Chapter 15
Perspectives for an Integrated Biomarker Approach to Drug Discovery and Development

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Abstract Today's psychopharmacological drugs remain focused on targets that were identified serendipitously more than half a century ago. As these targets have not proven to be at the core of the pathophysiology of the major psychiatric disorders, a better understanding of the disease biology seems a crucial step to identify more efficacious treatments. The tools to realize this goal include neuroendocrine, protein, transcription and genetic markers, neuroimaging and neurophysiological approaches. Obviously, the benefit for psychiatric patients of identifying a pattern of blood-based markers that combine information on the disease biology and treatment response would be enormous. Our transcription data from human blood cells suggest that this is a realistic possibility for the future. Ideally, markers identified in patients would be translated into distinct, hypothesis-driven animal models to facilitate conclusions on the potential therapeutic utility of novel compounds. The combined use of disease state and mechanistic models may characterize cellular and molecular mechanisms of various aspects of psychiatric disorders. This information, in turn, could help establish *in vitro* models that link cellular targets to (novel) pharmacological approaches. With the discussions around DSM-V, it can be hoped that the ambitious research agenda will guide and stimulate systematic research into the biology and biological markers of psychiatric disorders.

Abbreviations Akt: Serine/threonine-specific protein kinase; ANOVA: Analysis of Variance; BA: Broadman Area; Bcl-2: B-cell CLL/lymphoma 2; BDNF: Brain derived neurotrophic factor; CMS: Chronic mild stress; CRF: Corticotropin-releasing factor; DISC-1: Disrupted-in-schizophrenia 1; DNA: Deoxyribonucleic acid; DSM: Diagnostic and Statistical Manual; DST: Dexamethasone suppression test; FST: Forced swim test; GR: Glucocorticoid receptor; HAB rats: High anxiety bred rats; HPA: axis Hypothalamic–pituitary–adrenal axis; 5-HT: Serotonin; HUPO: Human Proteome Organization; ICD: International Classification of Diseases;

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LAB rats: Low anxiety bred rats; LC: Locus coeruleus; LH: Learned helplessness; mGlu2/3: Metabotropic glutamate 2/3 receptor; MPfc: Medial prefrontal cortex; MR: Mineralocorticoid receptor; NCE: New Chemical Entity; NIMH: National Institute of Mental Health; NRG1: Neuregulin-1; PBMC: Peripheral blood mononuclear cells; Pelora: Penalized logistic regression; ProDaC: Proteomics Data Collection; qPCR: Quantitative Polymerase-chain reaction; RNA: Ribonucleic acid; SGZ: Subgranular zone; siRNA: Small interfering ribonucleic acid; SLR: Stepwise logistic regression; SVZ: Subventricular zone; TST: Tail suspension test; VEGF: Vascular endothelial growth factor

15.1 Introduction

According to the National Institute of Mental Health (NIMH), mental disorders affect an estimated 26.2 percent of Americans aged 18 and older in a given year. Unlike many other chronic and disabling disorders, mental illnesses strike early in life, with unipolar depression and substance abuse accounting for 28% and 20%, respectively, of the disability from all medical causes in people aged 15–44 years (Insel and Scolnick, 2006). These data are in line with the Global Burden of Disease study, reporting that mental illness, including suicide, accounts for over 15% of the burden of disease in established market economies. This is more than the disease burden caused by all cancers.

Despite these alarming observations, biomedical research in the field of psychiatry has remained focused on treatment targets that were identified serendipitously more than half a century ago. Almost all available drugs target primarily monoamine transporter and receptors, in various combinations, leading to slightly different profiles. However, these differences rarely have a clinically relevant impact in terms of efficacy or safety. Moreover, the targets have not proven to be at the core of the pathophysiology of the major psychiatric disorders to this day, which may explain the modest efficacy of all available drugs when tested in not well defined patient populations. This is in contrast to research into other major chronic diseases, such as cancer and heart disease that has shed light on the biology, which has resulted in the successful development of new treatment targets.

However, it is tempting to look at the glass as half full in view of the ambitious research agenda for DSM-V, clearly stating the aim to develop ‘an etiologically based, scientifically sound (diagnostic) classification system’ (2002). Biomedical research that focuses on the disease rather than on treatment may improve our understanding of core biological alterations associated with psychiatric disorders. A better understanding of the disease biology, and the biological differences among patients, should advance the diagnostic classification, which today is entirely descriptive. In doing this, one would expect to also identify new targets that may yield more efficacious treatments, at least for subgroups of patients who share core biological disturbances.

A range of biological read-outs are mentioned in the DSM-V research agenda that may advance our understanding of the biology of mental disorders, including

neuroendocrine, protein, transcription and genetic markers, neuroimaging and neurophysiological approaches. Not surprisingly, the biological markers were and still are focused on the brain, where the pathophysiology of mental disorders is thought to occur. Although the brain certainly is a critical site to study the biology of mental disorders, there is increasing evidence for peripheral changes associated with mental disorders *per se* as well as with the response to treatment (Gold and Charney, 2002; Iga et al., 2007a; Maino et al., 2007). However, so far the majority of studies as well as the DSM-V research agenda focus on post-mortem assessment of brain tissue to discover molecular markers of psychiatric disorders, such as proteins and gene transcripts. While these studies can yield interesting molecular targets associated with a clinical syndrome and/or treatment response, several notes of caution are warranted: Firstly, one critical aspect related to biomarkers in psychiatry is their predictive value for either course of disease or treatment response. Obviously, markers identified in post-mortem brain tissue cannot address this need. Secondly, several variables can affect results of gene expression studies in post-mortem brain tissue and need to be carefully controlled for, as recently discussed in a publication by the members of the NIMH Conte Center and the Pritzker Neuropsychiatric Disorders Research Consortium (Atz et al., 2007).

Recently, multiple forms of blood markers as alternatives to brain markers have received significant attention (Avissar et al., 2004; Iga et al., 2007a, b; Ray et al., 2007b; Segman et al., 2005; Zieker et al., 2007).

Though quality control remains a very critical issue for all biomarker investigations, relatively simple procedures are available to ensure standardized blood collection with good preservation of RNA and reproducible transcription data in multi-center clinical trials. Obviously, the advantage of blood-based biomarkers that combine information on the disease biology and treatment response for psychiatric patients would be enormous.

In fact, the search for peripheral (blood) markers for affective states and treatment response is not new, but dates back several decades when publications showed differences in monoamines and related receptor levels in platelets from patients suffering from depression or schizophrenia, and effects of treatment (Garcia-Sevilla et al., 1981; Stahl et al., 1983). Though studies have not yielded a robust marker to date, advances in technology allow the study of multiple markers simultaneously and quantitatively at the transcriptional level in human peripheral leukocytes. Though the same is in principle true for protein biomarkers, there is no standardized procedure for sampling of plasma or serum in large clinical trials. Therefore, the detection and subsequent validation of protein biomarkers for psychiatric disorders will have to wait for agreed upon standards and guidelines by initiatives such as the EU funded 'Proteomics Data Collection' (ProDaC) workshops.

Meanwhile, data on gene transcription profiles from peripheral leukocytes showed interesting changes in patients suffering from an acute psychiatric disorder, suggesting that this approach is an encouraging route for the discovery of disease biomarkers in psychiatry (Segman et al., 2005; Zieker et al., 2007). As some of the very targeted transcription data also showed normalization with treatment, peripheral leukocyte transcription profiles could provide both disease as well as

treatment-related biomarkers (Iga et al., 2007a). In this chapter we discuss the use of gene transcription patterns as objective markers to identify biologically distinct subgroups of patients, to help understand the biology within these subgroups and to assess the treatment response. We will illustrate, using some of our own early data and experience, the possibilities transcription profiles from peripheral leukocytes offer in terms of add-ons to psychiatric diagnostic and efficacy markers.

As gene transcription analysis allows one to evaluate entire signaling pathways, this approach could discover patterns that distinguish between patients and controls as well as between subgroups of patients with high specificity and sensitivity. This, in turn, is a prerequisite for a biological marker to become a clinically relevant tool. Moreover, gene transcription related to cellular signaling and metabolism shows a high correlation between blood leukocytes and the brain in humans (Ray et al., 2007; Sullivan et al., 2006). Therefore, signaling alterations identified in human blood leukocytes can also guide hypotheses on the CNS pathophysiology of psychiatric disorders and provide critical information for the discovery of novel treatment targets.

The focus on patterns rather than individual markers should also help to establish complex relationships between biological markers and intermediate phenotypes. The importance of intermediate phenotypes is emphasized by the discussion put forward in the DSM-V research agenda on a dimensional vs. categorical classification system (2002). The dimensional approach includes an aspect often disregarded in psychiatric biomedical research, namely the examination of control populations. In this chapter we will provide data in support of the relevance of intermediate phenotypes by showing an association between transcription patterns and psychiatrically relevant clinical variables in a control population. Such data can be used to provide criteria for clinically relevant dimensions in a future classification system. Moreover, intermediate endophenotypes seem particularly relevant for drug development, as examination of drug effects in such 'control' subjects could provide early signs for efficacy in a patient population.

Another change in paradigm, put forward in the research agenda for DSM-V, is the importance of exploring biomarkers, including genetic markers, in smaller but biologically homogenous subject groups (2002). Therefore, a smaller group of subjects who share a distinct transcription profile could provide a stronger basis for detecting genetic markers than large but very heterogeneous populations, typically examined in pivotal clinical trials.

15.2 Clinical Drug Development

15.2.1 General Considerations

Due to the descriptive and purposefully non-etiological nature of today's psychiatric diagnostic classification (DSM-IV-TR and ICD-10), patients included in clinical trials are heterogeneous in terms of the clinical presentation and, as far as we know,

the underlying biology. One possibly extreme example of this unsatisfactory situation is the diagnosis of major depression, which encompasses patients with melancholic and atypical features, which are almost opposite in their clinical presentation and biology (Carroll et al., 2007; Gold and Chrousos, 2002). Moreover, there is evidence for a differential treatment response for these two subtypes (Quitkin et al., 1993; Stewart et al., 1993).

Another clinical discriminator likely to reflect biological differences is psychiatric co-morbidity. Many patients in clinical practice meet criteria for more than one disorder, particularly within the spectrum of affective disorders (Rush et al., 2005; Zimmerman et al., 2005). However, clinical trials examining safety and efficacy of a 'new chemical entity' (NCE) typically exclude patients with obvious psychiatric co-morbidity. This conundrum leads to an overrepresentation of patients with milder syndromes, but not necessarily a more homogenous patient population in terms of biology or treatment response (Khan et al., 2007; Souery et al., 2007). Support for this hypothesis comes from a recent analysis of antidepressant treatment trials performed between 1981 and 2000, showing an increase in the placebo response as well as highly variable responses to both placebo and active treatment (Walsh et al., 2002).

Hopefully, with further biological research, this unsatisfactory situation will be improved by identifying the biological signatures of disorders, including common co-morbid disorders, which may be re-classified as one disorder if a common biological basis for the symptoms is uncovered. One approach is to identify biological markers associated with certain features of psychiatric disorders both within and across toady's diagnostic entities. Thus, it can be presumed that distinct subtypes of major depression, such as depression with melancholic and atypical features, are distinguishable with regard to the (peripheral) biomarker profile (Gold and Chrousos, 2002). In fact, peripheral biomarker patterns may help to delineate core clinical features for these two depression subtypes, which remain a matter of debate (Parker, 2000; Parker et al., 2005; Posternak and Zimmerman, 2002). This would be an example of how biological markers contribute to a scientifically sound classification system.

An extension of the above approach is to address the biology of distinct clinical features across the boundaries of current diagnoses. The analysis of such complex relationships should help to characterize multiple intermediate phenotypes, which in turn may predispose to develop certain mental illnesses, e.g. when exposed to environmental stressors. Examples in psychiatry include impaired cognitive executive function, which can occur in schizophrenia, some forms of depression and in substance abuse. Another example is fatigue that can occur in different psychiatric disorders such as depression and anxiety, but also in disorders associated with a high incidence of depressive disorders, such as Parkinson's disease, Multiple Sclerosis and obesity (Heesen et al., 2006; Vgontzas et al., 2006; Weintraub and Stern, 2005). A focus on biological markers of distinct clinical features is in line with the DSM-V research agenda, which stresses the importance of studying (a) complex relationships between biological and clinical variables and (b) intermediate phenotypes (Charney et al., 2002). A better understanding of the biological basis of

certain clinical symptoms that are associated with different neuropsychiatric (and other) disorders will also help to improve treatment strategies for these complex disorders.

A prerequisite for the identification of such complex relationships is a thorough characterization of subjects, including clinical, environmental and biological factors. If taken seriously, this will markedly affect the information (clinical and biological) collected in clinical trials. The emphasis on biological markers and structured clinical information is rooted in the observation that information gathered through routine clinical methods is not reliable, leading to missing critical information such as previous diagnoses, that can markedly affect the treatment outcome (Ramirez et al., 2000).

Ultimately, the understanding of the biological underpinnings of core psychiatric syndromes will also advance the development of animal disease state models as well as cellular models to mimic specific aspects of a human disorder. This, in turn, will help profile new drug targets across today's diagnostic classification and should improve the predictive validity for novel drug targets in terms of efficacy (and safety) in humans.

15.2.2 Specific Biological Considerations

Ideally, by the time a NCE is first tested in man, preclinical data should have been collected that guide the clinical trials in terms of which target symptoms and biological features could be addressed with the potential new drug. While more specific clinical profiles are sometimes addressed as part of the life cycle management, leading to a broadening of the approved indication(s), reliance on biological read-outs is hampered by the lack of consensus parameters.

Though it is true that today no biological read-outs can be considered validated and of regulatory relevance, biological markers can (and should) be used for internal decision making. With some systematic guidance from the efforts around the DSM-V research agenda, biological read-outs can be selected from an abundance of (more ore less confirmed) data from decades of biological research in the field of psychiatry. This chapter will highlight some possibilities of how biological markers could address (biological) efficacy and selection of target populations in clinical development.

A starting point is the convergence of targets across current diagnostic entities. Thus, atypical antipsychotics are efficacious in treating depressive syndromes and recent drug development efforts target modulation of glutamate neurotransmission and inflammation for both affective disorders and schizophrenia. Also, there is growing evidence that risk factors, as well as alterations in neuronal circuitries, e.g. the limbic system and the basal ganglia, are shared between major psychiatric disorders such as schizophrenia and affective disorders. Moreover, some of the abnormalities observed in acutely ill patients can be detected in normal subjects who are or may be at risk to develop a disorder. Such intermediate phenotypes can

provide a useful enrichment strategy to support biologically based efficacy endpoints of NCEs in exploratory clinical trials.

In this regard, identification of biological markers which can provide reliable associations with distinct symptom clusters, treatment responses and safety profiles are needed to increase the probability of showing superior efficacy of novel drug treatments, and ultimately to improve patient care by hastening the process of identifying optimal treatment options for individual patients. The subsequent sections will focus on the opportunities that the disease biology provides for the discovery of diagnostic and efficacy biomarkers.

15.2.3 Disease Biology

Several biological alterations, including hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis, altered sleep EEG architecture, and metabolic and volumetric changes in limbic and prefrontal cortical brain areas have been identified in several major psychiatric disorders. Some of these changes also seem to confer a risk factor to develop a disorder rather than being themselves markers of the disease. The expansion of our knowledge into the molecular basis of these changes offers the chance to better understand the pathophysiology of the disorders and also to discover new treatment targets, including prophylactic treatments. As some biological alterations seem relevant for several disorders, systematic research into biomarker read-outs holds the promise to develop drugs that target specific biological changes across the current diagnostic boundaries. This approach should ultimately help to fulfill the goal of developing a scientifically sound classification system. The following sections will briefly review biological changes associated with major psychiatric disorders that could be used as early read-outs in drug development.

15.2.3.1 Stress and the Hypothalamic–Pituitary–Adrenal Axis

The HPA axis controls the release of glucocorticoids and is an essential component of an individual's response to stress. Several changes related to the HPA axis have been reported in psychiatric disorders, with most consistent changes described in patients with major depression. In general, changes related to the HPA axis can be divided into hyper- and hypoactive states, with the former being most consistently seen in patients with melancholic or psychotic features (Carroll et al., 1976, 2007; Gold and Chrousos, 2002; Holsboer, 1999; Kunzel et al., 2003). Several challenge tests have been developed, including the dexamethasone suppression test (DST), first employed more than 30 years ago, the refined dexamethasone-corticotropin-releasing factor (CRF) test, CRF and insulin tests. While none of these tests have reached the level of sensitivity and specificity to be considered as a diagnostic marker, some data suggest that the dexamethasone-CRF test could be an early indicator of the treatment response (Ising et al., 2007).

Non-pharmacological challenge tests have also been explored and have shown that early childhood experiences have a marked and long-lasting impact on the stress responses in adulthood (Heim et al., 2000, 2004; Pruessner et al., 2004). These studies are interesting for several reasons: (1) They highlight that differences in the stress response among patients with depression also impact the treatment response, thereby providing a tool to select patients for a specific treatment trial. (2) They offer the opportunity to study non-depressed subjects with an exaggerated stress response in early clinical development as a way to profile the impact of a new compound on this clinically very relevant biological system. The importance of this possibility is underscored by the lack of beneficial effects of most psychopharmacological treatments in a super healthy population, typically recruited when first testing a NCE in humans. By enriching the study population to exhibit some biological changes relevant for the indication, information of a NCE is obtained earlier and is more relevant for further profiling of its target population. In light of the fact that recent data have demonstrated HPA overactivity in first-onset psychosis and in patients at risk for schizotypal personality disorders (Pariante et al., 2005; Walker et al., 2001), a new drug effective at attenuating exaggerated HPA activity could be considered beyond an indication for affective disorders.

