of the 25 MA abusers, thalamic metabolism was lower in the 12 short-term abstinent MA

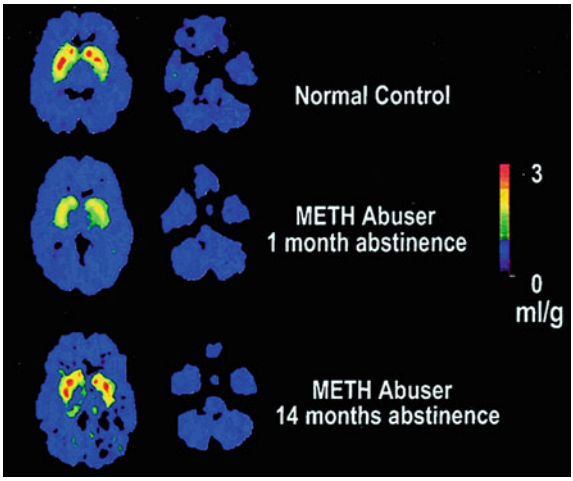

Fig. 10 Brain images of the distribution volume of [11C] *d-threo* methylphenidate in a control and a methamphetamine abuser evaluated twice, during short and protracted abstinence. Notice the significant increases in binding in striatum in the methamphetamine abuser with protracted abstinence. (Reprinted from Volkow et al. 2001b)

abusers compared to the 13 long-term abstinent MA abusers. Metabolism in the striatum did not differ between the two groups as a function of abstinence.

6.3 PET Findings and Cognitive Function

Many studies have assessed psychiatric symptoms and cognitive functioning (evaluated by a battery of cognitive tasks) in MA abusers in order to investigate whether the symptom severity or cognitive ability relate to PET findings. Volkow and colleagues administered a battery of motor and cognitive tasks to their participants who had abused MA (Volkow et al. 2001d). The motor tasks included the Timed Gait Task and Grooved Pegboard Task, the attentional tasks included the California Computerized Assessment Package, the Symbol Digit Modalities Test and the Stroop Interference. The Rey Auditory Verbal Learning Test was also administered to measure memory. The authors found that reductions of DAT in the striatum were associated with impairments in motor functioning (indicated by slowing on both motor tasks) and memory impairment, but not with any components of the administered attention tasks. Volkow et al. also found that higher parietal metabolism in MA abusers was associated with slowed motor performance on the grooved pegboard task and suggested that hyper metabolism in this region may also be linked to impairments in other cognitive functions, such as attention and visuospatial skills (Volkow et al. 2001d). McCann et al. found a negative correlation between DAT binding potential and memory assessed by the Wechsler Memory Scale-III (Wechsler 1981). In contrast to the Volkow study, no correlations were observed with motor impairment or measures of attention and executive control.

In a recent PET study that measured both DAT and VMAT2 within the striatum cognitive function was evaluated using tests of motor function, memory, learning,

attention and executive function (Johanson et al. 2006). The findings revealed that MA abusers exhibited performance deficits on only three of the 12 tasks. Failure to find more substantial changes in neurocognitive function may be attributed to the length of time that MA users were abstinent (ranging from 3 months to more than 10 years, mean 3 years).

6.4 PET Findings and Clinical Symptoms

Many studies have assessed psychiatric and clinical symptoms associated with long-term MA use. Clinical symptoms can be measured through psychiatric rating scales as well as questionnaires that measure drug related symptoms, such as craving. Such approaches can shed light on whether the severity of symptoms relates to changes in metabolic activity or dopamine transmission as measured by PET. Sekine et al. used the BPRS (Overall and Gorham 1962) to assess psychiatric symptoms in MA abusers who were at least 7 days abstinent. The authors discovered a negative relationship between DAT binding in the caudate/putamen and in the NAc with BPRS scores (Total and positive). There was no correlation between BPRS scores and DAT binding potential in the PFC. Iyo et al. replicated this finding in their PET study, determining that caudate/putamen and NAc (but not PFC) DAT binding potential correlated negatively with the positive symptom subscore of the BPRS while caudate/putamen binding only, correlated with the total BPRS score (Iyo et al. 2004). In addition to the correlations reported above, the severity of psychiatric symptoms often correlates with the duration of MA use (Sekine et al. 2001; Iyo et al. 2004). As may be expected, subjective craving also declines with increased amount of time abstinent from the drug (Sekine et al. 2001).

PET was used to assess the role of dopamine D2 receptors in the striatum and serotonin S2 receptors in the frontal cortex in the susceptibility to MA -induced psychosis. Subjects were six male MA abusers who had previously experienced MA psychosis and ten age- and sex-matched control subjects. The radiotracer used was 11C-N-methylspiperone. Although binding availability in the two regions did not differ between the two groups, the ratio of binding availability in the striatum to that in the frontal cortex significantly decreased in the MA subjects compared to the control subjects. These findings suggest that an imbalance in the activity of these two receptors may be related to the susceptibility to MA-induced psychosis (Iyo et al. 1993).

In a recent PET study self-reports of depression symptoms (as measured by the Beck Depression Inventory—BDI) correlated positively with glucose metabolism in the anterior cingulate and amygdala (London et al. 2004). The authors also reported that more recent MA use (measured by grams per week) was associated with greater levels of depression (measured by the BDI). Furthermore ratings of anxiety (measured by the State-Trait Anxiety Inventory) correlated negatively with activity in the anterior cingulate cortex, left insula, OFC and amygdala. The authors cited a study by Gur et al. to explain the negative correlation between

glucose metabolism in the insula and anxiety symptoms (Gur et al. 1987). In the Gur et al. study, the authors found an inverted U shape relationship between cortical blood flow and anxiety, such that cortical blood flow increased with anxiety in subjects with low levels of anxiety but that it decreased in those subjects with high anxiety (Gur et al. 1987). As the MA abusers in the London et al. study had a higher degree of anxiety symptoms, the authors suggest that the results in this study are consistent with being situated at the negative slope of the curvilinear function (p. 80). Sekine et al. found that rates of aggression (measured by the Aggression Questionnaire Scale) in MA abusers increased significantly with decreasing levels of SERT levels in most regions of interest studies; thalamus, caudate, putamen, anterior cingulate, OFC, temporal, cerebellar and dorsolateral cortex (Sekine et al. 2006). Whole brain statistical analysis supported this finding in OFC, ACC and temporal cortex.

7 Conclusion

There has been significant progress in our understanding of the human brain structure and function with imaging techniques. These advances in neuroimaging have greatly enhanced our knowledge of the neural correlates of addiction and allow scientists to relate these neural changes to patterns of drug use, drug abstinence and cognitive function. All of these approaches are critical to better understand the powerful effects that long-term substance abuse has on the human brain. Despite the powerful neurotoxic profile of MA (Davidson et al. 2001; Quinton and Yamamoto 2006), it appears that in some cases neuronal function does have the ability to recover following long-term MA use (Salo et al. 2011a; Volkow et al. 2001b; Wang et al. 2004; Nordahl et al. 2005; Kim et al. 2006; Ernst and Chang 2008; Salo et al. 2009b). This may be in part due to the fact that although MA is particularly damaging to the axonal processes, it may be that the cell body itself is relatively spared (Fowler et al. 2008). The findings that relate extended periods of drug sobriety to improvements in brain function have also been extended to measures of cognition (Simon et al. 2010; Salo et al. 2009b). Linking neuroimaging findings to behavior and clinical patterns of drug use is essential if there is to be a translational implementation of the science that occurs in the laboratory to the important clinical work that transpires in substance abuse centers. Improvements in MR have included not just enhanced resolution and contrast, but also brought forth new imaging techniques (i.e., MRS and DTI) both of which have clinical and research importance.