While HPA axis tests can be part of small clinical trials, these tests are not suitable for large multi-center trials and are not likely to be used as decision points in clinical practice. However, a modified HPA activity should alter transcription patterns in circulating blood leukocytes. So, if blood biomarkers are explored in parallel during early clinical development, one could correlate easily accessible blood markers with neuroendocrine markers. This approach could bridge the gap between complex markers explored in small and exploratory trials and the need for simple markers that help select patients for large pivotal trials.

While stress tests have been used by some companies in early clinical development, their value to correctly predict clinical efficacy, at least for a segment of the patient population, remains to be shown.

15.2.3.2 Sleep

Analogous to neuroendocrine markers, sleep EEG recordings are not easily conducted in large multi-center clinical trials. But, as in the case for neuroendocrine markers, sleep EEG changes in psychiatric disorders have been studied for more than three decades and have yielded some promising insights into disease biology, in particular for depressive syndromes.

While subjective sleep disturbances are present in many patients with psychiatric (and other) disorders, only a much more restricted group of patients show consistent and objective changes of the sleep architecture. Common sleep disturbances reported by many patients include prolonged sleep latency, early morning awakenings with inability to return to sleep and intermittent awakenings. While none of these symptoms is specific for a psychiatric (or other) disorder, patients with both prolonged sleep latency and increased nocturnal awakenings show greater

symptoms of depression when compared to patients with only one of these features (Taylor et al., 2005). Also, the presence of early morning awakening, increased REM density, short REM latency and reduced non-REM sleep is indicative of severe and recurrent depression (Kupfer et al., 1982, 1986). However, a short REM latency has also been noticed in schizophrenia, schizoaffective illness (Kupfer, 1976) and in elderly subjects (Lauer et al., 1991).

Therefore, recent data on the microarchitecture of sleep may be more promising in terms of specificity. Spectral sleep EEG analysis is considered state-of-the art today and using this method more subtle sleep EEG changes can be detected, which can help profile patients and treatment responses. Recent spectral EEG data have shown increased beta power in non-REM sleep in major depression. This sleep EEG marker of arousal was correlated with altered brain glucose metabolism in cortical areas (Nofzinger et al., 2000). As beta power in non-REM sleep was inversely correlated with subjective sleep quality, a NCE that reduces this beta activity and hence arousal may lead to both subjective and objective sleep improvements. Moreover, hyperarousal, thought to involve increased activity of afferent noradrenergic neurons, is a feature of some forms of depression, PTSD, and schizophrenia, which would be rational target indications for a drug that attenuates beta activity in non-REM sleep.

In addition, lower temporal coherence of left to right EEG synchronization has been reported in depression (Armitage et al., 1999, 2006; Robert et al., 2006) and seems to be a trait-like feature. Furthermore, an altered pattern of delta activity during sleep, namely a reduction of delta activity in the first sleep cycle, has been associated with depression, chronic course and vulnerability for recurrence if not normalized at clinical remission (Armitage et al., 2000, 2001; Kupfer, 1995). As few drugs increase delta activity, particularly during its physiological peak in the first sleep cycle, and commonly used hypnotics decrease delta activity but increase higher frequency activity, NCEs that positively affect delta activity could be a welcome add-on to the current portfolio of drugs to improve sleep (Walsh et al., 2007).

In summary, clinically relevant sleep markers can be identified from the existing data. If further explored systematically and confirmed, and linked to molecular markers, these could yield novel relevant treatment targets. Though the practical application of sleep EEG is restricted to studies with small numbers of patients, the similar physiology of sleep regulation in rodents and humans suggests that changes in sleep microarchitecture could be used as ‘translatable’ biomarkers for profiling of novel treatment targets.

15.2.3.3 Structural and Functional Brain Imaging

Considerable progress has been made in identifying the brain regions and neural circuits that underlie normal and abnormal cognitive-processing, ‘emotion-processing’ and mood regulation. Resting cerebral blood flow, glucose metabolism and structural neuroimaging studies point to a widespread network of frontal, limbic, and subcortical areas underlying the cardinal feature of negative emotionality, flat affect

and cognitive deficits observed in affective disorders and schizophrenia (Gur et al., 2007; Phillips et al., 2003). Interestingly, these studies also indicate some pathophysiological convergence at the functional neuroanatomical level, and particularly at the level of the hippocampus, for primary thought disorders, such as schizophrenia, and primary emotional disorders such as major depression and even primary cognitive disorders (Gur et al., 2007; Kitayama et al., 2005; Nemeroff et al., 2006; Vermetten et al., 2003). As reduced hippocampal volume has also been considered a marker of increased vulnerability to develop depression or PTSD (Gilbertson et al., 2002; Macmaster et al., 2007), small hippocampal volume could be another marker that confers a risk to develop a mental illness. As early treatment to prevent long-term disability is increasingly recognized, further longitudinal research in large populations of young subjects at risk, identified through a combination of clinical, e.g. family history, and biological, e.g. small hippocampi, factors, is needed.

However, differences are also noted: Unlike depression, schizophrenia is more consistently associated with reduced whole brain and amygdala volumes. In contrast, affective disorders seem to be associated with no change or an increase in amygdala volumes (Frodl et al., 2004; McDonald et al., 2004; Zetzsche et al., 2006). Another approach is to elucidate functional brain circuitries contributing to psychiatric syndromes. A recent elegant example provided by Milak and colleagues (Milak et al., 2005) demonstrated that regional changes in brain glucose metabolism can be linked to specific symptom clusters in patients with major depression. It will be interesting to see if these correlations hold true across today's diagnostic boundaries.

Furthermore, longitudinal studies in depression also provided evidence of the normalization of amygdala activation in response to negative facial expressions after successful antidepressant treatment, suggesting the feasibility of functional MRI studies to identify predictors of treatment outcome (Davidson et al., 2003; Fu et al., 2004; Sheline et al., 2001).

So far, drawbacks with available data from neuroimaging technology include (1) its applicability to small groups of subjects (and hence the question about confirmation) and (2) the lack of standardized data acquisition, that makes comparison (and hence confirmation) between studies often impossible. On the other hand, standardization, systematic extension and confirmation of existing knowledge can be used to evaluate NCEs in exploratory clinical trials and to explore correlations between neuroimaging and clinical findings. This would aid not only in optimal dose selection, but also in the identification of the optimal target population(s). With regard to dose finding, current trends in the development of novel antidepressants rely heavily on the translation of brain target occupancy derived from pharmacodynamic and pharmacokinetic relationships from animal models into the doses required to achieve similar occupancy of the drug target in brains of normal volunteers using selective PET ligands in Phase 1 studies (Frank and Hargreaves, 2003). Results from these studies are used to guide the therapeutic dose selection generally in the absence of information about efficacy. The validity of these assumptions is challenged by the fact that drugs that have no effect on mood in normal people relieve depression in those who are ill. The combination of target occupancy with functional read-outs such as fMRI responses to emotional stimuli

in depressed patients could provide an improved and reliable assessment of the relation between occupancy and target engagement.

Whether a detailed correlation of behavioral symptoms and neuroanatomical activation patterns can also be used reliably in rodents, remains to be confirmed. However, some early data support the hope that localized brain glucose metabolism in rodents can be linked to behavior as well as localized neurotransmitter release (Marsteller et al., 2007).

15.2.3.4 Transcription Markers

In evaluating neurochemical or molecular changes that occur in animal models and in the human disease state, the most commonly considered biomarkers to examine are proteins, metabolites or RNA transcripts. Each of these approaches offers specific advantages and limitations. Analysis of RNA transcripts is often a first choice because of the broad range of tools available for transcription profiling. Proteomic and metabolomic approaches offer great promise for identification and characterization of functional biomarkers for psychiatric disease, as they are able to detect patterns of changes that may be more directly linked to a disease state than transcription analysis. At the present time however, limitations in the technology (and cost), sampling standards and knowledge base limit the utility of these approaches for large scale biomarker identification projects.

The ideal marker set would be one that is altered in a disease state (diagnostic) and would be normalized following successful drug treatment and concomitant remission of symptoms (surrogate marker). Biomarkers for other aspects of treatment of psychiatric disorders, such as drug metabolism and toxicology, have been used successfully and are important for developing drugs and understanding drug metabolism (Searfoss et al., 2005; Suzuki et al., 2006). However, they are beyond the scope of this discussion.

One commonly used method for identification of biomarkers relevant to psychiatric disorders is RNA transcription analysis. RNA transcripts can be analyzed individually with high anatomical resolution using in situ hybridization, quantified in relatively small numbers (but with very high sensitivity) using quantitative PCR (qPCR) or analyzed on a genome wide scale using DNA microarrays. It can be successfully argued that analysis of protein distribution or function is more functionally relevant to the disease state than to the analysis of RNA levels, as transcript levels are not always closely coupled to the expression levels of a protein. Thus, smaller, more rapidly turned over proteins and enzymes, such as CRF and tyrosine hydroxylase (Ganguly et al., 2002; Wong and Tank, 2007; Xu et al., 2005) tend to have better correlation between RNA and protein levels compared to G-protein coupled receptors (Duncan et al., 1993; Frohna et al., 1995), where there are many examples of protein regulation without a concomitant increase (or decrease) of the RNA encoding that protein. Also, transcription analysis does not provide information about the functional state of the protein, e.g. phosphorylation of receptors or of signaling cascade elements. However, RNA transcription patterns provide valuable insights about distribution and regulation of RNA (and to some measure the proteins that they

encode) in the tissue of interest. Tools are available for examining (essentially) every gene expressed, whereas the same is not true for proteins at this time.

In the past decade vast strides have been made in improving methodology for assessing transcripts, especially real time qPCR and DNA microarrays. Initially, there were questions regarding replication of data across different array platforms (Jarvinen et al., 2004). However, improvements in arrays and analysis techniques have minimized these issues. Recent studies measuring expression across platforms and comparison with quantitative techniques confirm that current arrays have acceptable reliability, although confirmation of data by another method such as qPCR is still considered to be essential (Wang et al., 2006; Bosotti et al., 2007; Canales et al., 2006). Further validation and a reference data set come from the MicroArray Quality Control Project data set (Shi et al., 2006), as well as improved methods of analysis (Stafford and Brun, 2007). In an effort to establish reporting standards the FDA (in August 2007) issued a draft guidance for industry with regard to handling, preparation and analysis of RNA and microarray analysis. Despite the improvements in microarray platforms and sample preparation, microarrays cannot be used as the sole method for transcription analysis. qPCR, which is more accurate and precise, is still essential for the identification and confirmation of biomarker candidates.

Another very recent and attractive possibility with regard to transcript analysis is to examine micro RNA transcription. Micro RNAs fine-tune posttranscriptional expression changes of a large number of genes in mammals and have been suggested to significantly influence phenotypic variations (Georges et al., 2007; Schratt et al., 2006). Though the work today is focused on cancer indications, this approach holds great promise for complex traits common in psychiatric disorders.

Brain vs Blood Leukocyte Transcription Markers

The primary source of tissue for transcription profiling in psychiatric disorders has been postmortem brain samples. These have proved to be, and will likely continue to be, invaluable in collecting information about psychiatric disorders; however they have serious limitations. Using postmortem tissue introduces a large number of technical variables including varying post mortem intervals, agonal time prior to death and the resultant change in pH and subsequent alteration in RNA quality (Atz et al., 2007). The greatest limitation however, is the inability to assess changes in patients relative to symptoms and to assess the effects of treatment. Transcription profiling of blood or blood fractions is more desirable as samples are accessible and can be collected at multiple time points from the same individual, e.g. at different stages in the course of the disease. One approach is to isolate peripheral blood mononuclear cells (PBMCs). Although it is a commonly used methodology, it has drawbacks for multi-center clinical trials in that it is difficult to isolate PBMCs at the point of collection and there is good evidence for post collection changes in transcription (Tanner et al., 2002). An alternate approach is to draw whole blood from donors directly into tubes containing a chaotropic agent (example: PaxGene™, Becton Dickenson), which eliminates post collection changes in transcription, preserves RNA during transportation and storage and allows isolation of high quality RNA. Though the transcription profile will be different in

PBMCs as compared to whole blood, either will provide valid data (Feezor et al., 2004). As there are no standardized protocols available for protein or peptide preservation, transcription analysis is still the best available method for assessing regulation of multiple factors in clinical trials for comparison with animals and cellular systems.

As exemplified below, it seems reasonable to pursue a translational approach by examining transcription patterns identified in blood leukocytes from distinct patient populations in relevant animal brain areas, both in disease states and after pharmacological interventions.

Hypothesis-Driven vs Hypothesis-Free Approaches

The development of high throughput expression analysis methods using microarrays (Brenner et al., 2000; Schena et al., 1995, 1996; Velculescu et al., 1995), allowing a hypothesis-free data analysis, has had a profound impact on assessing gene expression changes. Microarrays will yield considerable information if the experiments have been designed and analyzed properly, and have been used successfully to probe CNS function and to assess changes in psychiatric disorders (Luo and Geschwind, 2001; Marcotte et al., 2003; Mirnics and Pevsner, 2004; Mirnics et al., 2006). A major benefit of using arrays is that patterns of change, or pathways affected, can be identified without prior knowledge or hypotheses. Careful experimental design considerations are key to any successful experiment, but are particularly important in array experiments. There is a tremendous differential expression of both RNA and proteins across regions of the brain, and altered regulation may be occurring in a small population of cells only. This can make detection of subtle but relevant changes difficult. Although arrays have been successfully used to profile changes in the brain in both animal models and in psychiatric disorders, it should be noted that a number of findings in array studies have not been replicated. The most extensively studied psychiatric disorder using microarrays and post-mortem CNS tissue is probably schizophrenia (Konradi, 2005; Mirnics et al., 2006). Changes were identified in genes related to presynaptic markers, mitochondria/energy homeostasis, myelination related genes and the GABA-Glutamate system. These have led to an increased understanding of the disorder, but have not yielded a usable biomarker or novel therapy up to this point.

Major depression, another heterogeneous psychiatric disorder, has been profiled using microarrays with mixed results. Evans et al. (Evans et al., 2004)], using arrays to profile BA46, have identified the FGF system as a factor in major depression. Sibille (Sibille et al., 2004) examined transcription in BA9 and BA47 and did not find changes in the FGF system, pointing either to regional differences in the cortex or lack of a strong association between unspecified major depression and the FGF system. Other studies (Altar et al., 2004; Newton et al., 2003; Shi et al., 2006; Sun et al., 2005) have identified that NPY and TRH RNA are increased in the CNS after electroconvulsive therapy. This is consistent with data that show that NPY administration has an antidepressant effect in the forced swim test (Husum et al., 2000). To date, there have been no studies on postmortem brain to examine generalized anxiety in humans.

An alternate method is to use a hypothesis driven approach and focus on a subset of genes that are likely to be involved in pathways related to the disease. Much of the data currently in the literature on psychiatric disorders has come from experiments looking at specific pathways or a few individual genes and not from genome-wide array studies. In fact, many of the gene classes that were found to be involved in schizophrenia using microarrays were predicted in earlier studies using technology other than large scale transcription profiling: e.g. changes in genes with a presynaptic function such as synapsin (Browning et al., 1993), synaptophysin (Glantz and Lewis, 1997), synaptic proteins (Karson et al., 1999), and decreased neuropil (Selemon and Goldman-Rakic, 1999). There are two major methodologies that can be implemented for a focused approach: Focused arrays and qPCR for multiple relevant genes have been used in various studies including expression profiling of schizophrenia (Lehrmann et al., 2003) as well as for identifying stress related genes relevant to depression (Ohmori et al., 2005; Rokutan et al., 2005b).

The throughput and data quality of qPCR methodologies have improved dramatically in recent years. Also, at present transcription data from microarrays are supposed to be confirmed using qPCR, as the latter offers greater sensitivity, dynamic range, accuracy and precision as compared to arrays. These can be critical factors as the expected changes in transcription levels are small. Using this approach, our early data show that it is possible to segment normal control populations as well as to identify distinct changes in different untreated patient segments. Our data also show that care must be exercised when evaluating changes in transcription levels, as even significant changes between two groups may not be a clinically relevant biomarker if variance of the readout is sufficiently high in a normal population.

Biomarkers that can be identified and measured in peripheral tissues such as blood have greater utility than those identified in CNS regions, as clinical samples are readily available. The underlying assumption is that changes seen in the brain will be reflected in the periphery and lymphocytes have been proposed as suitable neural probes (Gladkevich et al., 2004). Although not universally true for all genes, there is a good correlation between expression of many genes in the CNS and blood, especially genes related to cell signaling and metabolism (Ray et al., 2007; Sullivan et al., 2006). In previous studies of blood samples from schizophrenia patients a set of eight genes was identified that segregated populations of schizophrenic and bipolar patients from controls with high specificity (Tsuang et al., 2005). However, these studies must be interpreted with caution as other studies have identified different genes associated with schizophrenia (Middleton et al., 2002; Vawter et al., 2004a, b). There have also been several studies that examined expression patterns in peripheral blood in PTSD patients (Segman et al., 2005; Zieker et al., 2007) and patients with major depression (Kalman et al., 2005; Ohmori et al., 2005; Rokutan et al., 2005a). These studies have identified gene sets that are involved in the disease, and with additional confirmation, many may become clinically useful biomarkers. Taken as a whole these studies provide solid evidence that analysis of peripheral blood can be useful for the assessment of psychiatric disorders.