At the present time it is our position that imaging studies of an individual may not be diagnostic but rather their value lies in the ability to identify patterns of brain changes across groups with similar drug use characteristics. One of the challenges in measuring neural changes associated with long-term drug use is that one can never completely isolate the consequences of drug exposure on the brain from those that may predate the drug use itself. It is widely accepted that there

exists a genetic vulnerability to addiction and substance abuse. The combination of genetic and environmental influences is thought to contribute to 40–60% of the variability associated with the risk of addiction (Compton et al. 2005; Volkow 2005). However, even after the consideration of preexisting factors the majority of studies reviewed in this chapter point to significant consequences of the drug itself. The observed correlations between years use, dosage levels and recovery of neural chemistry and function following periods of sobriety suggest that observed brain changes are likely a synergistic effect between pre-existing factors and direct consequences of MA exposure. This complex interaction between risk factors and the direct effects of MA itself speaks to the need for more longitudinal imaging studies in substance abuse to track brain changes across periods of drug sobriety. Such studies will be of paramount importance to clinical treatment settings and will inform and guide future studies of substance abuse and addiction.

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Pharmacological MRI Approaches to Understanding Mechanisms of Drug Action

Michael J. Minzenberg

Abstract Functional neuroimaging is a novel technique for the study of drug action in the brain. The emerging role of this method is intimately tied to the unique challenges to advancing drug development for neuropsychiatric disorders. This chapter first presents a brief overview of the important treatment needs that remain to be met, which serve as clinical targets for drug development. Important factors that hinder progress in drug development, which arise from clinical, scientific and economic issues, are acknowledged. This sets the stage for the unique advantages of functional neuroimaging modalities such as functional MRI (fMRI) as a biomarker and drug development tool, in both clinical and preclinical phases. The physiological basis of the fMRI signal is briefly outlined, and aspects of neural signaling related to this signal change, with emphasis on implications for pharmacology studies. The utility of fMRI for evaluating the full anatomic extent of central neurotransmitter systems in a dynamic manner is then described. This is a critical advantage, and particularly important for studies of how systems such as the monoamines modulate distributed neural networks during cognitive processes in both health and illness, and how these actions are modified with pharmacological intervention. Central catecholamine systems are seen as paradigmatic targets amenable to pharmacologic fMRI. fMRI is observed to occupy a unique position in the armamentarium of methods available to the pharmacologist and the drug development process, and poised to play an expanding role in basic and clinical neuroscience.

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Contents

1 The Status and Challenges of Drug Development in Neuropsychiatric Illness

Any discussion of new methodologies available to advance neuropharmacology must first acknowledge the tremendous personal and public health impact of neuropsychiatric illness, and the substantial challenges to furthering our knowledge of how drugs work in the human brain. Psychiatric disorders are among the most debilitating illnesses that humans face. Contemporary global epidemiological surveys such as those conducted by the World Health Organization (Murray et al. 1994) have established a number of high-prevalence psychiatric conditions, including schizophrenia, mood disorders and substance abuse, as among the highest impact in medicine overall, reflected in measures of long-term disability. Not surprisingly, these conditions are associated with inordinate suffering on the part of the afflicted, their families and loved ones, and considerable economic cost incurred as direct costs of care and enormous levels of lost productivity.

A number of important unmet treatment needs persist among the more serious and chronic conditions encountered in psychiatry. These include symptom domains such as negative symptoms and cognitive dysfunction in schizophrenia, which confer the greatest impact on clinical outcome in this illness yet have no established treatment; treatment-refractory states and relapsing–remitting courses of illness, even for symptoms which serve as primary treatment targets, in mood, anxiety, psychotic and substance-use disorders; “secondary” symptoms that arise from these disorders and strongly modify outcome, such as impulsivity and

suicide; the deleterious effects of persistent symptoms on health-related behaviors and medical co-morbidity; and uncertain and variable effects of existing treatments on subjective well-being and long-term functional outcome, even for many patients who achieve full remission from primary target symptoms such as psychosis. The single-minded emphasis on monotherapy for disorders that are heterogeneous (at least as currently defined) may be one factor that tends to preclude attention to neglected clinical targets (Hyman and Fenton 2003). On the other hand, inappropriately narrow clinical indications are likely to be disapproved by the FDA as "pseudospecific" (Laughren and Levin 2006), leading to the need to "walk the tightrope" in adequately addressing these targets. More generally, there is little evidence that the morbidity or mortality associated with psychiatric illness has changed in the era of modern psychopharmacology (Insel and Scolnick 2006). Furthermore, the notion of either primary or secondary prevention remains a neglected goal in psychiatry (Insel and Scolnick 2006). Therefore, genuine advances in psychiatric treatment hold the promise to alleviate considerable suffering and global health burdens.

Unfortunately, the remote and recent history of drug development in psychiatry poses numerous challenges to achieving this aim. As is well known, the early history of drug discovery in mental health is characterized largely by serendipity (Ban 2006), with major successes initially arising from keen clinical observations made during use of certain compounds for unrelated medical conditions (such as surgical procedures, respiratory infections, hypertension and epilepsy). More recently, the industry has virtually uniformly pursued the strategy of mining existing targets (such as D2 receptors for antipsychotics, or monoamine transporters for depression) with a proliferation of so-called "me-too" products. One important consequence is that the newer agents available for major mental illness generally lack enhanced efficacy for existing treatment targets, nor clinically significant efficacy against new targets, but rather extend the range of adverse physiological effects that patients are exposed to, including some (e.g. metabolic syndrome) that appear likely to adversely affect long-term clinical outcomes such as life expectancy. Unfortunately for patients, families and clinicians, there may be a vicious cycle where the patients in most need of treatment advances (e.g., schizophrenia patients with prominent negative symptoms or cognitive dysfunction) are the least likely to benefit from new medications.

While some pharmaceuticals for central nervous system (CNS) indications have been among the most successful throughout medicine in recent years, generating staggering revenues for large pharmaceutical companies, with massive, expanding markets for these drugs, there has been a recent withdrawal overall of the pharmaceutical industry from CNS. The proximal causes appear to be the inordinate and expanding cost of bringing new drugs to market, particularly costly for CNS drugs, which show a relatively high failure rate compared to other classes of agents (Kola and Landis 2004). This scenario is accounted for in part by the increasing cost of obtaining regulatory approval. This trend away from CNS was set in motion even prior to the recent economic downturn, and appears likely to worsen, given the trend in costs (Breier 2005).

Many reasons for this inordinate cost dovetail with the scientific challenges to advancing drug development. There is now a considerable dialogue in the clinical/scientific and industry literatures addressing the myriad challenges facing the scientific, medical, regulatory and industry communities to advance the state of drug development for neuropsychiatric disorders (Agid et al. 2007; Conn and Roth 2008). These challenges, in the aggregate, provide a context for the emerging role of noninvasive functional neuroimaging in pharmacology, and are discussed in turn.