Regardless of which technology is used to identify a transcription (or, for that matter, a protein or metabolite) biomarker set, it is important to note that it is

unlikely to find an association between complex psychiatric disorders and a single marker. A more likely scenario is that there will be changes in multiple genes that must be examined as a pattern to achieve acceptable sensitivity and specificity.

Real World Data Using Focused Transcription Analysis from Blood Leukocytes

Both microarrays and qPCR have been widely used to measure gene expression changes in a variety of experimental systems and each method has its own benefits and challenges (Brazeau, 2004; Morey et al., 2006). However, given the larger dynamic range and sensitivity of qPCR (Mir, 2006), in addition to the speed and lower cost per sample, we have selected it as the platform of choice for our first studies. Blood samples are collected in the clinic with PaxGene™ RNA tubes.

While using qPCR to measure gene expression is more sensitive than using microarrays, one significant limitation is the restriction in the number of genes that can be assayed. We have addressed this limitation by using reports from the literature to enrich our list of target genes with those that have a high probability of being linked to the biochemical pathways that are affected in affective disorders.

Data Normalization

In order to effectively compare gene expression patterns between different samples, it is necessary to control for variables that could mask the underlying biological changes related to the psychiatric syndrome. The best way to minimize the influence of these variables is through the use of normalization genes (Andersen et al., 2004; Huggett et al., 2005; Jin et al., 2004; Vandesompele et al., 2002). Because it is unlikely that a "universal" normalization gene exists, we have identified several genes which, collectively, are stably expressed in blood samples derived from multiple groups of control subjects and different depressed populations.

Data Analysis and Statistics

Currently there is no consensus on what denotes a significant change in expression, since measurements are being made in different tissues using different techniques. Furthermore, while some expression changes might prove to be large between healthy and depressed individuals, others are likely to be more subtle, but not less important. For these reasons, especially in the early stages of the project, we believe that it is necessary to analyze the expression data using multiple approaches in order to have the best chance of uncovering patterns that are associated with a specific disease state, and/or correlate with response to treatment. Classical univariate methods such as *t* tests and ANOVA (or their non-parametric equivalents) are useful for comparing expressions between different groups, one gene at a time, or between one gene and one clinical variable within a group. When interpreting such analyses, it is prudent not to be too stringent with respect to p value cut-offs for fear of missing an important contributing gene during the exploratory phase. Confirmatory studies have to employ prospectively defined hypotheses and more rigorous analysis methods. We are also

actively investigating the use of multivariate approaches, such as MANOVA, stepwise logistic regression (SLR) and Pelora (penalized logistic regression) (Dettling and Buhlmann, 2004) to facilitate the identification of combinations of gene expression values that allow the discrimination between control and patient populations.

The combination of gene expression data and clinical information provides the opportunity to identify biomarkers that differentiate between the healthy and disease state and/or between different segments of diseased as well as healthy individuals. As described below for the analysis of a large set of control subjects, the latter has made it possible to examine intermediate phenotypes, which is an important step towards an improved understanding of the biology of psychiatric disorders. Whenever patients are being treated with medication, the opportunity exists to identify biomarkers that relate to treatment response and safety.

Analysis of Control Subjects

Many clinical trials, by design, do not contain a control group. Because our intention is to compare the gene expression patterns in healthy controls to those seen in depressed patients, it was important to generate gene expression data on a large number of control subjects. In addition, we speculated that the control group itself may be a valuable subject pool to investigate for intermediate phenotypes, since there is likely to be a continuum between completely healthy individuals and those with a clinically manifest psychiatric disorder. This approach is in line with suggestions raised in the context of DSM-V, emphasizing a dimensional rather than a categorical approach. Table 15.1 shows some combinations of gene expression and clinical parameters that produce the most significant p values ($p < 0.001$) in *t* tests or ANOVAs. Note that two genes, "G" and "Q", produce significant correlations with multiple parameters, making them strong candidates for genes whose expression may be linked to a clinically relevant feature of depression.

Analysis of Patients with Disease State 3

Comparison of gene expression patterns between matched control subjects and 21 patients with Disease state 3 demonstrates that 13 genes out of the 25 tested are differentially expressed between the two groups ($p < 0.01$)[Table 15.2]. Similar results are obtained if a larger number of unmatched controls are used for comparison. The expression data have also been analyzed using two multivariate approaches, stepwise logistic regression and Pelora. These techniques have begun to provide insight into combinations of gene expression values that allow the two groups to be differentiated.

Summary of Analysis of Multiple Patient Populations

Depression, as a complex disorder, will in all likelihood be associated with multiple, potentially subtle, gene expression alterations that may differ between subtypes of the disease. The heatmap shown in Table 15.2 summarizes the gene expression differences between controls and patients, derived from several patient populations involving different disease subtypes. The results depicted in this figure can be viewed in two

Table 15.1 Association of clinical parameters and gene expression in blood samples from healthy control subjects

	Gene X	Gene F	Gene G	Gene I	Gene K	Gene L	Gene Q
Clinical Variable							
Variable 1			■				■
Variable 2		■	■		■		■
Variable 3			■				■
Variable 4	▒						
Variable 5			■				■
Symptom							
Symptom 1			■				
Symptom 2			■	■			
Symptom 3			■			■	■

■ Increased ▒ Decreased

The expression of 29 genes in 299 control subjects was measured in blood using qPCR. ANOVAs or *t* tests, as appropriate, were used to correlate the expression values with responses to 29 items from the self-assessed questionnaires provided by the subjects. In some cases responses to multiple items were grouped to create additional composite scores for testing. For example, the scores for a combination of items related to symptoms of depression were summed to create a new category (Symptom 3). All of the combinations marked with a filled box in the table yielded a p value of $p < 0.001$ in the statistical test.

The heatmap shows gene expression changes between controls and depressed patients measured by quantitative PCR. The expression for each gene is compared between controls and patients using a *t* test. The direction and magnitude of gene expression change is shown by the colored bar and statistical significance is indicated by the number of stars (2 stars $p < 0.01$, 3 stars $p < 0.001$). Disease state one includes 172 patients and 59 control subjects. Disease state two contains 24 patients and 24 matched control subjects. Disease state three contains 21 patients and 21 matched controls. No correction was made for multiple testing.

different ways to extract different information. If viewed by rows across the different disease states, one can identify genes that display similar directional changes across multiple patient populations. For example, relative to the controls, the expression of genes “A”, “G”, “K”, and “P” are increased while genes “C”, “H”, and “J” tend to be decreased. These genes potentially represent common biomarkers for all depressed patients, regardless of disease subtype. Viewing the table by columns reveals that patients in each of the disease states display very different patterns of gene expression changes. For example, patients in disease state one have approximately twice as many genes that are increased in expression as are decreased. Disease states two and three almost exclusively display patterns of increased expression. However, even these two disease states exhibit different patterns and can be distinguished from one another based on several genes. It is our intention to utilize differences such as these to segment the patient populations in our studies.

Table 15.2 Summary of gene expression changes in different patient groups relative to control subjects (*See Color Plates*)

Comparison to Previous Reports

Publications have begun to appear in the literature in recent years reporting changes in peripheral gene expression in patients suffering from affective disorders (Iga et al., 2005, 2006, 2007a; Lima et al., 2005; Ohmori et al., 2005; Rocc et al., 2002; Segman et al., 2005; Tsao et al., 2006; Vogel et al., 2004; Zieker et al., 2007). However, meaningful comparisons between any of these studies and our work are very difficult for a variety of reasons. Each lab has used a different combination of experimental platforms (microarrays vs qPCR), normalization genes, source of tissue (PBMC vs whole blood), and ethnic background of the study group. This highlights the importance of standardizing the technological and methodological aspects as much as possible to lay the foundation for a systematic approach in the future.

In addition, because the previous studies have relied on a small sample size, usually 20 patients or less, all conclusions await confirmation in larger populations. Indeed, this last point also applies to some of our data and is actively being corrected

by the analysis of more patients. But similar profiles from different world regions point to the value of this approach. Ultimately, more experiments will clarify which observations reflect the real world.

15.2.3.5 Other Plasma Markers

Methods for characterizing changes in large numbers of proteins have improved in recent years. The advent of proteomics, including protein arrays, improved mass spectrometry technology (SELDI™, Ciphergen Inc) and better analysis of 2D gel electrophoresis have greatly augmented the quality and speed of protein analysis. It is however, far more difficult to assess large numbers of samples for changes in multiple proteins than it is to assess RNA transcripts. The need for standardization in proteomics has been recognized, as evidenced by initiatives such as the one of the Human Proteome Organization (HUPO). HUPO is seeking to characterize the human CNS proteome (HUPO brain proteome project) and to establish collection and analysis standards (HUPO Proteome Standards Initiative). Despite great strides in the field, there are still more tools for examining RNA transcription than currently exist for proteins. One other limitation of proteomic analysis is that samples collected in multi-center studies cannot be immediately processed, making RNA collection still the best available method for assessing regulation of multiple factors in blood samples in multi-center clinical trials.

On the other hand, if a focused approach is pursued, protein markers may provide a valuable translational bridge between human peripheral blood and animal CNS data. Thus, beta arrestin-1 protein levels have been shown to be reduced in leukocytes of depressed patients and this decrease is proportional to the severity of the symptoms (Avissar et al., 2004). In the same study it was shown that antidepressants raise beta arrestin-1 levels in relevant areas of the rodent brain, suggesting that markers identified in human blood could be the basis for studying therapeutic approaches in animal models. The relevance of plasma protein markers for CNS disorders has been recently illustrated in an elegant study using 18 signaling proteins that not only classified patients with Alzheimer's dementia with 90% accuracy, but also predicted progression to Alzheimer's disease over 2–6 years in patients with mild cognitive impairment.

In a similar vein, Kromer et al., (Kromer et al., 2005a) have identified glyoxalase as a marker of anxiety in mice bred for high or low anxiety. Animals with low anxiety compared to high anxiety animals had higher levels of glyoxalase 1 protein in the hypothalamus and amygdala. These changes were also seen in blood from these animals and the amount of glyoxalase 1 was associated with the degree of anxiety in the animals. The regulation of glyoxalase 1 as an anxiety marker has not been confirmed in human studies, although there is evidence for the linkage of the glyoxalase locus with unipolar depression (Tanna et al., 1989).

Also, biochemical markers derived from endogenous metabolic pathways that can be measured in plasma samples have the potential to be used in large patient groups. In this regard the application of a metabolomics approach to study geriatric depression illustrates the power of this technology, and shows great promise to aid in the elucidation of the biochemical pathways perturbed in disease and treatment (Paige et al., 2006). Besides its potential to yield diagnostic and efficacy markers,

this approach also holds promise related to safety, as shown by recently published metabolomics data related to lipid metabolism changes in schizophrenic patients treated with different atypical antipsychotics (Kaddurah-Daouk et al., 2007).

In addition, the assessment of peripheral monoamines and their metabolites met with little success in the past. However, metabolomics, by virtue of its ability to measure simultaneously multiple metabolites in biological fluids, shows great promise for the development of biomarkers, related to monoamines and beyond, for many psychiatric disorders. One example is inflammation-induced changes in serotonin metabolism, such as a reduction in plasma tryptophan and an increase in plasma kynurenine, which have been associated with incidence of depressive symptoms during interferon-α treatment (Bonaccorso et al., 2002; Capuron et al., 2003a). A more detailed exploration of plasma metabolites related to monoamine pathways in these patients could be a fruitful approach to identify robust biomarkers, at least for cytokine-induced depression (see also the following section).

15.2.4 Perspectives for Biomarkers in Drug Development

The widely accepted official NIH definition of a biomarker is 'a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' (Biomarkers Definitions Working Group, 2001). Based on this definition, all of the above mentioned technologies have the potential to yield a biomarker, but with differences in their applicability to clinical practice.

Obviously, a biomarker that can be measured in a single blood sample and which yields good specificity and sensitivity is more practical than a marker that requires MRI or PET scans. Blood transcription markers also offer the advantage of being able to explore multiple pathways and hence use patterns rather than single markers, which, as our early data suggest, seem capable of differentiating segments of patients with a high predictive value.

On the other hand, a conclusion on the pathophysiological relevance of such transcriptional markers would be greatly advanced by showing a correlation with functional alterations in brain circuitries. This, in turn, would also provide important guidance for animal studies and would facilitate translation between transcriptional changes in human blood leukocytes and animal brain areas. Finally, transcriptional changes (or, for that matter, protein, peptide or metabolite markers), that signal a relevant pathophysiological change and/or effect of treatment can also be explored in cellular assays. As the next sections will illustrate, such reductionist models could help profile novel drug targets early on, particularly if they are designed to address a specific (molecular) aspect of a disorder and if they use molecular read-outs, such as transcriptional changes, that are derived from human (patient) data.

Although it remains to be shown that such an approach is valid, the lack of substantial progress in treating psychiatric disorders for several decades also emphasizes the need to find alternative approaches. It has been recognized that the main reasons for the delay in the introduction of new antidepressants are the notoriously small effect sizes and large

placebo effects (Walsh et al., 2002). An alternative approach is needed that closes the gap between the patient symptoms and the pharmacodynamic profile of the drugs that are being developed. To achieve this goal the initial studies should aim to characterize the efficacy profile of the drug, by taking advantage of trait-markers for patient enrollment as well as the broad assessment of biomarkers that inform on the engagement of pathways relevant to the disease biology, and in the process enable the identification of relevant state-markers which predict treatment response and could be used in successive trials for enrichment of the trial with patients that respond to the treatment. In addition the assessment of biomarkers in Phase 3, the confirmatory phase of clinical development, is an important step towards the validation and qualification. Besides the necessary specificity and sensitivity of a biomarker an important feature is the easy access to source in order to enable their application to large clinical studies. The development of biochemical biomarkers that can be measured in blood samples have the potential to be used in large patient groups. This, in turn, is a prerequisite as the utility of biomarkers is directly related to their validation and qualification for decision-making. The qualification process involves a graded evidentiary process that links a biomarker with biological and clinical end points. The extensive efforts that such a process requires can only be achieved with a consortium approach that distributes the cost and risk and effectively drives the research and ultimately the regulatory acceptance of biomarkers (Wagner et al., 2007).

In summary, existing data and technologies allow for the identification of biological markers that can be used in exploratory clinical trials with NCEs. Although none of the markers can be considered validated today, they can be used for internal decision making, on dose, optimal target population(s) and target symptom(s). Importantly, the selection of biomarkers should be based on the preclinical data and the mechanism of action of a NCE, but should also take into account the discussions that have been initiated by several public-private partnerships as well as health authorities. In particular, consortia in both Europe and the USA have been set up, some of which include participation from health authorities, with the aim to establish standards and prioritize biomarkers, specifically for psychiatric disorders.

15.2.4.1 Examples Where Biomarkers Could Be Used Today

A recent example is major depression during cytokine therapy for cancer or hepatitis C (Capuron and Dantzer, 2003; Capuron and Miller, 2004; Wichers et al., 2005). It has been proposed that exaggerated production of inflammatory cytokines further stimulates the hypothalamic–pituitary–adrenal (HPA) axis and thereby contributes to the development of major depression (Leonard, 2005). The high incidence of depression under interferon-α treatment allows to prospectively collect samples and identify vulnerability markers (in subjects who do develop depression) and markers of resilience (in subjects who don't develop depression) (Capuron et al., 2003b). Such paradigms could aid in the understanding of the neurobiology of at least some forms of major depression. The clinical relevance of this approach is underscored by the high incidence of depression in disorders with known CNS inflammation, such as Multiple Sclerosis and Alzheimer's dementia (Ghaffar and Feinstein, 2007; Leonard, 2007; Lyketsos and Olin, 2002).

Another example is melancholic major depression. This old (fashioned) concept has recently received more attention, as it is a disorder with definable clinical signs and can be verified by laboratory tests and treatment responses (Taylor and Fink, 2007). The cardinal clinical manifestations of major depression with melancholic features include sustained and pervasive ahedonia and vegetative features, particularly early morning awakening, loss of appetite, weight loss and loss of libido. Also, there is evidence of physiological hyperarousal, which is thought to arise from the sustained stress system activation evidenced by hypercortisolism and sympathetic nervous system activation, and accompanied by suppression of the growth hormone and reproductive axes, insomnia, and loss of appetite. Another feature of melancholia is a diurnal variation in the severity of depressed mood, with greater severity in the morning than in the evening (Gold and Chrousos, 2002). Although only 25–30% of patients with major depression present with pure melancholic features, this subtype of major depression has been repeatedly associated with several laboratory findings, including dexamethasone non-suppression, high nocturnal cortisol, short REM latency, high REM density and reduced SWS and delta activity (Taylor and Fink, 2007). The biological features of melancholia should facilitate the identification of biomarkers related to the underlying pathophysiology. Furthermore, modeling of these abnormalities in animals provides ample opportunities for translational approaches to assess pathophysiological and etiological mechanisms as well as potential novel therapeutic targets.