The inevitable starting place for any discussion of these challenges is the system for classifying illness in psychiatry. The current standard, instantiated in the Diagnostic and Statistical Manual (DSM, now undergoing a revision to a fifth full edition), has a long and complex history, originating in committees of experts in clinical psychiatry. This system of diagnosis and classification remains based on clinical phenomenology, that is, reportable or clinically observable signs and symptoms, without reference to underlying theoretical models of mental illness, nor empirical knowledge about etiology or pathophysiology available from clinical or basic neuroscience. While more recent editions of the DSM have relied increasingly on empirical field trials to evaluate the measurement properties and performance of these criteria sets in discriminating among discrete illness types, there remains no rooting of categories in distinct etiologies or pathophysiological processes. In addition, it remains unclear whether individual syndromes (including schizophrenia-spectrum disorders, depression and personality disorders) are better represented as dimensional conditions. As a result, heterogeneity within categories, and co-morbidity across categories, are the rule for patients. The lack of correspondence between diagnoses and distinct pathophysiologies is a particular obstacle for drug development, as the resulting heterogeneity confounds efforts to establish molecular targets and useful biomarkers of drug action (see below).

The underlying pathophysiology also remains obscure for virtually all major mental illness. The biological events or fundamental cellular/molecular processes that form the basis for illness is essentially unknown for schizophrenia, autism, bipolar disorder, etc., despite a wealth of epidemiological clues about antecedents and risk states for many disorders, and a range of consistent biological and cognitive abnormalities observed in these disorders. This makes it hard to characterize and capitalize on treatment targets, as the field is to some extent "shooting in the dark." Beyond the nosological constraints, both the polygenic nature of mental illness, and the inherent complexity of the brain, may be root causes of this obscurity. While this is increasingly recognized in psychiatric genetics and systems neuroscience, there is no visible way yet around the roadblock.

The unique challenge of modeling the complex clinical phenomenology of psychiatric illness in animals remains an unresolved problem (Nestler and Hyman 2010). Many of the core features of mental illness, such as hallucinations, delusions, depressed mood and guilt, cannot be induced or detected in animals, including non-human primates. While cellular and molecular processes implicated in psychiatric disorders (such as monoamine transport, signaling via D2 receptors,

brain-derived neurotrophic factor, etc.) can be reliably and specifically manipulated with diverse methods, animal models of clinical phenomenology tend to express at best very partial (and typically very nonspecific) features of illness. For instance, animals with targeted gene knockouts or brain lesions that lead to social withdrawal are often suggested to variously model schizophrenia, autism and depression, which are distinct clinical conditions with widely divergent patterns of gene association, biological abnormalities, treatment response and clinical course. In addition, many cognitive processes that are important to psychiatric illness (e.g. language, abstract thought) are not found in any other species. Among treatment considerations, the precise drug treatment regimen (e.g. dose, duration) in animals that properly models treatment in clinical settings is typically uncertain. Furthermore, variation across species in both pharmacokinetics and pharmacodynamics confers hard limits on the utility of animal models for predicting the efficacy of drugs in humans. Not surprisingly then, there is poor predictive utility of animal models for effective treatments for cognition, for instance (Hagan and Jones 2005). Animal models remain an important pillar of both basic and clinical neuroscientific knowledge, given the inaccessibility of brain tissue in patients, the range of manipulations possible in animals, and the close correspondence of physiology across mammalian species. Nonetheless, significant refinement in how these models are developed and utilized will be necessary to improve their contribution (Nestler and Hyman 2010).

Interestingly, even for those drugs with clinical efficacy established beyond doubt, such as D2 antagonists for psychosis, or monoamine transport inhibitors for depression, the critical mechanism of action remains unclear. The consequences for neurotransmission of administering these drugs are well established; however, clinical effects tend to exhibit a considerable temporal delay beyond the onset of effects on neurotransmitter receptor activity. Delayed changes in neuronal signaling rates or patterns, intracellular signaling cascades, gene transcription and structural changes in synapses have each been addressed in recent work as likely mediators of clinical efficacy. Nonetheless, how an individual's brain can get from any of these changes relief from auditory hallucinations or suicidal thoughts remains essentially unknown. In addition, for many drugs (e.g. aripiprazole, clozapine, lithium, valproic acid, mirtazepine) there is evidence for a multiplicity of actions, each of which may be responsible for efficacy. This may be why investigators remain divided on whether more, versus less, specificity in the profile of drug action matters for clinical efficacy (Hopkins 2008; Roth et al. 2004). Indeed, the history of failed versus successful agents for major mental illness suggests that "magic shotguns" should be preferred to "magic bullets" (Roth et al. 2004). The trouble with this observation follows from the complexity of effects associated with a single drug that has multiple actions on numerous highly interacting neurotransmitter systems. This may necessitate both non-conventional small-molecule screening approaches to identify promising agents, and significant advances in direct testing in humans by a variety of experimental methods, including functional neuroimaging.

2 Functional Magnetic Resonance Imaging as a Biomarker

The question of which molecular process is most essential for clinical efficacy, and therefore most worthy of investigation and development, is often framed as a problem of "target validation." This notion is intimately tied to the construct of "biomarkers" in drug development. The NIH Biomarker Definitions Working Group has defined a biomarker as "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention" (Lesko and Atkinson 2001). The FDA has proposed a similar definition of a "marker" or surrogate endpoint for testing medical treatments (US Senate Bill 830), and the FDA Critical Path Initiative (http://www.fda.gov/oc/initiatives/criticalpath/) has identified Imaging as a critical technology to surmount the drug development roadblock. A typical role for a biomarker is to properly "measure the delivery of drugs to their intended targets, and to understand and predict pathophysiology, and how it is altered by therapy, through monitoring variables known to have clinical relevance" (Frank and Hargreaves 2003). The NIH Working Group defines three levels of biomarkers: those that track the natural course of illness (type 0); those that examine intervention effects, where the drug's mechanism of action is known but where a strict relationship to clinical outcome has not been established (type 1); and those where changes in the biomarker are truly predictive of clinical outcome (type 2). Wong and colleagues suggest that at present, most neuroimaging measures can be considered to have the status of either type 0 or type 1 biomarker (Wong et al. 2009). Useful biomarkers can uniquely enable proof-of-concept studies for novel agents early in the drug discovery process, and thus reduce risk in safety and efficacy determinations in the course of drug development. They can be used to directly compare competing interventions, to evaluate pharmacokinetics, to stratify clinical populations, to ascertain treatment considerations such as dose and duration. They are also often sensitive predictors of adverse events associated with a given agent, including the detection of central effects of agents with primarily peripheral actions. As a surrogate for a clinical endpoint, they ideally provide earlier (and less expensive) measures of clinical efficacy and side effects. Furthermore, useful biomarkers may support the extension of a known compound into new therapeutic areas as its effects in the human brain are elaborated, and importantly, may also permit the "rescue" of drugs that have failed a primary CNS indication (O'Connor and Roth 2005; Shorter 2002). Biomarkers are now ubiquitous in drug development for indications throughout medicine, with variable levels of success which depends in a large part on the degrees of validation of the measure as a biomarker.