15.3 Animal Models

There are a multitude of animal models that have been employed to study particular aspects of major depression. A comprehensive overview of these models has been covered elsewhere and is beyond the scope of this text. However, a selection of these models will be highlighted here in relation to their practical use for identifying novel compounds with therapeutic antidepressant efficacy. It is important to keep in mind that the ultimate goal of any animal model is to achieve construct validity by sharing homologous etiology and pathophysiology to the disease state in humans. Unlike neurological diseases in which the disease biology is well-established, the biological underpinnings of major psychiatric disorders are varied and still relatively poorly understood, thus making the development of such models, e. g. for major depression, rather rare.

15.3.1 Animal Models of Depression

Most of the animal models for major depression in use today are designed to simulate some aspect of the disorder itself by exposing the animal to a single or series of stressful conditions. While this approach has proven useful, caution must be used in interpreting results from these studies since the biological substrate(s) involved

in producing the response may not reflect the human condition. Still other models base their utility on predictive validity, or the ability of the model to distinguish compounds that are clinically useful, while often times having no direct relation to depression per se.

The choice of an animal model for any drug discovery research program presents a number of unique technical and theoretical challenges. Most notably, routine use of a model requires that it be reliable, well-suited to high-throughput testing and offer predictive validity. To this end, the most widely used assays employ modifications of a rodent model based on 'learned helplessness' (LH). In its original form, the LH paradigm described the inability of animals to escape a stressor when given the option following a session in which the same stressor was presented in an inescapable fashion (Seligman et al., 1980). Subsequent iterations of the LH model involve stress-induced coping strategies expressed as the eventual acquisition of an immobile posture brought about by acute inescapable stress. These models are among the most widely used in animal research for detecting antidepressant-like activity and they include the forced swim test (FST) and the tail suspension test (TST).

15.3.1.1 Forced Swim Test

The FST is the most widely used animal model in depression research (Cryan and Holmes, 2005). The utility of the FST has been outlined extensively (Borsini, 1995; Cryan and Holmes, 2005b; Nestler et al., 2002; Petit-Demouliere et al., 2005). In short, the FST involves placing a rodent in an inescapable cylindrical water bath where it swims around the container searching for a means of escape. Eventually, the animal assimilates an immobile posture with intermittent bouts of specific activities (swimming and struggling/climbing). The test measure focuses on the time spent immobile, which is thought to reflect a change in coping strategy akin to a state of behavioral despair (Cryan and Mombereau, 2004; Porsolt et al., 1977), an alteration from active to passive [(Holmes, 2003), a learned adaptation (De Pablo et al., 1989; West, 1990) and/or a means to conserve energy (Holmes, 2003; Petit-Demouliere et al., 2005).

The FST represents an obvious advantage in discovery research due to its ability to detect acute and chronic antidepressant-like activity over a large range of clinically relevant antidepressant drug treatments including tricyclics, monoamine oxidase inhibitors and atypical antidepressants, as well as non-drug based paradigms such as electroconvulsive shock and exercise. (Cryan et al., 2005b). More recently, modifications to the FST have enabled the reliable detection of selective serotonin reuptake inhibitors and monitoring specific behaviors associated with serotonergic and/or noradrenergic activities (Detke et al., 1995; Lucki, 1997). Thus, the FST offers good predictive validity and is used routinely as a 'frontline' assay for screening compounds for antidepressant-like activity. The ability to use this test in both mouse and rat rodent species has also added to the utility of this assay.

15.3.1.2 Tail Suspension Test

The tail suspension test (TST) is based on the same theoretical coping strategy as the FST that is thought to come about in the advent of an animal being presented with an inescapable stressor. The utility of the TST has been described elsewhere, and detailed comparisons between the TST and the FST have been discussed (Cryan et al., 2005a). In this test, a mouse is hung upside down by the tail, where it initially engages in escape-related behavior and eventually adopts an immobile posture. Like the FST, the TST is sensitive to a number of clinically used antidepressant compounds, although specific differences have been noted between TST and FST with respect to sensitivity and efficacy among classes of antidepressant agents (Cryan and Mombereau, 2004; Cryan et al., 2005a; Porsolt et al., 1987). The TST is almost always carried out in mice, but other rodent species have been used, and it is generally considered to offer good predictive validity for antidepressant-like activity.

15.3.1.3 Limitations of Current Animal Models

As two of the most widely used animal model tests in use today to detect antidepressant-like activity, the FST and the TST share some common limitations. Notably, these tests have been developed largely based on monoamine-based therapeutics. While monoamine-based drugs have been successful in the clinic, it is becoming increasingly apparent that novel targets must be identified to improve efficacy over currently used therapies. It seems unlikely that future iterations of monoaminergic drugs will sufficiently lack unwanted side-effects in addition to providing superior efficacy to those compounds already in use. To this end, experimental paradigms must be employed that detect novel mechanisms of action.

An important limitation of the FST and TST models stems from the lack of understanding of the immobility behavior itself. For instance, one must consider whether the immobility behavior has direct relevance to the human symptoms of depression, and whether the actions of antidepressant drugs in these models is reflective of their therapeutic actions per se. Perhaps the major criticism of the FST and TST arises from the acute actions of antidepressant compounds in these models. It is well known that clinically effective antidepressant drugs require approximately 3–4 weeks of administration to achieve their therapeutic effect. From this standpoint, it seems unlikely that acute effects of these compounds in the FST or TST are reflective of potential ‘therapeutic’ efficacy. These questions highlight the concern that non-monoaminergic targets will be adequately identified using these models.

Another limitation of FST and TST is that the measured behavioral endpoint for these models is confounded by potential locomotor effects of the test compound. Thus, false positives may be identified based on a compound’s ability to nonspecifically increase spontaneous locomotor activity. While this has not generally been an issue for monoamine-based drugs, this possibility raises a valid issue for using these models to assess potential novel targets for potential antidepressant-like activity. Ultimately, the value for models like the FST and TST in demonstrating

antidepressant-like effects of compounds with novel mechanisms awaits the clinical validation of the target in question. In the meantime, one approach for preclinical research on depression is to begin to address the need to improve efficacy read-outs by employing mechanistic and disease-state models of the disorder.

15.3.1.4 Future Directions for Depression Research

Appropriate diagnoses and corresponding successful treatment of specific depression segments has been hampered by our lack of clear understanding of the biological underpinnings that are responsible for the various symptoms of depressive disorders. However, research in depression has recently emphasized the advantage of focusing on these individual segments of depression to parse out specific phenotypes, rather than on depression as a whole (Cryan and Slattery, 2007). It is well known that the experience of significant life stressors is a clear risk factor for depression, and anxiety is often co-morbid with depression. In this context, it is proposed that efforts to utilize disease state and mechanistic models that specifically focus on stress and stress/HPA neurocircuitry (as it relates to depression) may serve as a valuable way forward in the drug development process. By taking this focused approach on the stress axis, it is hoped that depression-related neuronal pathways will be characterized and that molecular aspects of this circuitry can be more readily identified through translational methods.

A convenient framework with which to conceptualize this notion for the following discussion is to define the stress axis according to a modified version of the Gould and Chrousos model (Gold and Chrousos, 2002). In this framework, the stress axis consists of the complex and integrated neuronal interplay between the CRF-containing neurons of the hypothalamus (HPA axis), the amygdala and the noradrenergic containing locus coeruleus (LC). Emphasis for potential mechanistic studies will be placed on terminal fields of the LC such as the medial prefrontal cortex (mPfc) and the ventral hippocampus. The specific segment of depression that will be addressed by such mechanistic models is melancholia, which is characterized by a hyperarousal, associated with hyperactivity of the HPA axis and the LC.

Disease State Models

A number of tests have employed chronic stress paradigms to model certain aspects of depression and, more recently, schizophrenia. One of the depression models, chronic mild stress (CMS), is induced by exposing animals to intermittent, unpredictable and uncontrollable stressors over a period of several weeks to achieve the desired behavioral endpoint (Willner, 1997). Animals exposed to CMS exhibit a reduction in the consumption of a normally palatable sweet solution, and this is considered to model the ahedonia that is a hallmark feature of major depressive disorders. Additional changes are noted in a number of physiological and neuroendocrine parameters that are thought to model depressive symptoms in humans. Moreover,

exposure to CMS increases immobility in the FST (Molina et al., 1994). An attractive feature of the CMS model is the fact that the effects of repeated stress are reversed by antidepressant agents over a time-course that mimics their time-course to clinical efficacy. However, a major hindrance to the widespread use of the CMS model is the fact that between-laboratory reproducibility is poor, possibly related to differences in frequency, duration and stressors employed across experimental protocols.

A different approach to exploiting the stress axis as it relates to depression is through the use of genetic models via selective breeding. For instance, high anxiety bred (HAB) rats and mice exhibit enhanced immobility in FST, HPA hyperactivity and increased anxiety compared to their low anxiety bred (LAB) counterparts (Kromer et al., 2005b; Landgraf et al., 1999; Liebsch et al., 1998a, b). The observation of elevated levels of glyoxalase- 1 in a number of brain regions has been identified as a potential marker in LAB mice, suggesting that low expression of this protein may identify trait anxiety in HAB animals. HAB rats display HPA dysregulation (Keck et al., 2002, 2003) that is accompanied by an over expression of hypothalamic vasopressin (Landgraf and Wigger, 2002) and LC hyperactivity (Salchner et al., 2006). Interestingly, the HPA dysregulation noted in HAB rats is normalized by a V1 receptor antagonist, suggesting that the HAB model of anxiety and depressive-like behavior may lend itself to identifying activity of novel therapeutic targets.

Disease state models offer the unique advantage of modeling specific (endo-) phenotypes of depression. In addition to detecting the activity of potential novel therapeutics, it is desirable to gain knowledge about the molecular underpinnings of the response being measured. To this end, it is of particular interest to investigate the responders and non-responders to treatment in these paradigms and to translate those findings to the human condition as they may relate to the clinical observations that note differences between treatment responsive and non-responsive patients. Such an understanding would aid in identifying candidate genes, at the genetic and expression level, that may confer a response to treatment.

Mechanistic Models of Stress Axis Activity

Mechanistic models are valuable in discovery research as they allow for the desired aspects of a particular model to be relatively isolated for in-depth study. While portions of the neurochemical approach outlined below are rather broad in nature, the scope of mechanistic models is mostly limited to the LC hyperactivity component and the subsequent effect on cortical function. As is the case for disease state models, the goal for these mechanism-based assays is to allow for the detection of novel compound activity while simultaneously facilitating an understanding of the molecular mechanisms involved in the behavioral endpoint of the model. An understanding of the molecular mechanisms involved in the behavioral and neurochemical sequelae associated with isolated preparations may begin to bridge the gap between preclinical and clinical outcomes and will help establish cellular models to begin to address the causal molecular factors contributing to psychiatric disorders.

Adult Neurogenesis

Adult neurogenesis involves the turnover and maturation of progenitor cells into new neurons in the dendate gyrus of the hippocampus (Gross, 2000). Neurogenesis has been demonstrated to occur in rodent and human dentate gyrus (Eriksson et al., 1998), and much attention has been given to the relevance of the interplay between stress and neurogenesis in rodent models (Malberg and Duman, 2003; McEwen, 2001). Activation of the HPA axis decreases neurogenesis in rodents and clinically effective antidepressant therapies have been shown to reverse these deleterious effects on cellular proliferation (Malberg and Duman, 2003; Van Praag, 1999). From the drug discovery perspective, the potential utility of the neurogenesis approach is buttressed by its apparent ability to detect novel compounds of potential therapeutic relevance (David et al., 2007). However, it should be cautioned that neurogenesis does not seem a requisite for behavioral effects of all antidepressant-like compounds (David et al., 2007).

At the molecular level, chronic HPA activation has been associated with alterations in genes known to regulate cell proliferation, and these alterations can be prevented with tricyclic antidepressant treatment (Alfonso et al., 2004). These findings are consistent with antidepressant-mediated reversal of chronic stress effects on features of neurogenesis and are of particular importance for supporting a translational research approach in attempts to link alterations in gene expression to disease states.

Neurochemistry

The notion that monoaminergic neurotransmission is implicated in the regulation of mood is a decades-old concept. Moreover, the fact that monoaminergic transporter blockers represent the most widely prescribed antidepressant treatment underscores the importance of enhanced monoamine transmission in the treatment of depression. The fact that antidepressant drugs enhance monoamine levels acutely while their clinical efficacy takes weeks to develop precludes the temptation to overestimate the value of data from acute neurochemical studies. Nonetheless, the fact that protracted monoamine enhancement is thought to mediate the plasticity necessary to achieve therapeutic effects suggests that acute neurochemical data can be useful in estimating a drug's antidepressant potential following long-term treatment. Specifically, the assessment of monoamine release in corticolimbic structures with *in vivo* microdialysis following acute administration offers a valid approach to compound assessment (Millan, 2004; Millan et al., 2000). While the utility of this approach for monoamine-based therapies is obvious, it can also aid in evaluating a number of novel targets since it has been demonstrated that several of these can affect monoamine transmission either directly or indirectly. For instance, NMDA receptor blockers (Millan et al., 2000), metabotropic glutamate 2/3 receptor ($mGlu_{2/3}$) agonists and antagonists (Cartmell and Schoepp, 2000), CRF_1 receptor antagonists and melatonin receptor agonists (Millan, 2003) all seem to increase corticolimbic monoamine release in some capacity. A number of studies also support a role for additional neurotransmitter

(Swanson et al., 2005b) and peptidergic systems in mood disorders (David et al., 2007; Swanson et al., 2005a). The current need for novel therapeutics underscores the potential utility of an integrated drug discovery approach that includes *in vivo* microdialysis assessment of glutamatergic, GABAergic and peptidergic function in colimbic areas, in addition to the traditional survey of monoamine transmitter systems. Furthermore, evaluation of compound effects on corticolimbic acetylcholine transmission is also of great interest given the supposed pro-cognitive benefits of enhancing this neurotransmitter system in the cortex.

In keeping within the framework of the stress axis defined earlier, the *in vivo* microdialysis approach can be useful for assessing mechanistic read-outs of novel target function in the LC-mPfc pathway. mPfc, which receives noradrenergic input from LC (Ungerstedt, 1971), is an important brain structure implicated in mood disorders (Glavin, 1985a, b). A consequence of aberrant LC hyperactivity and the subsequent noradrenergic elevation that results in melancholia is the deleterious inhibition of mPfc function (Gold and Chrousos, 2002), and it has been well demonstrated that various acute stressors increase norepinephrine turnover/release in this structure (Glavin, 1985a). It is proposed that the mechanistic effects of novel compounds may be assayed by exploiting the effects of acute stress on increased mPfc norepinephrine. For example, $mGlu_{2/3}$ receptor agonists have recently been identified as useful agents of therapeutic potential in anxiety disorders (Swanson et al., 2005b). In this light, it has been demonstrated that $mGlu_{2/3}$ receptor agonists can reduce immobilization- (Swanson et al., 2004) and elevated platform stress-induced enhancement (Lorrain et al., 2005) of mPfc norephinephrine in rats. $mGlu_{2/3}$ agonist-mediated reversal of the NE increase in response to elevated platform stress has also been demonstrated in a mouse elevated platform exposure model [Fig. 15.1]. It is important to note that Lorrain et al. (Lorrain et al., 2005) also demonstrated a similar reversal in platform-induced increases in mPfc norephinephrine with the clinically effective anxiolytic diazepam, and a putative anxiolytic-like CRF_1 receptor antagonist (Koob, 1999). Interestingly, a recent study showed clinical efficacy of $mGlu_{2/3}$ agonists to treat positive and negative symptoms of schizophrenia (Patil et al., 2007).

Collectively these data suggest that mechanistic models that effectively isolate some aspect of the stress axis may be useful in identifying brain regions of interest for translational studies that are useful to cross current diagnostic boundaries. Thus, it is hoped that mechanistic assays may eventually guide the identification of novel biomarkers relevant to disease biology.

In Vivo Electrophysiology

Mechanistic models at the cellular level are also potentially valuable for parsing out mechanisms related to endophenotypes of depression. Again turning to the LC-NE system, a number of stressful stimuli increase LC neuronal activity (Abercrombie and Jacobs, 1987) and exogenous stimulation of LC activity has been associated with stress responses in non-human primates and humans (Charney et al., 1984). Interestingly, acute administration of known (Draper et al., 2007; Salchner and Singewald, 2002) antidepressant agents has been reported to elicit anxiety-like

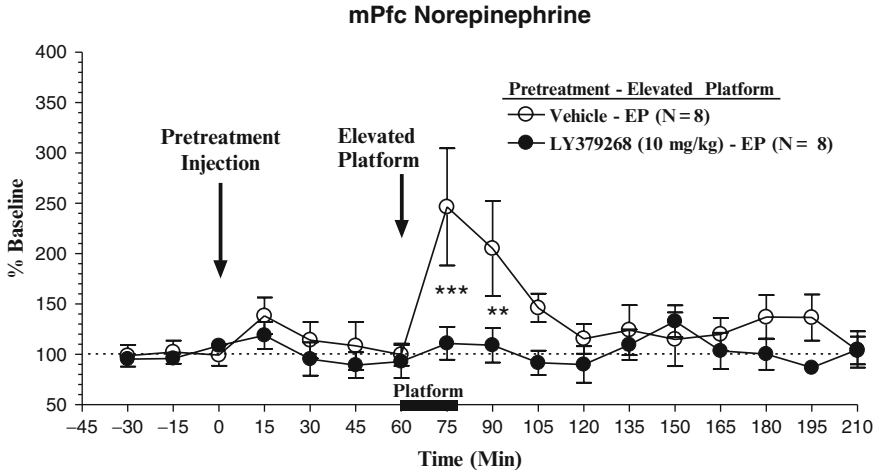

Fig. 15.1 Effects of the $mGlu_{2/3}$ receptor agonist, LY379268, on stress-induced increases in extracellular medial prefrontal cortex norepinephrine levels.Following baseline sample collection, freely moving CD-1 mice were injected with saline vehicle or LY379268 (10 mg/kg, IP; 10 mg/kg) 1 h prior to exposure to elevated platform exposure. The elevated platform consisted of an open, elevated platform made of clear plexiglass top (15 × 7.5 cm) supported 18 cm from the test cage floor by an aluminum tripod. Microdialysis samples were collected every 15 min by an automated fraction collector and norepinephrine concentrations were measured from the dialysate via HPLC with electrochemical detection. A within subjects design was used to generate percent baseline data by expressing each time point for each animal as a percentage of the average of its last 3 baseline samples. Data are then expressed as the mean ± SEM for all animals at each time point within each treatment group.Two-way ANOVA reveals significant effect of time (stress) [$F = 2.505$; $P < 0.0015$] and treatment [$F = 17.05$; $P < 0.0001$] with a significant interaction [$F = 1.897$; $P < 0.0216$]. ** $P < 0.01$; *** $P < 0.001$ compared to vehicle-EP with Bonferroni Post-test. $N = 8$ per group.

behaviors in rodents and this effect has been proposed to arise from enhanced LC activity (Salchner and Singewald, 2002).

Precipitated withdrawal from morphine administration is a model system for studying the effects of compounds on single unit activity of LC neurons and the associated somatic (Frenois et al., 2002) and behavioral (Schulteis et al., 1998) anxiety it produces. In this model, morphine can be administered to rodents over 1–2 days at different doses and a withdrawal syndrome elicited by treating them with an opiate antagonist. The withdrawal syndrome varies in intensity depending on the dose of morphine administered, duration of treatment and dose of opiate antagonist, and this behavioral syndrome is accompanied by enhanced single unit LC firing (Rasmussen et al., 1990). It has been determined that enhanced LC firing upon morphine withdrawal is the result of increased glutamatergic input to this region (Akaoka and Aston-Jones, 1991). In line with microdialysis results mentioned above and the proposed presynaptic mechanism of $mGlu_{2/3}$ receptor function (Cartmell and Schoepp, 2000), $mGlu_{2/3}$ receptor agonists reduce both the enhanced LC firing and behavioral effects of morphine withdrawal (Vandergriff and Rasmussen, 1999). Again, consistent with microdialysis results using platform stress, CRF_1 antagonists block morphine withdrawal-enhanced norepinephrine release in cortex (Funada et al., 2001).

15.3.1.5 Conclusion on Animal Models

The above data highlight the synergistic utility of utilizing distinct, hypothesis-driven mechanistic models based on a conceptual framework of segmentation to draw parallel and supporting conclusions on the potential therapeutic utility of novel compounds. Thus, the combined use of disease state and mechanistic models may offer an advantage to identifying and characterizing cellular and molecular mechanisms involved in various aspects of major psychiatric disorders, including depression, anxiety and schizophrenia.

15.4 Cellular Models

A comprehensive approach to the identification of predictive biomarkers in neuropsychiatric disorders (for both disease and treatment) should include *in vitro* cellular models to augment the clinical and preclinical approaches already discussed here. Cellular models by virtue of their simplicity may seem to represent a (detrimentally) reductionist interpretation of psychiatric disorders and the real human suffering they entail. However it is therein that their promise lies —for out of simplicity can emerge clarity, if the right components of the system are maintained (and unnecessary components abandoned). As symptom clusters and their underlying biochemistries become better defined, it is possible to envision *in vitro* models that could capture key elements of the morphological and molecular processes that emerge in the organism as dimensions of psychiatric illness. In the best embodiment, *in vitro* models can serve as abridged versions of the larger system, displaying emergent properties but providing the practical advantages of working at the cellular rather than at the organismal level. Moreover, cellular models do not need to recapitulate all aspects of a tissue/network/pathway/illness to be both instructive and predictive. When a model has been shown to provide outputs consonant with inputs (such as treatment benchmarks), it may be used to test novel treatments and generate hypotheses for testing in more complex systems. For example, changes in gene expression in response to treatment in clinical samples can be investigated *in vitro*. Similarly, dysregulated biochemical pathways associated with pathology *in vivo* (such as hypercortisolemia in melancholic depression) are more easily defined *in vitro* and may yield novel biomarkers or therapeutic targets. *In vitro* systems are also highly amenable to hypothesis testing since tools for dissecting association from causation, such as selective blockers and siRNAs, are practical to use. Finally, *in vitro* models may help diminish the gap between preclinical and clinical data by providing early confirmation in human cells of expected/desired outcomes. We believe that development of cellular models aimed at common symptom clusters and their underlying biochemistries (representing disease segments of the future) will augment and complement the larger effort to identify biomarkers that is the primary focus of this chapter. An overview of reported efforts in this direction is provided below, with a focus on cell culture models rather than acute

ex vivo slices. While the following sections are not a comprehensive review, they illustrate the possibilities that cellular assays hold in terms of shedding light onto molecular aspects of disease-related processes, and particularly HPA axis (dys-) regulation and neuronal proliferation, survival and remodeling.

15.4.1 In Vitro Models of HPA Axis Dysregulation in Neuronal Cells

Many of the effects of chronic stress are believed to be mediated by the sustained elevation in glucocorticoid levels. Groups working on *in vitro* models of HPA axis dysfunction have primarily used hippocampal neurons, hippocampal-derived cell lines, or neural stem cells and have focused on the respective roles of the two corticosteroid receptors, the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR): Treatment with a GR agonist triggers neuronal apoptosis, which is counteracted by a MR agonist (Crochemore et al., 2005) hence reflecting the *in vivo* situation (Rozeboom et al., 2007; Sousa et al., 2007).

Another regulator associated with stress and depression is CRF (Charmandari et al., 2005; Claes, 2004). CRF regulation and signaling pathways have been studied in primary amygdalar cultures (Kasckow et al., 1999), on a cell line derived from primary amygdalar cultures (Mulchahey et al., 1999) as well as in hypothalamic primary cultures (Emanuel et al., 1990; Hillhouse and Milton, 1989; Kasckow et al., 2003). Altogether, these *in vitro* studies have indicated that CRF levels in the amygdala are affected by some of the same stimuli that regulate hypothalamic CRF.

Recently, the neuroblastoma cell line Neuro 2A has been used to show that a variety of antidepressants inhibit CRF gene transcription (Budziszewska et al., 2004). Interestingly, similar results were obtained when the same cells were exposed to some antipsychotic treatments (Basta-Kaim et al., 2005).

Physiological stress is also associated with increased firing rate of LC neurons (Bremner et al., 1996), possibly mediated by CRF (Smagin et al., 1996). Mechanistic studies of the modulatory effects that corticosteroids and CRF exert on receptor signaling have been done by Bundley and collaborators (Bundey et al., 1997; Bundey and Kendall, 1999) using a LC-like neuronal cell line (Suri et al., 1993). In the same cell line, Thiel et al (Thiel and Cibelli, 1999) have shown that CRF triggers a PKA-dependent signaling cascade that leads to activation of gene transcription.

15.4.2 In Vitro Models of Monoaminergic Function

The brainstem raphe nuclei comprise the major source of serotonin (5-HT) to the rest of the brain. Primary cultures of raphe neurons have been employed to study the regulation of serotonin release and of presynaptic receptors on serotoninergic

neurons (Birthelmer et al., 2007). These cells were also recently used by Scheuch (Scheuch et al., 2007) to link specific tryptophan hydroxylase 2 promoter polymorphisms observed in humans to gene expression abnormalities.

In addition, a raphe-derived cell line was developed and used to study regulation mechanisms of serotonin synthesis, uptake and 5-HT1A coupling mechanisms (Koldzic-Zivanovic et al., 2006; Kushwaha and Albert, 2005). The same cell line has been used to study the polymorphism of 5-HT1A receptor as well as of the serotonin transporter gene promoter (Czesak et al., 2006; Mortensen et al., 1999). In addition, Zhu (Zhu et al., 2006) showed that proinflammatory cytokines activate serotonin transporters, providing an *in vitro* model to study molecular mechanisms related to inflammation-induced depression (Capuron and Miller, 2004; Wichers et al., 2005).

The septal nuclei rich with cholinergic neurons represent a component of the limbic system that regulates emotions and impulses. In a co-culture system of septal with raphe neurons it was shown that the regulation of presynaptic receptor function strongly depends on the concentrations of endogenous transmitter in the neuronal environment (Ehret et al., 2007). These findings open up the possibility that pathological conditions or therapeutic drug treatment could be modeled using this *in vitro* approach.

15.4.3 Neurogenesis and Neuroplasticity

It is now well established that antidepressants increase hippocampal neurogenesis in experimental animals (D'Sa and Duman, 2002). Moreover, cell proliferation has been shown to be necessary for the action of many antidepressant drugs in animal models (Sahay and Hen, 2007).

Though antipsychotics can increase neurogenesis in the subventricular zone (SVZ) as well as the subgranular zone (SGZ) of the hippocampus, conflicting results have been reported (Newton and Duman, 2007).

15.4.3.1 Antidepressants and Neurogenesis

Numerous studies focused on understanding the molecular mechanisms of antidepressants have employed models of *in vitro* neurogenesis mostly using neural stem cells derived from the adult hippocampus. These studies have indicated that in addition to increasing proliferation, antidepressants also promote survival and/or differentiation of neural stem cells (Chiou et al., 2006a, b; Peng et al., 2007). Intracellular signaling underlying these processes has also been analyzed in these studies.

RNA interference *in vitro* studies demonstrated that both neuroprotective and differentiation effects were dependent on BDNF (brain derived neurotrophic factor)

expression and Bcl-2 (B-cell leukemia/lymphoma 2) activation (Huang et al., 2007; Peng et al., 2007), confirming *in vivo* findings (D'Sa and Duman, 2002; Schmidt and Duman, 2007). Besides BDNF, vascular endothelial growth factor (VEGF) has also been found to exert direct mitogenic effects on neural progenitors *in vitro* (Warner-Schmidt and Duman, 2007). VEGF is induced by multiple classes of antidepressants at time points consistent with the induction of cell proliferation and therapeutic action of these treatments.

An alternative *in vitro* model to study the effects of antidepressants on cell proliferation has been developed by Zusso (Zusso et al., 2004) using cerebellar granule neurons in culture. The authors reported an increase in proliferation after treatment with the antidepressant fluoxetine. The newly generated cells can be expanded and induced to differentiate into neurons, astrocytes, and oligodendrocytes.

15.4.3.2 Antipsychotics and Neurogenesis

Antipsychotic drugs increase the proliferation of non-neuronal cell types in the prefrontal cortex (Newton and Duman, 2007), consistent with the emerging idea that neurogenesis may be disturbed in schizophrenia (Toro and Deakin, 2007). There is growing evidence that the action of antipsychotic drugs could be mediated at least in part by increased proliferation of neuronal as well as glial cells (Newton and Duman, 2007; Wang et al., 2004). However, while effects for antidepressants are consistent, the results obtained with antipsychotics so far are conflicting (Halim et al., 2004; Kodama et al., 2004; Wang et al., 2004).

15.4.3.3 Neuroplasticity in Mood Disorders and Schizophrenia

In vitro models have been shown to be valuable for clarifying the biological function of specific genes that are found to be linked to psychiatric disorders. Thus, multiple genes such as neuregulin-1 (NRG1), Akt (a serine/threonine-specific protein kinase), disrupted-in-schizophrenia 1 (DISC-1) and dysbindin-1, have recently been associated with schizophrenia although their role in the pathophysiology of the disease is not clear (Owen et al., 2005). A variety of *in vitro* neuronal assays showed that some of these genes are implicated in the process of neurite formation. A comprehensive review of these *in vitro* cellular assays can be found in Bellon et al, (Bellon, 2007). These data lend support to clinical findings of abnormal cytoarchitecture and disconnectivity in several regions of the brains of schizophrenic patients (Gur et al., 2007; Harrison and Weinberger, 2005). Interestingly, some of the genes reported above, namely DISC-1 and NRG, have also been described as genetic susceptibility factors for depression and bipolar disorders (Blackwood et al., 2007; Maier et al., 2006), pointing to a common neurobiological process that contributes to major psychiatric disorders.

15.4.4 Non-Neuronal Cells

In vitro cellular models from peripheral cells (e.g. blood cells, fibroblasts) have been used to probe for gene expression abnormalities that might be present in patients with psychiatric disorders. The identification of altered genes or signaling pathways in readily available human cells is particularly useful in the context of biomarkers to facilitate diagnosis and segmentation of patients as well as to monitor response to medication. Signaling pathways show a correlation between brain and peripheral cells, and hence peripheral cell read-outs in humans may be used to monitor treatment response (Ray et al., 2007; Sullivan et al., 2006). Peripheral blood cells are of obvious interest as biomarkers and have been discussed elsewhere in the chapter. Skin fibroblasts are also relatively accessible and show promise as "windows" into the brain. As several biochemical changes observed in neural tissue of depressed patients have also been observed in fibroblasts, they may be useful for *in vitro* models. Fibroblasts from patients with melancholic major depression confirmed the abnormalities in 5-HT2A receptor-stimulated signaling previously observed in the brain (Shelton et al., 2004). Furthermore, it has been reported that patients with melancholia show distinctly different gene expression compared to controls and patients with depression but without melancholic features (Shelton et al., 2004). In conclusion, non-neuronal cell models can also begin to provide data with which to segment patients for appropriate treatment and track therapeutic efficacy.

15.4.5 Conclusion on Cellular Models

True cellular models of psychiatric disorders do not (yet) exist. The progress described here represents the first stages in developing *in vitro* models relevant to human neuropsychiatric disorders and treatments. Indeed, *in vitro* models have proved useful to unravel some of the molecular mechanisms and to attribute direct cellular targets to specific therapeutic treatments. Furthermore, *in vitro* models have provided functional information on human gene polymorphisms, and also hints on the potential function of some genes, i.e. by knocking down or over-expressing the gene of interest. As clinicians increasingly seek to define biologically-driven endophenotypes in schizophrenia and mood disorders, cellular models may begin to play a larger role in deciphering the underlying biochemistries as well as enabling the discovery of novel targets, treatments, and biomarkers for these disorders.

15.5 Summary and Perspectives

In order to achieve the ambitious goal of the DSM-V research agenda, namely to develop a scientifically sound classification system, a better understanding of the biological underpinnings of psychiatric disorders seems to be a crucial first step. In this regard biomarkers should play a pivotal role.

The previous sections have outlined perspectives on how research into biomarkers, if pursued systematically, could advance our understanding of the biology of psychiatric disorders. Besides the obvious advantages of objective markers for the determination of a diagnosis in psychiatry, biomarkers could also become pivotal for the choice of treatment for a particular patient. In fact, biomarkers that could help make a diagnosis, could relate to the pathophysiology, and could be used to monitor treatment effects could have a major impact on drug development. Animal models could be constructed that mimic a specific biomarker profile of a human disease state and allow testing of novel drug effects on such a circumscribed clinically relevant biomarker read-outs. This approach provides an opportunity to assess effects of new drug targets on multiple distinct biological abnormalities relevant to one or several patient segments. Such a biologically focused evaluation could also help identify multiple therapeutic indications for a novel compound. In addition, these mechanistic constructs could form the basis for cellular assays to assess drug targets very early.

Beforehand, however, the available biomarker data should be critically reviewed and prioritized for future research. This seems important, as there are no confirmed and validated biomarkers available despite the abundant literature on biological findings in psychiatric disorders. This unsatisfying situation reflects the lack of both a systematic approach and use of standardized evaluation methods for clinical and biological factors. Also, if biomarkers are to address diagnostic uncertainty and support treatment selection and novel drug development, they should be assessable in humans and rodents and, at least in humans, at multiple time-points for the same individual. In addition, the biomarker data must yield a consistent result if (practical) standardized procedures are followed and should show high specificity and sensitivity. The ideal biomarker would also show, in individuals at risk, an intermediate level between that seen in acutely ill and super-healthy subjects.

Although many biomarkers explored in scientific publications are not useful for general psychiatric practice, e.g. functional neuroimaging markers or neuroendocrine challenge tests, this could change if they can be correlated with highly accessible markers from blood samples. In this regard, transcription profiling from peripheral leukocytes seems more valuable today than proteomics and metabolomics approaches, as standards exist with regard to sampling, preservation, storage and analysis. In addition, transcription profiling, particularly if a pattern based on a limited number of genes with satisfactory predictive value is identified, can be applied to animal and cellular models. Such mechanistic constructs will also facilitate the discovery of new molecular targets. Once a transcription pattern has been confirmed, it seems reasonable to expect that related protein and metabolite markers will be identified and turned into a commercial biomarker kit.