Given the foregoing review, it should be clear why the development of biomarkers in psychiatry has lagged considerably behind that of the rest of medicine. Nonetheless, CNS may be the vanguard in the use of imaging biomarkers in medicine, with other specialties following this lead, and it is reasonable to assume that each of the goals of a good biomarker outlined above will be attained with

functional neuroimaging measures in the foreseeable future. Wong et al. (2009) emphasize two critical phases in the course of drug development where noninvasive neuroimaging has emerging potential. First, clinical experiments that are conducted relatively early in development that aim to demonstrate "proof of biology" by testing a novel hypothesis by associating target engagement with a biological change that is proposed to lead to a therapeutic response. Second, subsequent clinical studies that demonstrate "proof of concept", defined as proof that this engagement of the target is linked to a clinically significant change in a clinical endpoint, or in other words that this target engagement by the drug has a true clinical impact that is likely to change how patients with the given condition are treated. This is typically established in phase II clinical trials.

A number of other practical advantages are observed with pharmaco-fMRI in drug development. The method does not involve exposure to ionizing radiation, rendering it more useful for repeated imaging studies in individual subjects. The capacity for easy, reliable repeat scanning is a significant advantage, as this is necessary to permit full washout of drugs with a long elimination half-life (which is a desirable feature of agents for clinical use), and to evaluate effects that may be region- and time-sensitive, such as those underlying a latency to clinical efficacy, as well as tolerance, sensitization and withdrawal effects (Stein 2001). Time-sensitive effects may be important to identify limits to effective drug action. Repeat studies also allow within-subject study designs, to confer greater statistical power for the evaluation of dose–response relationships (and dose–response interactions with brain region) and for the comparative efficacy of different agents, by varying doses or agents within subjects across sessions (Honey and Bullmore 2004).

3 Functional Magnetic Resonance Imaging in Preclinical Drug Development

The utility of fMRI is in fact observed even prior to clinical phases of drug development. One distinct advantage of this methodology is that it can be used noninvasively and in a repeated manner across diverse species, yet it is also feasible to combine with more invasive measures of neural activity in animal models. Rodents and primates alike are now readily investigated with fMRI, including with MRI scanners with small bores to accommodate rodents and particularly high magnetic field strengths (e.g. 12 Tesla) that are not yet approved for use in humans. The use of high field strength and combination with invasive measures such as intracranial electrical recordings or microdialysis has afforded insights into the relationship between BOLD signal change and cellular processes, to inform the use of fMRI in clinical populations. fMRI in animal models provides "functional signatures" by identifying distributed neural circuits engaged by a certain drug. It may be more sensitive to changes in a circuit underlying behavioral or cognitive effects than the overt behaviors themselves that are measured in traditional behavioral assays. Active circuits modulated by drugs may also remain

undetermined by overt behaviors in behavioral assays, yet may nonetheless have clinically relevant effects. It is less dependent on a limited set of receptor ligands, which significantly restricts the range of pharmacological assessments available in positron emission tomography (PET). fMRI may also have a particular advantage for specific classes of agents. For instance, full receptor agonists, partial agonists and inverse agonists are now entering the pharmacopoeia as novel agents for a variety of neuropsychiatric conditions (with aripiprazole as a successful example). Agents with these pharmacological actions, or potent agonists acting at low-density CNS receptors, are difficult to evaluate with other modalities such as PET. fMRI is uniquely capable of evaluating the neural effects of agents such as these, as it primarily indexes post-synaptic membrane polarization states, without relying on competition between a given agent and a (radiolabelled) competitor for ligand binding (see below). fMRI may in fact be the only existing method which can provide proof of target (at the circuit level), and target engagement, for these classes of agents (Borsook et al. 2006).

4 The Biophysical and Physiological Basis of Signal in fMRI

Because a fundamental concern of neuropharmacology is the effects of chemical compounds on signaling processes in the brain, it is essential to consider the types of signaling processes that can be measured by fMRI. As with all MR-based imaging procedures used in humans, the fMRI signal from which neural activity is inferred is derived from complex biophysical phenomena, and dependent on a particular set of physiological features of neural and hemodynamic properties of the brain and cerebrovasculature. While a full treatment of this is precluded by length considerations, a proper basic understanding is necessary in order to consider how fMRI measures brain activity, and the challenges of elucidating the action of drugs which act on both the neurons and the vasculature of the brain. Many excellent, detailed overviews of these phenomena are available elsewhere (Logothetis and Wandell 2004; Norris 2006); these are briefly outlined here as an introduction, along with some basic procedural aspects of MRI. First, a large, doughnut-shaped magnet with a circular electric current (maintained at very low temperatures to perpetuate the current) establishes a static magnetic field with relatively high strength, on the order of 40,000 times that of the earth's magnetic field. Subjects are placed inside, with humans typically reclining awake and relaxed in a supine position on a table inserted into the bore of the magnet; they typically maintain both visual contact with a monitor to present visual stimuli during cognitive tasks, and auditory contact with the investigator at the MRI console (though other tasks and monitoring are feasible, such as auditory, gustatory or somatosensory stimuli, physiological monitoring, eye tracking/pupillometry, etc.). The magnetic field is experienced in the subjects' soft tissues, with the most important effect being the alignment of protons with the direction of the static field. These protons are primarily available as hydrogen ions in water;

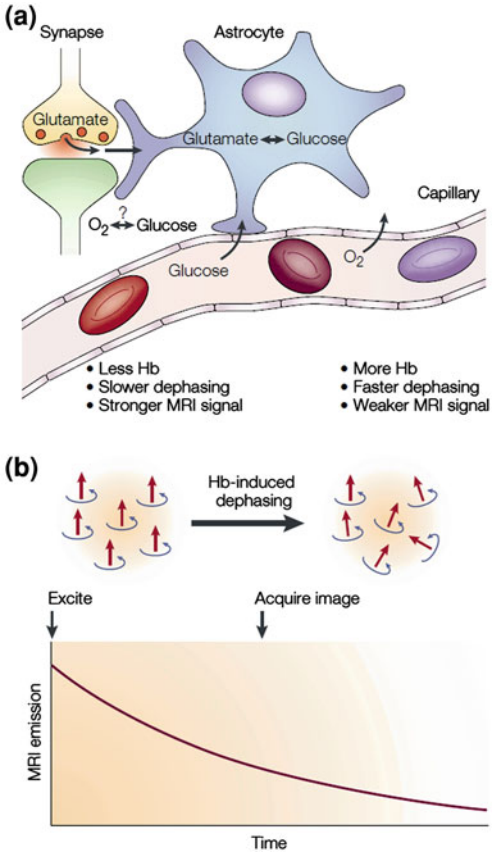

Fig. 1 BOLD functional magnetic resonance imaging (fMRI)

at relatively low field strength this source is primarily intravascular, but as the field strength increases, an increased proportion of protons from water are contained within the parenchyma of the brain. A radiofrequency pulse is then triggered in a repetitive manner during the course of image acquisition. This pulse alters the angle of the protons' spin in a manner that is controlled by the pulse sequence (the "flip angle") set by the investigator. With the brief cessation of the pulse, the original angle of the protons' spin is assumed again. This is referred to as "relaxation", and during this process energy is released which is detected by the scanner. A gradient coil distributes the field in a graded manner across space (i.e., the subject's head); this creates signal variation in two spatial dimensions that permits a two-dimensional image "slice" of the brain to be acquired, and these slices are in turn acquired in a progressive, repetitive manner across the third spatial dimension so that a three-dimensional image can be constructed. The signal acquired is maximized with the proportion of protons that resonate, that is, spin in phase with each other. Protons that spin out of phase tend to cancel each other's contribution to the signal recovered by the scanner. This "dephasing" is strongly

affected by inhomogeneity in the local tissue environment; in the vasculature, this is determined most critically by the oxidation state of hemoglobin contained in erythrocytes that course through the tissue.

Here, the physiology of the cerebrovasculature comes into play. The vasculature of the brain has complex autoregulatory processes to maintain blood flow and oxygen delivery to the brain (Heeger and Ress 2002) (see Fig. 1).

Among these, as relative increases in local neural activity occur (e.g. in response to a sensory stimulus or another information processing demand), there is a transient increase in flow to the local area. While the increased neural activity elicited in this manner may rely primarily on "aerobic" glycolysis, the vasculature tends to deliver more oxygen than needed locally. Therefore, the oxygen extraction fraction tends to decrease with increased local neural activity, and the fraction of oxygenated hemoglobin coursing through this vascular bed increases. As the paramagnetism of hemoglobin decreases with increasing oxygen content, the local field inhomogeneity is transiently decreased, permitting a relatively higher rate of signal recovery by the scanner, due to relatively less dephasing of the signal among the affected protons. Thus, the signal measured in fMRI is referred to as the Blood Oxygen Level-Dependent contrast, or BOLD signal (Bandettini et al. 1992; Kwong et al. 1992; Ogawa et al. 1992) [reviewed in (Logothetis and Wandell 2004)]. This signal is measured as a relative signal, as it does not strictly quantify blood flow, oxygen content, or energetics in the neurons or local tissues (though other emerging MR-based methods are now available to quantify flow, such as arterial spin-labeling). Therefore, the degree of neural activity determined with this method and attributed to the experimental condition(s) must be measured relative to a "baseline" condition. This baseline can be either an implicit baseline, typically determined as the residual (i.e. the error term) in a statistical model of experimental effects, or alternatively, the signal associated with another explicit (baseline) experimental condition.

A critical feature of this complex physiological process, with particular implications for the use of fMRI for pharmacology, is the link between neural activity and hemodynamics. This functional link, between neurons/glia and the adjacent vasculature, is referred to as *neurovascular coupling*. The precise cellular and molecular mechanisms that form the basis of this coupling remain incompletely elucidated (Raichle and Mintun 2006). However, a number of distinct yet interrelated biochemical processes and mediators have been implicated. These include vasoactive ions, nitric oxide, glutamate, adenosine, lactate, prostaglandins and other endogenous compounds, with a special role for astrocytes (Girouard and Iadecola 2006). Most of the energy need of neurons, especially in primates, appears budgeted for postsynaptic activity (Attwell and Iadecola 2002). However, other investigators have found evidence for presynaptic activity as a major energy consumer as well, and alternatively, there is evidence that neurotransmission may directly drive changes in hemodynamic activity, not dependent per se on energy utilization [see (Logothetis and Pfeuffer 2004) for review]. What is common to each of these models of neurovascular coupling is an emphasis on signaling activity by neurons as the basis for changes in local hemodynamics. Importantly, for the

present discussion, a considerable range of pharmacological agents, including numerous therapeutic CNS drugs, may have either direct or indirect effects on one or more of these biochemical processes. There is evidence for cerebrovascular regulation by a range of neurotransmitter systems, including glutamate [via NMDA receptors: (Faraci and Breese 1993; Lovick et al. 1999)], dopamine (Krimer et al. 1998), norepinephrine (Palmer 1986), serotonin (Palmer 1986) and acetylcholine (Sato et al. 2002). How the precise sites of action of these neurotransmitters in the vasculature may affect neurovascular coupling, or other hemodynamic responses that affect BOLD signal change, remain to be elucidated. This is an active area of research, not merely because of the implications for interpreting BOLD signal change in pharmaco-fMRI studies, but also because the neurochemical regulation of the cerebrovasculature is important to numerous disorders of this system, including migraine, stroke and Alzheimer's disease (Girouard and Iadecola 2006). In the meantime, particular experimental methods have been recommended to support disambiguation of neural versus vascular sources of drug effects on the BOLD signal. These include fMRI experimental design that includes proper control conditions that are not expected to show neurally mediated drug effects; direct measures of blood flow and vascular reactivity, other physiological monitoring such as oxygen consumption, and complementary measures of neural activity that are not dependent on blood flow, such as electroencephalography (Iannetti and Wise 2007). It should be appreciated that the use of these measures is not necessarily straightforward, as drug-induced changes in flow for instance may arise from changes in local central neural activity rather than directly on the local vasculature. Nonetheless, convergent evidence for a neural mechanism of action of drugs evaluated by fMRI remains an important goal.

5 Features of Neural Signaling that are Measured by fMRI

5.1 Local Field Potentials Versus Action Potential Generation

Recent studies have begun to elucidate which of the diverse types of signaling processes manifest by neurons may be measured in BOLD signal change. A very influential study conducted by Logothetis and colleagues (Logothetis et al. 2001) investigated electrical activity in awake monkeys concurrently with measurement of BOLD contrast by fMRI. Microelectrode recordings were made in the V1 visual cortex to derive measures of both local field potentials (LFP) and multi-unit activity (MUA). The LFP is a measure of slow electrical signals and subthreshold activity in neuronal membranes (not action potentials), reflecting primarily a weighted average of synchronous dendritic and somatic components of synaptic signals of a local neuronal population, with LFP amplitude a function of the extent and geometry of the recorded dendrites. MUA, in contrast, is a measure of overt spiking (action potential) activity in a recorded neuronal population, with cell size a critical determinant of amplitude, and the MUA is variably heterogeneous as a

result, especially across brain regions. Both the LFP and MUA are measured in a local population of neurons adjacent to the recording electrode, though the LFP probably is detected from a larger expanse of space than the MUA. Logothetis evaluated how each of these measures compared in their relationship to BOLD signal change in the monkeys, first in the absence of visual stimulation, to determine how to model the relationship of each to the BOLD signal. Then, they compared visually evoked BOLD responses with those predicted by the models derived from LFP and MUA. The authors found that both the LFP and the MUA predicted BOLD signal change very well in visual cortex, with a slight but significant advantage for the LFP. The difference appeared due to the transient nature of the MUA, which returned to baseline prior to stimulus offset, in contrast to both the LFP and BOLD signal, which were sustained for up to 20 s. The authors concluded that BOLD signal primarily reflects input and processing within a local area rather than spiking output. In contrast, a different study addressing this problem found that LFP and MUA were comparable in the strength of their relationship with BOLD signal change, measured with intracranial recording in awake humans (Mukamel et al. 2005). However, other studies may provide definitive evidence in favor of the LFP account of BOLD signal change (Mathiesen et al. 1998; Thomsen et al. 2004). Using a rat cerebellar preparation that permits the uncoupling of LFP with MUA, these investigators employed electrical stimulation of parallel fibers that leads to both a monosynaptic excitatory postsynaptic potential, and a disynaptic inhibitory postsynaptic potential in the Purkinje output neurons of the cerebellar cortex. With strong synaptic excitation but minimal change in the net spiking activity of these neurons, they found that blood flow and LFP nevertheless increased. This critically suggests that the mechanism underlying BOLD contrast is more directly determined by the LFP than spiking activity (though it is important to acknowledge that under most conditions, LFP and MUA are themselves highly correlated). These and other studies have also suggested that there may not be a simple linear relationship between LFP and the BOLD signal, and that the association of BOLD with both LFP and spiking activity may vary with the design of experiments, including stronger associations among all measures seen with more transient stimulus presentations that are typical of rapid event-related designs in fMRI (see Heeger and Ress 2002). More generally, with these studies BOLD signal change is presently thought to primarily reflect the postsynaptic integrative processes that occur in a local brain region, which are more diverse and arguably more informative of neural integration than spiking activity (Logothetis and Wandell 2004).