Implicit in the above paragraph are at least two paradigm shifts, which appear needed to progress from several decades of small and incremental improvements of drug development in psychiatry: Firstly, biological findings used for mechanistic constructs in animals should be based on objectively measured data in patients. Secondly, the link between biological findings and clinical characteristics should not be confined to the current diagnostic entities.

With regard to the first point, some pharmaceutical companies have followed this route by using human genetic mutations in the target populations to discover new drug targets. Whether this effort will prove fruitful for polygenetic disorders, such as psychiatric disorders, remains to be seen. This approach may, in fact, become more useful once biological findings help to delineate homogenous patient segments which are then used for the discovery of genetic polymorphisms.

The second point is addressed in the DSM-V conferences and in some recent publications. Thus, specific symptom clusters rather than an entire disorder are linked to biological findings and can help uncover the biology of distinct phenotypes. This point may be of particular importance when one aims to address early onset of disorders or prophylactic treatments. As the specific symptoms an individual develops depend on the individual's genetic makeup and the environmental context, objective markers that allow recognition of endophenotypes associated with increased vulnerability will help select individuals for prophylactic treatment.

With the discussions around DSM-V, it can be hoped that the process and the discussions will continue to guide and stimulate systematic research into the biology and biological markers of psychiatric disorders long after DSM-V has been realized.

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References

A Research Agenda for DSM-V™. Washington, D.C. American Psychiatric Association (2002)

Abercrombie, E. D., Jacobs, B. L.: Single-unit response of noradrenergic neurons in the locus coeruleus of freely moving cats. I. Acutely presented stressful and nonstressful stimuli. J Neurosci 7, 2837–2843 (1987)

Akaoka, H., Aston-Jones, G.: Opiate withdrawal-induced hyperactivity of locus coeruleus neurons is substantially mediated by augmented excitatory amino acid input. J Neurosci 11, 3830–3839 (1991)

Alfonso, J., Pollevick, G. D., van der Hart, M. G., Flugge, G., Fuchs, E., Frasch, A. C.: Identification of genes regulated by chronic psychosocial stress and antidepressant treatment in the hippocampus. Eur J Neurosci 19, 659–666 (2004)

Altar, C. A., Laeng, P., Jurata, L. W., Brockman, J. A., Lemire, A., Bullard, J., Bukhman, Y. V., Young, T. A., Charles, V., Palfreyman, M. G.: Electroconvulsive seizures regulate gene expression of distinct neurotrophic signaling pathways. J Neurosci 24, 2667–2677 (2004)

Andersen, C. L., Jensen, J. L., Orntoft, T. F.: Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res 64, 5245–5250 (2004)

Armitage, R., Hoffmann, R. F., Rush, A. J.: Biological rhythm disturbance in depression: temporal coherence of ultradian sleep EEG rhythms. Psychol. Med. 29, 1435–1448 (1999)

Armitage, R., Hoffmann, R., Trivedi, M., Rush, A. J.: Slow-wave activity in NREM sleep: sex and age effects in depressed outpatients and healthy controls. Psychiatry Res. 95, 201–213 (2000)

Armitage, R., Emslie, G. J., Hoffmann, R. F., Rintelmann, J., Rush, A. J.: Delta sleep EEG in depressed adolescent females and healthy controls. J. Affect. Disord. 63, 139–148 (2001)

Armitage, R., Hoffmann, R., Emslie, G., Rintelmann, J., Robert, J.: Sleep microarchitecture in childhood and adolescent depression: temporal coherence. Clin. EEG. Neurosci. 37, 1–9 (2006)

Atz, M., Walsh, D., Cartagena, P., Li, J., Evans, S., Choudary, P., Overman, K., Stein, R., Tomita, H., Potkin, S., Myers, R., Watson, S. J., Jones, E. G., Akil, H., Bunney, W. E., Jr., Vawter, M. P.: Methodological considerations for gene expression profiling of human brain. J. Neurosci. Methods 163, 295–309 (2007)

Avissar, S., Matuzany-Ruban, A., Tzukert, K., Schreiber, G.: Beta-arrestin-1 levels: reduced in leukocytes of patients with depression and elevated by antidepressants in rat brain. Am. J. Psychiatry 161, 2066–2072 (2004)

Basta-Kaim, A., Budziszewska, B., Jaworska-Feil, L., Tetich, M., Kubera, M., Leskiewicz, M., Lason, W.: Inhibitory effect of imipramine on the human corticotropin-releasing-hormone gene promoter activity operates through a PI3-K/AKT mediated pathway. Neuropharmacology 49, 156–164 (2005)

Bellon, A.: New genes associated with schizophrenia in neurite formation: a review of cell culture experiments. Mol Psychiatry 12, 620–629 (2007)

Biomarkers Definitions Working Group: Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin. Pharmacol. Ther. 69, 89–95 (2001)

Birthelmer, A., Ehret, A., Riegert, C., Rothmaier, A. K., Leemhuis, J., Jackisch, R.: Modulation of electrically evoked serotonin release in cultured rat raphe neurons. J Neurochem 100, 1613–1625 (2007)

Blackwood, D. H., Pickard, B. J., Thomson, P. A., Evans, K. L., Porteous, D. J., Muir, W. J.: Are some genetic risk factors common to schizophrenia, bipolar disorder and depression? Evidence from DISC1, GRIK4 and NRG1. Neurotox. Res 11, 73–83 (2007)

Bonaccorso, S., Marino, V., Puzella, A., Pasquini, M., Biondi, M., Artini, M., Almerighi, C., Verkerk, R., Meltzer, H., Maes, M.: Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. J. Clin. Psychopharmacol. 22, 86–90 (2002)

Borsini, F.: Role of the serotonergic system in the forced swimming test. Neurosci Biobehav. Rev 19, 377–395 (1995)

Bosotti, R., Locatelli, G., Healy, S., Scacheri, E., Sartori, L., Mercurio, C., Calogero, R., Isacchi, A.: Cross platform microarray analysis for robust identification of differentially expressed genes. BMC Bioinformatics 8 Suppl 1, S5 (2007)

Brazeau, D. A.: Combining genome-wide and targeted gene expression profiling in drug discovery: microarrays and real-time PCR. Drug Discov. Today. 9, 838–845 (2004)

Bremner, J. D., Krystal, J. H., Southwick, S. M., Charney, D. S.: Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. Synapse. 23, 28–38 (1996)

Brenner, S., Johnson, M., Bridgham, J., Golda, G., Lloyd, D. H., Johnson, D., Luo, S., McCurdy, S., Foy, M., Ewan, M., Roth, R., George, D., Eletr, S., Albrecht, G., Vermaas, E., Williams, S. R., Moon, K., Burcham, T., Pallas, M., DuBridge, R. B., Kirchner, J., Fearon, K., Mao, J., Corcoran, K.: Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. Nat. Biotechnol. 18, 630–634 (2000)

Browning, M. D., Dudek, E. M., Rapier, J. L., Leonard, S., Freedman, R.: Significant reductions in synapsin but not synaptophysin specific activity in the brains of some schizophrenics. Biol Psychiatry 34, 529–535 (1993)

Budziszewska, B., Jaworska-Feil, L., Tetich, M., Basta-Kaim, A., Kubera, M., Leskiewicz, M., Lason, W.: Regulation of the human corticotropin-releasing-hormone gene promoter activity by antidepressant drugs in Neuro-2A and AtT-20 cells. Neuropsychopharmacology 29, 785–794 (2004)

Bundey, R. A., Iredale, P. A., Kendall, D. A.: Corticosteroid modulation of signal transduction in the CATH.a cell line. J Neurochem 68, 362–369 (1997)

Bundey, R. A., Kendall, D. A.: Inhibition of receptor-mediated calcium responses by corticotrophin-releasing hormone in the CATH.a cell line. Neuropharmacology 38, 39–47 (1999)

Canales, R. D., Luo, Y., Willey, J. C., Austermiller, B., Barbacioru, C. C., Boysen, C., Hunkapiller, K., Jensen, R. V., Knight, C. R., Lee, K. Y., Ma, Y., Maqsodi, B., Papallo, A., Peters, E. H., Poulter, K., Ruppel, P. L., Samaha, R. R., Shi, L., Yang, W., Zhang, L., Goodsaid, F. M.: Evaluation of DNA microarray results with quantitative gene expression platforms. Nat. Biotechnol. 24, 1115–1122 (2006)

Capuron, L., Dantzer, R.: Cytokines and depression: the need for a new paradigm. Brain Behav. Immun. 17 Suppl 1, S119–S124 (2003)

Capuron, L., Miller, A. H.: Cytokines and psychopathology: lessons from interferon-alpha. Biol. Psychiatry 56, 819–824 (2004)

Capuron, L., Neurauter, G., Musselman, D. L., Lawson, D. H., Nemeroff, C. B., Fuchs, D., Miller, A. H.: Interferon-alpha-induced changes in tryptophan metabolism. relationship to depression and paroxetine treatment. Biol. Psychiatry 54, 906–914 (2003a)

Capuron, L., Raison, C. L., Musselman, D. L., Lawson, D. H., Nemeroff, C. B., Miller, A. H.: Association of exaggerated HPA axis response to the initial injection of interferon-alpha with development of depression during interferon-alpha therapy. Am. J. Psychiatry 160, 1342–1345 (2003b)

Carroll, B. J., Curtis, G. C., Mendels, J.: Neuroendocrine regulation in depression. II. Discrimination of depressed from nondepressed patients. Arch. Gen. Psychiatry 33, 1051–1058 (1976)

Carroll, B. J., Cassidy, F., Naftolowitz, D., Tatham, N. E., Wilson, W. H., Iranmesh, A., Liu, P. Y., Veldhuis, J. D.: Pathophysiology of hypercortisolism in depression. Acta Psychiatrica Scandinavica 115, 90–103 (2007)

Cartmell, J., Schoepp, D. D.: Regulation of neurotransmitter release by metabotropic glutamate receptors. J Neurochem 75, 889–907 (2000)

Charmandari, E., Tsigos, C., Chrousos, G.: Endocrinology of the stress response. Annu. Rev Physiol 67, 259–284 (2005)

Charney, D. S., Heninger, G. R., Breier, A.: Noradrenergic function in panic anxiety. Effects of yohimbine in healthy subjects and patients with agoraphobia and panic disorder. Arch. Gen. Psychiatry 41, 751–763 (1984)

Charney DS, Barlow DH, Botteron K, Cohen JD, Goldman D, Gur RE, Lin K-M, Lopez JF, Meador-Woodruff JH, Moldin SO, Nestler EJ, Watson SJ, Zalcman SJ. Neuroscience Research Agenda to Guide Development of a Pathophysiologically Based Classification System. In: Kupfer DJ, First MB, Regier DA, editors. A Research Agenda for DSM-V™. Washington, D.C.: American Psychiatric Association, 2002. p. 31–84.

Chiou, S. H., Chen, S. J., Peng, C. H., Chang, Y. L., Ku, H. H., Hsu, W. M., Ho, L. L., Lee, C. H.: Fluoxetine up-regulates expression of cellular FLICE-inhibitory protein and inhibits LPS-induced apoptosis in hippocampus-derived neural stem cell. Biochem Biophys. Res Commun. 343, 391–400 (2006a)

Chiou, S. H., Ku, H. H., Tsai, T. H., Lin, H. L., Chen, L. H., Chien, C. S., Ho, L. L., Lee, C. H., Chang, Y. L.: Moclobemide upregulated Bcl-2 expression and induced neural stem cell differentiation into serotoninergic neuron via extracellular-regulated kinase pathway. Br. J Pharmacol 148, 587–598 (2006b)

Claes, S. J.: Corticotropin-releasing hormone (CRH) in psychiatry: from stress to psychopathology. Ann Med 36, 50–61 (2004)

Crochemore, C., Lu, J., Wu, Y., Liposits, Z., Sousa, N., Holsboer, F., Almeida, O. F.: Direct targeting of hippocampal neurons for apoptosis by glucocorticoids is reversible by mineralocorticoid receptor activation. Mol Psychiatry 10, 790–798 (2005)

Cryan, J. F., Holmes, A.: The ascent of mouse: advances in modelling human depression and anxiety. Nat. Rev Drug Discov. 4, 775–790 (2005)

Cryan, J. F., Mombereau, C.: In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. Mol Psychiatry 9, 326–357 (2004)
Cryan, J. F., Slattery, D. A.: Animal models of mood disorders: Recent developments. Curr. Opin. Psychiatry 20, 1–7 (2007)
Cryan, J. F., Mombereau, C., Vassout, A.: The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci Biobehav. Rev 29, 571–625 (2005a)
Cryan, J. F., Valentino, R. J., Lucki, I.: Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav. Rev 29, 547–569 (2005b)
Czesak, M., Lemonde, S., Peterson, E. A., Rogaeva, A., Albert, P. R.: Cell-specific repressor or enhancer activities of Deaf-1 at a serotonin 1A receptor gene polymorphism. J Neurosci 26, 1864–1871 (2006)
David, D. J., Klemenhagen, K. C., Holick, K. A., Saxe, M. D., Mendez, I., Santarelli, L., Craig, D. A., Zhong, H., Swanson, C. J., Hegde, L. G., Ping, X. I., Dong, D., Marzabadi, M. R., Gerald, C. P., Hen, R.: Efficacy of the MCHR1 antagonist N-[3-(1-{[4-(3,4-difluorophenoxy) phenyl]methyl}(4-piperidyl))-4-methylphen yl]-2-methylpropanamide (SNAP 94847) in mouse models of anxiety and depression following acute and chronic administration is independent of hippocampal neurogenesis. J Pharmacol Exp Ther 321, 237–248 (2007)
Davidson, R. J., Irwin, W., Anderle, M. J., Kalin, N. H.: The neural substrates of affective processing in depressed patients treated with venlafaxine. Am. J. Psychiatry 160, 64–75 (2003)
De Pablo, J. M., Parra, A., Segovia, S., Guillamon, A.: Learned immobility explains the behavior of rats in the forced swimming test. Physiol Behav 46, 229–237 (1989)
Detke, M. J., Rickels, M., Lucki, I.: Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology. (Berl.) 121, 66–72 (1995)
Dettling, M., Buhlmann, P.: Finding predictive gene groups from microarray data. J. Multivariate Analysis 90, 106–131 (2004)
Draper, I., Kurshan, P. T., McBride, E., Jackson, F. R., Kopin, A. S.: Locomotor activity is regulated by D2-like receptors in Drosophila: an anatomic and functional analysis. Dev Neurobiol 67, 378–393 (2007)
D'Sa, C., Duman, R. S.: Antidepressants and neuroplasticity. Bipolar Disord. 4, 183–194 (2002)
Duncan, G. E., Breese, G. R., Criswell, H. E., Johnson, K. B., Schambra, U. B., Mueller, R. A., Caron, M. G., Fremeau, R. T., Jr.: D1 dopamine receptor binding and mRNA levels are not altered after neonatal 6-hydroxydopamine treatment: evidence against dopamine-mediated induction of D1 dopamine receptors during postnatal development. J Neurochem 61, 1255–1262 (1993)
Ehret, A., Birthelmer, A., Rutz, S., Riegert, C., Rothmaier, A. K., Jackisch, R.: Agonist-mediated regulation of presynaptic receptor function during development of rat septal neurons in culture. J Neurochem 102, 1071–1082 (2007)
Emanuel, R. L., Girard, D. M., Thull, D. L., Majzoub, J. A.: Second messengers involved in the regulation of corticotropin-releasing hormone mRNA and peptide in cultured rat fetal hypothalamic primary cultures. Endocrinology 126, 3016–3021 (1990)
Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., Gage, F. H.: Neurogenesis in the adult human hippocampus. Nat. Med 4, 1313–1317 (1998)
Evans, S. J., Choudary, P. V., Neal, C. R., Li, J. Z., Vawter, M. P., Tomita, H., Lopez, J. F., Thompson, R. C., Meng, F., Stead, J. D., Walsh, D. M., Myers, R. M., Bunney, W. E., Watson, S. J., Jones, E. G., Akil, H.: Dysregulation of the fibroblast growth factor system in major depression. Proc Natl Acad Sci U S A 101, 15506–15511 (2004)
Feezor, R. J., Baker, H. V., Mindrinos, M., Hayden, D., Tannahill, C. L., Brownstein, B. H., Fay, A., MacMillan, S., Laramie, J., Xiao, W., Moldawer, L. L., Cobb, J. P., Laudanski, K., Miller-Graziano, C. L., Maier, R. V., Schoenfeld, D., Davis, R. W., Tompkins, R. G.: Whole blood and leukocyte RNA isolation for gene expression analyses. Physiol Genomics 19, 247–254 (2004)

Frank, R., Hargreaves, R.: Clinical biomarkers in drug discovery and development. Nat. Rev. Drug Discov. 2, 566–580 (2003)

Frenois, F., Cador, M., Caille, S., Stinus, L., Le, M. C.: Neural correlates of the motivational and somatic components of naloxone-precipitated morphine withdrawal. Eur J Neurosci 16, 1377–1389 (2002)