5.2 Oscillatory Brain Activity

One of the more interesting features of this synaptic integration which relates to BOLD signal change is the oscillations observed among populations of neurons. A wide range of oscillatory phenomena is seen across a wide frequency band and

throughout the brain of mammals (Buzsaki and Draguhn 2004). A number of oscillatory frequency bands have been implicated in information processing, and more generally, they may serve as signatures of dynamic functional assemblies of neurons that are responsible for cognition. Oscillatory activity can be detected by subjecting the LFP to filters to restrict the electrical potentials to certain oscillatory ranges. It can also be detected in the EEG recorded non-invasively from the scalp, when the electrical signal recorded is subjected to signal processing methods such as wavelet deconvolution. While scalp-recorded oscillatory activity maintains a lower frequency limit due to attenuation and filtering of the signal by tissues intervening between brain and scalp, brain oscillations detected at the scalp nonetheless correspond well to that measured intracranially, in their relationship with other biological and cognitive processes. Like the LFP more generally, brain oscillations are not strictly associated with spiking activity. Single neurons can oscillate, typically as a function of a set of coordinated cell membrane ion conductances; however, large-scale oscillations tend to emerge from complex dynamics between multiple neuronal types. A critical element appears to be the activity of inhibitory interneurons, which in the cortex use gamma amino-butyric acid (GABA) as a neurotransmitter. Oscillation-based synchrony is the most energy-efficient mechanism for temporal coordination (Buzsaki and Draguhn 2004). Oscillation may be an important mechanism of gain control in the nervous system (Salinas and Sejnowski 2001), as well as supporting resonance (Hutcheon and Yarom 2000), which can be seen as a form of tuning of neurons. Gain control and resonance are two important features of neuronal activity that confer sensitivity and specificity to neuronal signals and codes. One high-frequency oscillation that appears particularly related to BOLD signal change is in the gamma range (typically defined as 30–80 Hz, though higher gamma frequencies can be detected in intracranial recordings). A number of research groups have now reported strong and specific associations between BOLD signal change and gamma-range oscillations in the LFP, with diverse experiments such as the comparison of intracranial responses of neurosurgical patients to BOLD signal change in healthy adults in response to the identical visual/auditory stimulus (Mukamel et al. 2005; Nir et al. 2007); combined fMRI/EEG recording in epileptic patients performing a semantic decision task (Lachaux et al. 2007); and simple visual stimulation of V1 in monkeys (Goense and Logothetis 2008). It thus appears that BOLD signal change may reasonably index changes in power in the gamma range. This is fortuitous given the diverse cognitive processes that are also associated with gamma oscillations. These include perceptual integrative processes such as binding of sensory representations into a coherent percept (Fries 2009); episodic memory (Herrmann et al. 2004); working memory (Tallon-Baudry et al. 1998); and cognitive control processes (Cho et al. 2006; Minzenberg et al. 2010). These cognitive processes are generally highly dependent on prefrontal cortical function and ascending modulation by catecholamines and other neurotransmitter systems. Gamma oscillations (measured primarily by scalp EEG) are disrupted in a number of neuropsychiatric conditions (Herrmann and Demiralp 2005; Uhlhaas and Singer 2006) which are also characterized by cognitive deficits in these processes and their neural

substrates, measured by functional and structural imaging and post-mortem histopathology. This critically suggests that altered brain oscillations may represent a physiological signature of neural circuit dysfunction that is expressed in impaired cognition and perhaps even clinical symptomatology. While brain oscillations are fundamentally dependent on the complex temporal-spatial dynamic interactions between GABAergic interneurons and glutamatergic principal cells in regions such as neocortex and hippocampus, nonetheless there are a number of other neurotransmitter systems that modulate the power and frequency of these oscillations, and perhaps the neuronal membership in an active assembly that participates in a given oscillation (Whittington et al. 2000). These observations suggest the potential for fMRI to characterize the sites and mechanisms of action of pharmacological agents (including monoaminergic and peptidergic agents, anesthetics, and other drugs) which affect brain oscillations, and the relationship of these oscillatory effects with clinical efficacy.

5.3 Negative BOLD Response

In typical traditional fMRI studies, investigators process the acquired images and derive the signal used to infer brain activity using a linear statistical model of BOLD signal change. Statistical inference regarding the predicted experimental effects is made by contrasting the targeted experimental manipulation against the (implicit or explicit) baseline, with increased activity (typically beta values derived from the regression model of BOLD signal change) above the baseline leading to rejection of a null hypothesis. However, approximately 10 years ago, various research teams began to appreciate certain experimental conditions associated with changes from baseline in a *negative* direction, i.e. phenomena where local neural activity appeared higher in the baseline, or "resting" state and subsequently decreased during certain cognitive demands. Directed interrogation of brain regions that exhibited this pattern of task-related deactivations from baseline revealed a previously unidentified functional network in humans, centered on midline cortical regions in the medial prefrontal and parietal cortices, and lateral cortical areas including the inferior parietal lobule and lateral temporal lobe. Remarkably, this same network has been consistently identified in functional connectivity analyses of so-called "resting state" BOLD fluctuation, where subjects are awake and alert during fMRI but unengaged in any externally directed cognitive task. This network is now commonly referred to as the "default mode network", which implies a significant level of activity in the absence of an overt or externally directed task, and it is observed in both humans and non-human primates (Buckner et al. 2008; Raichle and Mintun 2006). More recently, there is evidence that the default mode network is negatively correlated with the fronto-parietal "task-positive" network, implying a reciprocal relationship that may be controlled in accordance with the behavioral state or current goals of the organism (Fox et al. 2005). In the context of the present discussion, one important implication of this discovery is that BOLD signals may be used to meaningfully

evaluate neuronal inhibition with certain cognitive processes, especially those with significant demands on attention or control processes.