Frodl, T., Meisenzahl, E. M., Zetzsche, T., Hohne, T., Banac, S., Schorr, C., Jager, M., Leinsinger, G., Bottlender, R., Reiser, M., Moller, H. J.: Hippocampal and amygdala changes in patients with major depressive disorder and healthy controls during a 1-year follow-up. J. Clin. Psychiatry 65, 492–499 (2004)

Frohna, P. A., Neal-Beliveau, B. S., Joyce, J. N.: Neonatal 6-hydroxydopamine lesions lead to opposing changes in the levels of dopamine receptors and their messenger RNAs. Neuroscience 68, 505–518 (1995)

Fu, C. H., Williams, S. C., Cleare, A. J., Brammer, M. J., Walsh, N. D., Kim, J., Andrew, C. M., Pich, E. M., Williams, P. M., Reed, L. J., Mitterschiffthaler, M. T., Suckling, J., Bullmore, E. T.: Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. Arch. Gen. Psychiatry 61, 877–889 (2004)

Funada, M., Hara, C., Wada, K.: Involvement of corticotropin-releasing factor receptor subtype 1 in morphine withdrawal regulation of the brain noradrenergic system. Eur J Pharmacol 430, 277–281 (2001)

Ganguly, S., Coon, S. L., Klein, D. C.: Control of melatonin synthesis in the mammalian pineal gland: the critical role of serotonin acetylation. Cell Tissue Res 309, 127–137 (2002)

Garcia-Sevilla, J. A., Zis, A. P., Hollingsworth, P. J., Greden, J. F., Smith, C. B.: Platelet alpha 2-adrenergic receptors in major depressive disorder. Binding of tritiated clonidine before and after tricyclic antidepressant drug treatment. Arch. Gen. Psychiatry 38, 1327–1333 (1981)

Georges, M., Coppieters, W., Charlier, C.: Polymorphic miRNA-mediated gene regulation: contribution to phenotypic variation and disease. Curr. Opin. Genet. Dev. 17, 166–176 (2007)

Ghaffar, O., Feinstein, A.: The neuropsychiatry of multiple sclerosis: a review of recent developments. Curr. Opin. Psychiatry 20, 278–285 (2007)

Gilbertson, M. W., Shenton, M. E., Ciszewski, A., Kasai, K., Lasko, N. B., Orr, S. P., Pitman, R. K.: Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. Nat. Neurosci. 5, 1242–1247 (2002)

Gladkevich, A., Kauffman, H. F., Korf, J.: Lymphocytes as a neural probe: potential for studying psychiatric disorders. Prog. Neuropsychopharmacol. Biol Psychiatry 28, 559–576 (2004)

Glantz, L. A., Lewis, D. A.: Reduction of synaptophysin immunoreactivity in the prefrontal cortex of subjects with schizophrenia. Regional and diagnostic specificity. Arch. Gen. Psychiatry 54, 943–952 (1997)

Glavin, G. B.: Selective noradrenaline depletion markedly alters stress responses in rats. Life Sci 37, 461–465 (1985a)

Glavin, G. B.: Selective noradrenaline depletion markedly alters stress responses in rats. Life Sci 37, 461–465 (1985b)

Glavin, G. B.: Stress and brain noradrenaline: a review. Neurosci Biobehav. Rev 9, 233–243 (1985c)

Gold, P. W., Charney, D. S.: Diseases of the mind and brain: depression: a disease of the mind, brain, and body. Am. J. Psychiatry 159, 1826 (2002)

Gold, P. W., Chrousos, G. P.: Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. Mol. Psychiatry 7, 254–275 (2002)

Gross, C. G.: Neurogenesis in the adult brain: death of a dogma. Nat. Rev Neurosci 1, 67–73 (2000)

Gur, R. E., Keshavan, M. S., Lawrie, S. M.: Deconstructing psychosis with human brain imaging. Schizophr. Bull. 33, 921–931 (2007)

Halim, N. D., Weickert, C. S., McClintock, B. W., Weinberger, D. R., Lipska, B. K.: Effects of chronic haloperidol and clozapine treatment on neurogenesis in the adult rat hippocampus. Neuropsychopharmacology 29, 1063–1069 (2004)

Harrison, P. J., Weinberger, D. R.: Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 10, 40–68 (2005)

Heesen, C., Nawrath, L., Reich, C., Bauer, N., Schulz, K. H., Gold, S. M.: Fatigue in multiple sclerosis: an example of cytokine mediated sickness behaviour? J. Neurol. Neurosurg. Psychiatry 77, 34–39 (2006)

Heim, C., Newport, D. J., Heit, S., Graham, Y. P., Wilcox, M., Bonsall, R., Miller, A. H., Nemeroff, C. B.: Pituitary–adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. JAMA 284, 592–597 (2000)

Heim, C., Plotsky, P. M., Nemeroff, C. B.: Importance of studying the contributions of early adverse experience to neurobiological findings in depression. Neuropsychopharmacology 29, 641–648 (2004)

Hillhouse, E. W., Milton, N. G.: Effect of acetylcholine and 5-hydroxytryptamine on the secretion of corticotrophin-releasing factor-41 and arginine vasopressin from the rat hypothalamus *in vitro*. J Endocrinol. 122, 713–718 (1989)

Holmes, P. V.: Rodent models of depression: reexamining validity without anthropomorphic inference. Crit. Rev Neurobiol 15, 143–174 (2003)

Holsboer, F.: The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. J. Psychiatr. Res. 33, 181–214 (1999)

Huang, Y. Y., Peng, C. H., Yang, Y. P., Wu, C. C., Hsu, W. M., Wang, H. J., Chan, K. H., Chou, Y. P., Chen, S. J., Chang, Y. L.: Desipramine activated Bcl-2 expression and inhibited lipopolysaccharide-induced apoptosis in hippocampus-derived adult neural stem cells. J Pharmacol Sci 104, 61–72 (2007)

Huggett, J., Dheda, K., Bustin, S., Zumla, A.: Real-time RT-PCR normalisation; strategies and considerations. Genes Immun. 6, 279–284 (2005)

Husum, H., Mikkelsen, J. D., Hogg, S., Mathe, A. A., Mork, A.: Involvement of hippocampal neuropeptide Y in mediating the chronic actions of lithium, electroconvulsive stimulation and citalopram. Neuropharmacology 39, 1463–1473 (2000)

Iga, J., Ueno, S., Yamauchi, K., Motoki, I., Tayoshi, S., Ohta, K., Song, H., Morita, K., Rokutan, K., Ohmori, T.: Serotonin transporter mRNA expression in peripheral leukocytes of patients with major depression before and after treatment with paroxetine. Neurosci Lett. 389, 12–16 (2005)

Iga, J., Ueno, S., Yamauchi, K., Numata, S., Motoki, I., Tayoshi, S., Kinouchi, S., Ohta, K., Song, H., Morita, K., Rokutan, K., Tanabe, H., Sano, A., Ohmori, T.: Gene expression and association analysis of LIM (PDLIM5) in major depression. Neurosci Lett. 400, 203–207 (2006)

Iga, J., Ueno, S., Yamauchi, K., Numata, S., Kinouchi, S., Tayoshi-Shibuya, S., Song, H., Ohmori, T.: Altered HDAC5 and CREB mRNA expressions in the peripheral leukocytes of major depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 628–632 (2007a)

Iga, J., Ueno, S., Yamauchi, K., Numata, S., Tayoshi-Shibuya, S., Kinouchi, S., Nakataki, M., Song, H., Hokoishi, K., Tanabe, H., Sano, A., Ohmori, T.: Gene expression and association analysis of vascular endothelial growth factor in major depressive disorder. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 658–663 (2007b)

Insel, T. R., Scolnick, E. M.: Cure therapeutics and strategic prevention: raising the bar for mental health research. Mol. Psychiatry 11, 11–17 (2006)

Ising, M., Horstmann, S., Kloiber, S., Lucae, S., Binder, E. B., Kern, N., Kunzel, H. E., Pfennig, A., Uhr, M., Holsboer, F.: Combined dexamethasone/corticotropin releasing hormone test predicts treatment response in major depression - a potential biomarker? Biol. Psychiatry 62, 47–54 (2007)

Jarvinen, A. K., Hautaniemi, S., Edgren, H., Auvinen, P., Saarela, J., Kallioniemi, O. P., Monni, O.: Are data from different gene expression microarray platforms comparable? Genomics 83, 1164–1168 (2004)

Jin, P., Zhao, Y., Ngalame, Y., Panelli, M. C., Nagorsen, D., Monsurro, V., Smith, K., Hu, N., Su, H., Taylor, P. R., Marincola, F. M., Wang, E.: Selection and validation of endogenous reference genes using a high throughput approach. BMC Genomics 5, 55 (2004)

Kaddurah-Daouk, R., McEvoy, J., Baillie, R. A., Lee, D., Yao, J. K., Doraiswamy, P. M., Krishnan, K. R.: Metabolomic mapping of atypical antipsychotic effects in schizophrenia. Mol. Psychiatry 1–12 (2007)

Kalman, J., Palotas, A., Juhasz, A., Rimanoczy, A., Hugyecz, M., Kovacs, Z., Galsi, G., Szabo, Z., Pakaski, M., Feher, L. Z., Janka, Z., Puskas, L. G.: Impact of venlafaxine on gene expression profile in lymphocytes of the elderly with major depression–evolution of antidepressants and the role of the "neuro-immune" system. Neurochem Res 30, 1429–1438 (2005)

Karson, C. N., Mrak, R. E., Schluterman, K. O., Sturner, W. Q., Sheng, J. G., Griffin, W. S.: Alterations in synaptic proteins and their encoding mRNAs in prefrontal cortex in schizophrenia: a possible neurochemical basis for 'hypofrontality'. Mol Psychiatry 4, 39–45 (1999)

Kasckow, J. W., Regmi, A., Sheriff, S., Mulchahey, J., Geracioti, T. D., Jr.: Regulation of corticotropin-releasing factor messenger RNA by nicotine in an immortalized amygdalar cell line. Life Sci 65, 2709–2714 (1999)

Kasckow, J. W., Aguilera, G., Mulchahey, J. J., Sheriff, S., Herman, J. P.: *In vitro* regulation of corticotropin-releasing hormone. Life Sci 73, 769–781 (2003)

Keck, M. E., Wigger, A., Welt, T., Muller, M. B., Gesing, A., Reul, J. M., Holsboer, F., Landgraf, R., Neumann, I. D.: Vasopressin mediates the response of the combined dexamethasone/CRH test in hyper-anxious rats: implications for pathogenesis of affective disorders. Neuropsychopharmacology 26, 94–105 (2002)

Keck, M. E., Welt, T., Muller, M. B., Uhr, M., Ohl, F., Wigger, A., Toschi, N., Holsboer, F., Landgraf, R.: Reduction of hypothalamic vasopressinergic hyperdrive contributes to clinically relevant behavioral and neuroendocrine effects of chronic paroxetine treatment in a psychopathological rat model. Neuropsychopharmacology 28, 235–243 (2003)

Khan, A., Schwartz, K., Kolts, R. L., Ridgway, D., Lineberry, C.: Relationship between depression severity entry criteria and antidepressant clinical trial outcomes. Biol. Psychiatry 62, 65–71 (2007)

Kitayama, N., Vaccarino, V., Kutner, M., Weiss, P., Bremner, J. D.: Magnetic resonance imaging (MRI) measurement of hippocampal volume in posttraumatic stress disorder: a meta-analysis. J. Affect. Disord. 88, 79–86 (2005)

Kodama, M., Fujioka, T., Duman, R. S.: Chronic olanzapine or fluoxetine administration increases cell proliferation in hippocampus and prefrontal cortex of adult rat. Biol Psychiatry 56, 570–580 (2004)

Koldzic-Zivanovic, N., Seitz, P. K., Cunningham, K. A., Thomas, M. L., Hughes, T. K.: Serotonin regulation of serotonin uptake in RN46A cells. Cell Mol Neurobiol 26, 979–987 (2006)

Konradi, C.: Gene expression microarray studies in polygenic psychiatric disorders: applications and data analysis. Brain Res Brain Res Rev 50, 142–155 (2005)

Koob, G. F.: Corticotropin-releasing factor, norepinephrine, and stress. Biol Psychiatry 46, 1167–1180 (1999)

Kromer, S. A., Kessler, M. S., Milfay, D., Birg, I. N., Bunck, M., Czibere, L., Panhuysen, M., Putz, B., Deussing, J. M., Holsboer, F., Landgraf, R., Turck, C. W.: Identification of glyoxalase-I as a protein marker in a mouse model of extremes in trait anxiety. J Neurosci 25, 4375–4384 (2005a)

Kromer, S. A., Kessler, M. S., Milfay, D., Birg, I. N., Bunck, M., Czibere, L., Panhuysen, M., Putz, B., Deussing, J. M., Holsboer, F., Landgraf, R., Turck, C. W.: Identification of glyoxalase-I as a protein marker in a mouse model of extremes in trait anxiety. J Neurosci 25, 4375–4384 (2005b)

Kunzel, H. E., Binder, E. B., Nickel, T., Ising, M., Fuchs, B., Majer, M., Pfennig, A., Ernst, G., Kern, N., Schmid, D. A., Uhr, M., Holsboer, F., Modell, S.: Pharmacological and nonpharmacological factors influencing hypothalamic–pituitary–adrenocortical axis reactivity in acutely depressed psychiatric in-patients, measured by the Dex-CRH test. Neuropsychopharmacology 28, 2169–2178 (2003)

Kupfer, D. J.: REM latency: a psychobiologic marker for primary depressive disease. Biol. Psychiatry 11, 159–174 (1976)
Kupfer, D. J.: Sleep research in depressive illness: clinical implications--a tasting menu. Biol. Psychiatry 38, 391–403 (1995)
Kupfer, D. J., Targ, E., Stack, J.: Electroencephalographic sleep in unipolar depressive subtypes: support for a biological and familial classification. J. Nerv. Ment. Dis. 170, 494–498 (1982)
Kupfer, D. J., Reynolds, C. F., III, Grochocinski, V. J., Ulrich, R. F., McEachran, A.: Aspects of short REM latency in affective states: a revisit. Psychiatry Res. 17, 49–59 (1986)
Kushwaha, N., Albert, P. R.: Coupling of 5-HT1A autoreceptors to inhibition of mitogen-activated protein kinase activation via G beta gamma subunit signaling. Eur J Neurosci 21, 721–732 (2005)
Landgraf, R., Wigger, A.: High vs low anxiety-related behavior rats: an animal model of extremes in trait anxiety. Behav. Genet. 32, 301–314 (2002)
Landgraf, R., Wigger, A., Holsboer, F., Neumann, I. D.: Hyper-reactive hypothalamo-pituitary–adrenocortical axis in rats bred for high anxiety-related behaviour. J. Neuroendocrinol. 11, 405–407 (1999)
Lauer, C. J., Riemann, D., Wiegand, M., Berger, M.: From early to late adulthood. Changes in EEG sleep of depressed patients and healthy volunteers. Biol. Psychiatry 29, 979–993 (1991)
Lehrmann, E., Hyde, T. M., Vawter, M. P., Becker, K. G., Kleinman, J. E., Freed, W. J.: The use of microarrays to characterize neuropsychiatric disorders: postmortem studies of substance abuse and schizophrenia. Curr. Mol Med 3, 437–446 (2003)
Leonard, B. E.: The HPA and immune axes in stress: the involvement of the serotonergic system. Eur. Psychiatry 20 Suppl 3, S302–S306 (2005)
Liebsch, G., Linthorst, A. C., Neumann, I. D., Reul, J. M., Holsboer, F., Landgraf, R.: Behavioral, physiological, and neuroendocrine stress responses and differential sensitivity to diazepam in two Wistar rat lines selectively bred for high- and low-anxiety-related behavior. Neuropsychopharmacology 19, 381–396 (1998a)
Liebsch, G., Montkowski, A., Holsboer, F., Landgraf, R.: Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety-related behaviour. Behav Brain Res 94, 301–310 (1998b)
Lima, L., Mata, S., Urbina, M.: Allelic isoforms and decrease in serotonin transporter mRNA in lymphocytes of patients with major depression. Neuroimmunomodulation. 12, 299–306 (2005)
Lorrain, D. S., Baccei, C. S., Correa, L. D., Bristow, L. J.: Comparison of the effects of diazepam, the CRF1 antagonist CP-154,526 and the group II mGlu receptor agonist LY379268 on stress-evoked extracellular norepinephrine levels. Neuropharmacology 48, 927–935 (2005)
Lucki, I.: The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav Pharmacol 8, 523–532 (1997)
Luo, Z., Geschwind, D. H.: Microarray applications in neuroscience. Neurobiol Dis 8, 183–193 (2001)
Lyketsos, C. G., Olin, J.: Depression in Alzheimer's disease: overview and treatment. Biol. Psychiatry 52, 243–252 (2002)
Macmaster, F. P., Mirza, Y., Szeszko, P. R., Kmiecik, L. E., Easter, P. C., Taormina, S. P., Lynch, M., Rose, M., Moore, G. J., Rosenberg, D. R.: Amygdala and Hippocampal Volumes in Familial Early Onset Major Depressive Disorder. Biol. Psychiatry (2007)
Maier, W., Zobel, A., Wagner, M.: Schizophrenia and bipolar disorder: differences and overlaps. Curr. Opin. Psychiatry 19, 165–170 (2006)
Maino, K., Gruber, R., Riedel, M., Seitz, N., Schwarz, M., Muller, N.: T- and B-lymphocytes in patients with schizophrenia in acute psychotic episode and the course of the treatment. Psychiatry Res. 152, 173–180 (2007)
Malberg, J. E., Duman, R. S.: Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. Neuropsychopharmacology 28, 1562–1571 (2003)
Marcotte, E. R., Srivastava, L. K., Quirion, R.: cDNA microarray and proteomic approaches in the study of brain diseases: focus on schizophrenia and Alzheimer's disease. Pharmacol Ther 100, 63–74 (2003)