An important consideration for fMRI studies of pharmacology is how the "negative BOLD response" (NBR) may inform the nature of neuronal membrane polarization states or signaling activity in the brain, and how these may be sensitive to pharmacological intervention. The metabolic budget associated with inhibitory neuronal processes is incompletely known at present, and it has been argued that quantification of the energy cost of inhibition cannot be meaningfully isolated from that of excitation in the brain (Buzsaki et al. 2007). Nonetheless, recent fMRI studies in animals have offered some intriguing hints that the NBR may be associated with increased neuronal inhibition in a local brain region. These studies have found that NBR is associated with decreased blood flow and blood volume and increased deoxyhemoglobin locally, and decreases in both the LFP and MUA (Boorman et al. 2010); and that cortical hyperpolarization occurs with local decreases in oxygenation, as would be expected with the NBR (Devor et al. 2007). fMRI studies in humans are consistent with these observations (Shmuel et al. 2002; Wade and Rowland 2010) and suggest that the NBR is not merely due to vascular steal or altered neurovascular coupling (Lin et al. 2011). Furthermore, the NBR in humans has been associated with cortical GABA concentration measured by magnetic resonance spectroscopy (Northoff et al. 2007). These various observations suggest that fMRI has utility for detecting inhibitory neuronal processes, and therefore potential for the evaluation of pharmacological effects that are manifest in neuronal hyperpolarization or decreased neuronal activity. Neuronal inhibitory processes are not only essential to brain oscillations (as indicated above), but form a fundamental feature of the operation of neural networks in general, both at the local circuit level and in long-range intracortical and cortical-subcortical network operations as well. Indeed, the "small-world" nature of cerebral circuitry that is increasingly apparent in the mammalian brain is critically dependent on these inhibitory processes, which are probably mediated primarily by GABA (Buzsaki et al. 2004). Furthermore, the actions of modulatory neurochemical systems (including those at virtually all monoamine receptor subtypes) are predominantly inhibitory. These observations suggest that for a neuroimaging method to comprehensively interrogate the effects of drugs on signaling in the brain, a valid and sensitive measure of inhibitory processes is essential. fMRI is promising in this regard, and cellular model-based tests of neuronal inhibitory effects of pharmacological agents have recently appeared (Minzenberg et al. 2008, 2011).

6 fMRI as a Tool to Evaluate the Functional Role of Central Neurotransmitter Systems

fMRI has at least three key methodological features which confer important advantages over other neuroimaging and non-imaging methods used in human pharmacology. First, the spatial resolution with the currently standard fMRI

methods for image acquisition and processing allows the discrimination of activity in local brain regions at approximately millimeter spatial resolution. While this is not yet a degree of spatial resolution that can discriminate cortical columns, or subregions within small subcortical nuclei, for instance (at least not with standard methods), it is increasingly adequate for the resolution of adjacent subcortical regions in tightly packed subcortical regions such as the thalamus or midbrain dopaminergic areas. Second, the temporal resolution of fMRI is critical to monitoring the time course of information processing as it unfolds in typical cognitive task paradigms. Virtually all experimental tasks that are in use to evaluate cognitive processes comprise few to many subcomponent processes. These include investigations of perception, attention, various forms of memory, control processes, incentive and reward processes, and motor output, not to mention more complex phenomena such as social and emotion processes. While fMRI is constrained by the hemodynamic response from the millisecond sampling rate of EEG, for instance, it still can be easily used to isolate neural activity associated with discrete events ("event-related" fMRI) or processing stages within single trials. This stands in contrast to either PET or the older approach in fMRI, which both rely on blocked sequences of trials to compare brain activity under different experimental conditions. This older approach does not permit the isolation of the varied subcomponent cognitive processes that might be demanded in a single trial: for instance, a typical working memory task paradigm requires a subject to encode a stimulus, maintain a representation of the stimulus or its associated information across a delay, perceive the subsequent target stimulus and reactivate the stored information and use it to make a decision and an associated overt motor response. This entire sequence of information processing stages typically occurs over a span of 3–20 s. Because it is entirely possible, and of great theoretical and practical interest, that a given drug modulates the maintenance of information and not the motor response that follows from the use of that information, event-related fMRI represents a clear advance over other noninvasive methods in achieving specificity in drug modulatory effects on cognition and its neural basis. The third major advantage of fMRI is the lack of dependence on the development of brain-penetrating, potent and selective ligands for neurotransmitter receptors of sufficient density in the brain, to evaluate effects on signaling. Many important neurotransmitter systems are not yet amenable to interrogation with ligand-PET in humans (e.g. noradrenergic system), yet these systems remain fully amenable to the study of drug effects on signaling processes using brain-penetrating compounds such as beta adrenergic receptor antagonists and norepinephrine transporter inhibitors. In principle, any chemical agent that enters the brain and has psychological effects can be evaluated by fMRI, both in the presence or absence of information-processing demands.

As a consequence of these particular methodological features, fMRI represents an advance in the evaluation of drug action on neurotransmitter systems in modulating distributed neural networks and the discrete cognitive processes that they support. The capacity to interrogate small structures located deep in the brain is a major advantage over EEG for instance, which only measures electrical

activity arising from large cortical regions. In order to fully understand how neurochemical systems operate, and how they are affected by drug interventions, it is critical to have a method that can evaluate drug effects on activity at the cell bodies of neurons that give rise to these systems. This is because of a single-minded focus on terminal fields (e.g. in the cortex) of projections arising from these subcortical areas, while representing the "business end" of the system as the local influence on signal throughput, nonetheless cannot account for how a drug may affect neurotransmission by modulating its major determinant, which is activity arising from the cell body. In addition, these subcortical structures have numerous reciprocal projections with their projection targets, and therefore drug effects on cognition may result from changes in descending influence on subcortical nuclei. For these reasons, it is important to interrogate the full anatomic extent of these systems, that is, the activity from the origin of cells in subcortical regions to their full terminal fields throughout the brain. A recent and exciting development in functional neuroimaging therefore is the emerging capability of fMRI to measure activity in subcortical regions such as the ventral tegmental area, substantia nigra and locus coeruleus, which give rise to monoamine neurotransmitter systems which are of great importance to both the etiology and treatment of much neuropsychiatric illness.

6.1 fMRI Studies of Subcortical Catecholamine Systems

The neurochemical systems that arise from the human brain stem and use dopamine (DA) or norepinephrine (NE) as a neurotransmitter subserve critical roles in a number of cognitive and behavioral processes. For DA, these include incentive and reward-based learning (Haber and Knutson 2010), behavioral and cognitive control (Montague et al. 2004), attention, integration of related affective information such as pleasurable and aversive features of environmental stimuli to modulate cognition and guide behavior, and integration of brain activity as a function of general overt activity levels and the sleep–wake cycle. Cognitive and behavioral processes subject to modulation by NE overlap with those of DA to a larger degree than has been traditionally appreciated. Reward-based learning may be the only one of these processes that has been largely unaddressed with respect to the central NE system. NE additionally has long been recognized for its role in arousal, elementary aspects of perception and autonomic functions. In general, these systems, arising from the ventral tegmental area (VTA) and the substantia nigra (SN) of the ventral midbrain for DA, and the pontine locus coeruleus (LC) for NE, serve as critical links between the environment and the organism, for the valuation and use of information to guide information storage and goal-directed action (Aston-Jones and Cohen 2005; Bouret and Sara 2005; Montague et al. 2004; Sara 2009) [see Figure from Sara (2009) below for projection patterns arising from VTA and LC]. In keeping with this general role, the circuitry that these areas participate in is extensive, involving a large share of the major functional areas in

cortical and subcortical regions. For the VTA, this primarily includes limbic regions (e.g. hippocampus, hypothalamus, amygdala, ventral striatum, basal forebrain cholinergic nuclei), association neocortices, and other monoamine systems. For the SN, this includes dorsal striatum, thalamus, and more distant targets in the motor cortices, other cortical areas and cerebellum. For the LC-NE system, virtually the entire brain serves as a target, with the dorsal striatum as the only region in the mammalian brain that contains a paucity of NE or adrenergic receptors. These networks are organized as discrete functional loops which give rise to much of what the brain can achieve in cognition and behavior. As might be expected, these networks are also disrupted in a wide range of neuropsychiatric illnesses, including Parkinson's disease, schizophrenia, obesity and drug addiction, psychopathy, dementias, mood and anxiety disorders, attention-deficit disorder and many others. Accordingly, these catecholamine systems are important targets for the action of existing and candidate drugs for the treatment of these disorders, currently including l-DOPA, DA and NE transporter inhibitors, adrenergic antagonists, and DA receptor agonists and antagonists.

It is therefore of great interest to interrogate the functional dynamics of catecholamine systems during cognitive and behavioral processes, as model systems in which to evaluate the potential of new and existing drugs to modulate these processes and remediate the clinical syndromes arising from disturbances in these processes.

Recent work from several research groups has shown that these subcortical areas can be reliably evaluated during experimental cognitive tasks in humans, and that the functional role of these areas in humans is consistent with those determined in animal models (Duzel et al. 2009). While the LC-NE system has been relatively less well studied in humans, there is evidence that drug effects on this system can be tested in a manner that is informed by physiological and cognitive model-based predictions (Minzenberg et al. 2008). In addition, concurrent pupillometry can be acquired in the MRI environment, both to aid in topographic localization of the LC in BOLD images, as well as to provide a novel concurrent behavioral measure of LC-NE system functioning that can be related to the fMRI measure of LC activity during cognitive processes (Sterpenich et al. 2006). Among the midbrain DA regions, there are a number of distinct and important species differences between rodents and humans or non-human primates (Duzel et al. 2009). These species differences highlight the need for a valid and sensitive method to study these systems directly in humans. In addition, in humans, the functional and anatomic distinctions between the VTA and SN in particular are also much less well drawn than is commonly appreciated. For example, the midbrain region most representative of the rat VTA resides in the dorsal part of the primate SN; these two nuclei are more continuous with each other in humans than in the rat, leading some anatomists to distinguish dorsal/ventral tiers of the midbrain DA complex rather than VTA versus SN (and the retrorubral field); the projection patterns of VTA versus SN are not easily distinguished in humans; and the response properties of VTA and SN neurons show no significant differences (reviewed in Duzel et al. 2009). Due to these observations, plus the limits on

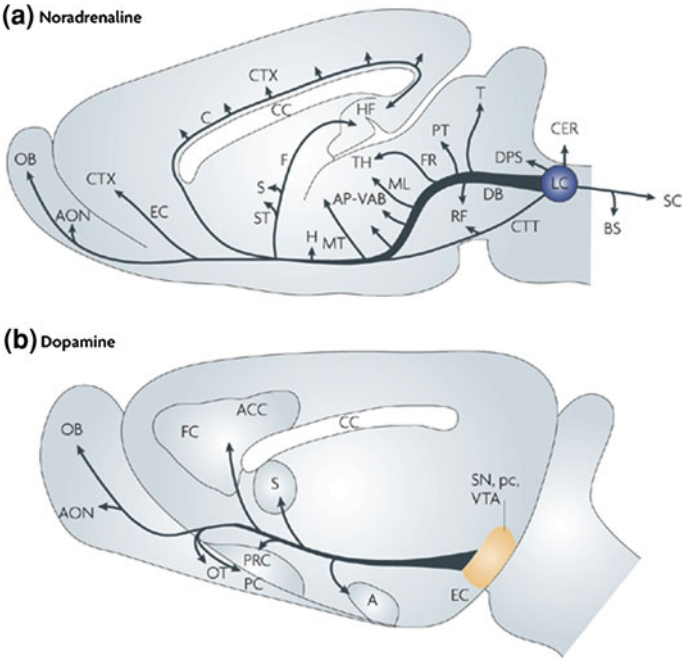

Fig. 2 Comparative anatomy: **a** Noradrenaline. **b** Dopamine

spatial discrimination between adjacent, small subcortical structures, many investigators who study these regions in humans with functional neuroimaging refer generally to the "dopaminergic midbrain." In these studies, the VTA/SN complex is typically localized in BOLD images by reference to structural scans acquired as either magnetization transfer scans, proton density scans or T1-weighted scans sensitive to neuromelanin, which in the brain stem is restricted to catecholamine neurons.

It is important to consider the various possible underlying physiological sources of BOLD signal change in the neurons found in this area [see (Duzel et al. 2009) Fig. 2]. These could include LFPs arising from either glutamatergic or GABAergic inputs to tonically active DA neurons, glutamatergic inputs to silent DA neurons, or inhibition of GABAergic input to DA neurons. BOLD signal change could also arise from burst firing of DA neurons, or local DA release from DA neurons in either burst or tonic firing modes. While the relationship of LFPs in these DA-rich areas to discharge of these neurons remains to be characterized in animals, the most parsimonious account, based on single-unit studies in monkeys, suggests that DA neuron discharge is primarily a function of direct excitatory glutamatergic input from the neocortex or elsewhere in the midbrain. In addition, fMRI studies in animals have supported a relationship between BOLD signal change and DA neurotransmission. These studies find a strong association of DA release (induced

with stimulants) and BOLD signal change in limbic terminal fields of midbrain DA projections e.g. nucleus accumbens; (Chen et al. 1997), and that this relationship persists with DA lesions and fetal DA neuron grafts (Chen et al. 1999). Stimulant-induced changes in blood volume (which itself is highly-related to BOLD signal change) show similar associations (reviewed in (Knutson and Gibbs 2007), and these stimulant effects show predictable interactions with co-administered D2 antagonists (Chen et al. 2005; Schwarz et al. 2004). An elegant study of healthy human subjects, using fMRI during a reward-based learning paradigm and ligand-PET, found BOLD signal change in the VTA/SN to correlate with [^{11}C] raclopride displacement as a measure of increased DA release in the nucleus accumbens (Schott et al. 2008). These various studies indicate that BOLD signal change closely follows DA neurotransmission, at least in some brain areas that receive strong input from the VTA/SN. Nonetheless, a number of physiological features of these systems may have important implications for interpreting studies of cognition and pharmacology in humans, including the role of local inhibition in the VTA/SN, the role of silent DA neurons, and the temporal relationship of BOLD signal change to discharge of these neurons. Studies combining functional neuroimaging with genetics or pharmacology in humans, or invasive measures in animals, will further elucidate the underlying basis of BOLD signal change in these brain areas to optimize the utility of neuroimaging in studies of human pharmacology.

7 Summary

The emergence of noninvasive functional neuroimaging methods such as fMRI for neuropharmacology is intimately tied to the status of drug development for CNS disorders, and the importance of currently unmet treatment needs among patients with these disorders. These imaging methods are seen to have a number of unique advantages to meet these goals, as well as serving as a tool for basic neuroscience. The physiological basis for signal change in fMRI is complex, and the readout in BOLD signal change is a function of a number of signaling processes in the brain, each of which are of interest for pharmacology. Widely distributed neurotransmitter systems such as the monoamines are readily amenable to study using fMRI, and the dynamics of these systems' operations over space and time can be interrogated in a uniquely advantageous manner. fMRI and related methods therefore stand poised to occupy a unique position in the armamentarium of experimental methods available to the basic and clinical scientist, to achieve progress in our understanding of the pharmacology of the brain and how we may attain greater success in relieving the massive burden of neuropsychiatric illness.

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Index

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