Marsteller, D. A., Barbarich-Marsteller, N. C., Patel, V. D., Dewey, S. L.: Brain metabolic changes following 4-week citalopram infusion: Increased (18)FDG uptake and gamma-amino butyric acid levels. Synapse 61, 877–881 (2007)

McDonald, C., Zanelli, J., Rabe-Hesketh, S., Ellison-Wright, I., Sham, P., Kalidindi, S., Murray, R. M., Kennedy, N.: Meta-analysis of magnetic resonance imaging brain morphometry studies in bipolar disorder. Biol. Psychiatry 56, 411–417 (2004)

McEwen, B. S.: Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. Ann N Y Acad Sci 933, 265–277 (2001)

Middleton, F. A., Mirnics, K., Pierri, J. N., Lewis, D. A., Levitt, P.: Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. J Neurosci 22, 2718–2729 (2002)

Milak, M. S., Parsey, R. V., Keilp, J., Oquendo, M. A., Malone, K. M., Mann, J. J.: Neuroanatomic correlates of psychopathologic components of major depressive disorder. Arch. Gen. Psychiatry 62, 397–408 (2005)

Millan, M. J.: The neurobiology and control of anxious states. Prog. Neurobiol 70, 83–244 (2003)

Millan, M. J.: The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. Eur J Pharmacol 500, 371–384 (2004)

Millan, M. J., Gobert, A., Bervoets, K., Rivet, J. M., Veiga, S., Brocco, M.: Induction of spontaneous tail-flicks in rats by blockade of transmission at N-methyl-D-aspartate receptors: roles of multiple monoaminergic receptors in relation to the actions of antipsychotic agents. J Pharmacol Exp Ther 292, 672–683 (2000)

Mir, K. U.: Ultrasensitive RNA profiling: counting single molecules on microarrays. Genome. Res 16, 1195–1197 (2006)

Mirnics, K., Pevsner, J.: Progress in the use of microarray technology to study the neurobiology of disease. Nat. Neurosci 7, 434–439 (2004)

Mirnics, K., Levitt, P., Lewis, D. A.: Critical appraisal of DNA microarrays in psychiatric genomics. Biol Psychiatry 60, 163–176 (2006)

Molina, V. A., Heyser, C. J., Spear, L. P.: Chronic variable stress enhances the stimulatory action of a low dose of morphine: reversal by desipramine. Eur J Pharmacol 260, 57–64 (1994)

Morey, J. S., Ryan, J. C., Van Dolah, F. M.: Microarray validation: factors influencing correlation between oligonucleotide microarrays and real-time PCR. Biol Proced. Online. 8, 175–193 (2006)

Mortensen, O. V., Thomassen, M., Larsen, M. B., Whittemore, S. R., Wiborg, O.: Functional analysis of a novel human serotonin transporter gene promoter in immortalized raphe cells. Brain Res Mol Brain Res 68, 141–148 (1999)

Mulchahey, J. J., Regmi, A., Sheriff, S., Balasubramaniam, A., Kasckow, J. W.: Coordinate and divergent regulation of corticotropin-releasing factor (CRF) and CRF-binding protein expression in an immortalized amygdalar neuronal cell line. Endocrinology 140, 251–259 (1999)

Nemeroff, C. B., Bremner, J. D., Foa, E. B., Mayberg, H. S., North, C. S., Stein, M. B.: Posttraumatic stress disorder: a state-of-the-science review. J. Psychiatr. Res. 40, 1–21 (2006)

Nestler, E. J., Gould, E., Manji, H., Buncan, M., Duman, R. S., Greshenfeld, H. K., Hen, R., Koester, S., Lederhendler, I., Meaney, M., Robbins, T., Winsky, L., Zalcman, S.: Preclinical models: status of basic research in depression. Biol Psychiatry 52, 503–528 (2002)

Newton, S. S., Collier, E. F., Hunsberger, J., Adams, D., Terwilliger, R., Selvanayagam, E., Duman, R. S.: Gene profile of electroconvulsive seizures: induction of neurotrophic and angiogenic factors. J Neurosci 23, 10841–10851 (2003)

Newton, S. S., Duman, R. S.: Neurogenic actions of atypical antipsychotic drugs and therapeutic implications. CNS. Drugs 21, 715–725 (2007)

Nofzinger, E. A., Price, J. C., Meltzer, C. C., Buysse, D. J., Villemagne, V. L., Miewald, J. M., Sembrat, R. C., Steppe, D. A., Kupfer, D. J.: Towards a neurobiology of dysfunctional arousal in depression: the relationship between beta EEG power and regional cerebral glucose metabolism during NREM sleep. Psychiatry Res. 98, 71–91 (2000)

Ohmori, T., Morita, K., Saito, T., Ohta, M., Ueno, S., Rokutan, K.: Assessment of human stress and depression by DNA microarray analysis. J Med Invest. 52 Suppl, 266–271 (2005)

Owen, M. J., Craddock, N., O'donovan, M. C.: Schizophrenia: genes at last? Trends Genet 21, 518–525 (2005)

Pariante, C. M., Dazzan, P., Danese, A., Morgan, K. D., Brudaglio, F., Morgan, C., Fearon, P., Orr, K., Hutchinson, G., Pantelis, C., Velakoulis, D., Jones, P. B., Leff, J., Murray, R. M.: Increased pituitary volume in antipsychotic-free and antipsychotic-treated patients of the AEsop first-onset psychosis study. Neuropsychopharmacology 30, 1923–1931 (2005)

Parker, G.: Classifying depression: should paradigms lost be regained? Am. J. Psychiatry 157, 1195–1203 (2000)

Parker, G., Malhi, G., Mitchell, P., Wilhelm, K., Austin, M. P., Crawford, J., Hadzi-Pavlovic, D.: Progressing a spectrum model for defining non-melancholic depression. Acta Psychiatr. Scand. 111, 139–143 (2005)

Patil, S. T., Zhang, L., Martenyi, F., Lowe, S. L., Jackson, K. A., Andreev, B. V., Avedisova, A. S., Bardenstein, L. M., Gurovich, I. Y., Morozova, M. A., Mosolov, S. N., Neznanov, N. G., Reznik, A. M., Smulevich, A. B., Tochilov, V. A., Johnson, B. G., Monn, J. A., Schoepp, D. D.: Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat. Med. (2007)

Peng, C. H., Chiou, S. H., Chen, S. J., Chou, Y. C., Ku, H. H., Cheng, C. K., Yen, C. J., Tsai, T. H., Chang, Y. L., Kao, C. L.: Neuroprotection by Imipramine against lipopolysaccharide-induced apoptosis in hippocampus-derived neural stem cells mediated by activation of BDNF and the MAPK pathway. Eur Neuropsychopharmacol. (2007)

Petit-Demouliere, B., Chenu, F., Bourin, M.: Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology. (Berl.) 177, 245–255 (2005)

Phillips, M. L., Drevets, W. C., Rauch, S. L., Lane, R.: Neurobiology of emotion perception II: Implications for major psychiatric disorders. Biol. Psychiatry 54, 515–528 (2003)

Porsolt, R. D., Le, P. M., Jalfre, M.: Depression: a new animal model sensitive to antidepressant treatments. Nature 266, 730–732 (1977)

Porsolt, R. D., Chermat, R., Lenegre, A., Avril, I., Janvier, S., Steru, L.: Use of the automated tail suspension test for the primary screening of psychotropic agents. Arch. Int Pharmacodyn. Ther 288, 11–30 (1987)

Posternak, M. A., Zimmerman, M.: Partial validation of the atypical features subtype of major depressive disorder. Arch. Gen. Psychiatry 59, 70–76 (2002)

Pruessner, J. C., Champagne, F., Meaney, M. J., Dagher, A.: Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C]raclopride. J. Neurosci. 24, 2825–2831 (2004)

Quitkin, F. M., Stewart, J. W., McGrath, P. J., Tricamo, E., Rabkin, J. G., Ocepek-Welikson, K., Nunes, E., Harrison, W., Klein, D. F.: Columbia atypical depression. A subgroup of depressives with better response to MAOI than to tricyclic antidepressants or placebo. Br. J. Psychiatry Suppl 30–34 (1993)

Ramirez, B. M., Bostic, J. Q., Davies, D., Rush, A. J., Witte, B., Hendrickse, W., Barnett, V.: Methods to improve diagnostic accuracy in a community mental health setting. Am. J. Psychiatry 157, 1599–1605 (2000)

Rasmussen, K., Beitner-Johnson, D. B., Krystal, J. H., Aghajanian, G. K., Nestler, E. J.: Opiate withdrawal and the rat locus coeruleus: behavioral, electrophysiological, and biochemical correlates. J Neurosci 10, 2308–2317 (1990)

Ray, S., Britschgi, M., Herbert, C., Takeda-Uchimura, Y., Boxer, A., Blennow, K., Friedman, L. F., Galasko, D. R., Jutel, M., Karydas, A., Kaye, J. A., Leszek, J., Miller, B. L., Minthon, L., Quinn, J. F., Rabinovici, G. D., Robinson, W. H., Sabbagh, M. N., So, Y. T., Sparks, D. L., Tabaton, M., Tinklenberg, J., Yesavage, J. A., Tibshirani, R., Wyss-Coray, T.: Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. Nat. Med (2007)

Robert, J. J., Hoffmann, R. F., Emslie, G. J., Hughes, C., Rintelmann, J., Moore, J., Armitage, R.: Sex and age differences in sleep macroarchitecture in childhood and adolescent depression. Sleep 29, 351–358 (2006)

Rocc, P., De, L. C., Eva, C., Marchiaro, L., Milani, A. M., Musso, R., Ravizza, L., Zanalda, E., Bogetto, F.: Decrease of the D4 dopamine receptor messenger RNA expression in lymphocytes from patients with major depression. Prog. Neuropsychopharmacol. Biol Psychiatry 26, 1155–1160 (2002)

Rokutan, K., Morita, K., Masuda, K., Tominaga, K., Shikishima, M., Teshima-Kondo, S., Omori, T., Sekiyama, A.: Gene expression profiling in peripheral blood leukocytes as a new approach for assessment of human stress response. J Med Invest. 52, 137–144 (2005a)

Rokutan, K., Morita, K., Masuda, K., Tominaga, K., Shikishima, M., Teshima-Kondo, S., Omori, T., Sekiyama, A.: Gene expression profiling in peripheral blood leukocytes as a new approach for assessment of human stress response. J Med Invest. 52, 137–144 (2005b)

Rozeboom, A. M., Akil, H., Seasholtz, A. F.: Mineralocorticoid receptor overexpression in forebrain decreases anxiety-like behavior and alters the stress response in mice. Proc Natl Acad Sci U S A 104, 4688–4693 (2007)

Rush, A. J., Zimmerman, M., Wisniewski, S. R., Fava, M., Hollon, S. D., Warden, D., Biggs, M. M., Shores-Wilson, K., Shelton, R. C., Luther, J. F., Thomas, B., Trivedi, M. H.: Comorbid psychiatric disorders in depressed outpatients: demographic and clinical features. J. Affect. Disord. 87, 43–55 (2005)

Sahay, A., Hen, R.: Adult hippocampal neurogenesis in depression. Nat. Neurosci 10, 1110–1115 (2007)

Salchner, P., Singewald, N.: Neuroanatomical substrates involved in the anxiogenic-like effect of acute fluoxetine treatment. Neuropharmacology 43, 1238–1248 (2002)

Salchner, P., Sartori, S. B., Sinner, C., Wigger, A., Frank, E., Landgraf, R., Singewald, N.: Airjet and FG-7142-induced Fos expression differs in rats selectively bred for high and low anxiety-related behavior. Neuropharmacology 50, 1048–1058 (2006)

Schena, M., Shalon, D., Davis, R. W., Brown, P. O.: Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270, 467–470 (1995)

Schena, M., Shalon, D., Heller, R., Chai, A., Brown, P. O., Davis, R. W.: Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. Proc Natl Acad Sci U S A 93, 10614–10619 (1996)

Scheuch, K., Lautenschlager, M., Grohmann, M., Stahlberg, S., Kirchheiner, J., Zill, P., Heinz, A., Walther, D. J., Priller, J.: Characterization of a Functional Promoter Polymorphism of the Human Tryptophan Hydroxylase 2 Gene in Serotonergic Raphe Neurons. Biol Psychiatry (2007)

Schmidt, H. D., Duman, R. S.: The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. Behav Pharmacol 18, 391–418 (2007)

Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., Kiebler, M., Greenberg, M. E.: A brain-specific microRNA regulates dendritic spine development. Nature 439, 283–289 (2006)

Schulteis, G., Yackey, M., Risbrough, V., Koob, G. F.: Anxiogenic-like effects of spontaneous and naloxone-precipitated opiate withdrawal in the elevated plus-maze. Pharmacol Biochem Behav 60, 727–731 (1998)

Searfoss, G. H., Ryan, T. P., Jolly, R. A.: The role of transcriptome analysis in pre-clinical toxicology. Curr. Mol Med 5, 53–64 (2005)

Segman, R. H., Shefi, N., Goltser-Dubner, T., Friedman, N., Kaminski, N., Shalev, A. Y.: Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. Mol. Psychiatry 10, 500–13, 425 (2005)

Selemon, L. D., Goldman-Rakic, P. S.: The reduced neuropil hypothesis: a circuit based model of schizophrenia. Biol Psychiatry 45, 17–25 (1999)

Seligman, M. E., Weiss, J., Weinraub, M., Schulman, A.: Coping behavior: learned helplessness, physiological change and learned inactivity. Behav Res Ther 18, 459–512 (1980)

Sheline, Y. I., Barch, D. M., Donnelly, J. M., Ollinger, J. M., Snyder, A. Z., Mintun, M. A.: Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. Biol. Psychiatry 50, 651–658 (2001)

Shelton, R. C., Liang, S., Liang, P., Chakrabarti, A., Manier, D. H., Sulser, F.: Differential expression of pentraxin 3 in fibroblasts from patients with major depression. Neuropsychopharmacology 29, 126–132 (2004)

Shi, L., Reid, L. H., Jones, W. D., Shippy, R., Warrington, J. A., Baker, S. C., Collins, P. J., de, L. F., Kawasaki, E. S., Lee, K. Y., Luo, Y., Sun, Y. A., Willey, J. C., Setterquist, R. A., Fischer, G. M., Tong, W., Dragan, Y. P., Dix, D. J., Frueh, F. W., Goodsaid, F. M., Herman, D., Jensen, R. V., Johnson, C. D., Lobenhofer, E. K., Puri, R. K., Schrf, U., Thierry-Mieg, J., Wang, C., Wilson, M., Wolber, P. K., Zhang, L., Amur, S., Bao, W., Barbacioru, C. C., Lucas, A. B., Bertholet, V., Boysen, C., Bromley, B., Brown, D., Brunner, A., Canales, R., Cao, X. M., Cebula, T. A., Chen, J. J., Cheng, J., Chu, T. M., Chudin, E., Corson, J., Corton, J. C., Croner, L. J., Davies, C., Davison, T. S., Delenstarr, G., Deng, X., Dorris, D., Eklund, A. C., Fan, X. H., Fang, H., Fulmer-Smentek, S., Fuscoe, J. C., Gallagher, K., Ge, W., Guo, L., Guo, X., Hager, J., Haje, P. K., Han, J., Han, T., Harbottle, H. C., Harris, S. C., Hatchwell, E., Hauser, C. A., Hester, S., Hong, H., Hurban, P., Jackson, S. A., Ji, H., Knight, C. R., Kuo, W. P., LeClerc, J. E., Levy, S., Li, Q. Z., Liu, C., Liu, Y., Lombardi, M. J., Ma, Y., Magnuson, S. R., Maqsodi, B., McDaniel, T., Mei, N., Myklebost, O., Ning, B., Novoradovskaya, N., Orr, M. S., Osborn, T. W., Papallo, A., Patterson, T. A., Perkins, R. G., Peters, E. H., Peterson, R., Philips, K. L., Pine, P. S., Pusztai, L., Qian, F., Ren, H., Rosen, M., Rosenzweig, B. A., Samaha, R. R., Schena, M., Schroth, G. P., Shchegrova, S., Smith, D. D., Staedtler, F., Su, Z., Sun, H., Szallasi, Z., Tezak, Z., Thierry-Mieg, D., Thompson, K. L., Tikhonova, I., Turpaz, Y., Vallanat, B., Van, C., Walker, S. J., Wang, S. J., Wang, Y., Wolfinger, R., Wong, A., Wu, J., Xiao, C., Xie, Q., Xu, J., Yang, W., Zhang, L., Zhong, S., Zong, Y., Slikker, W., Jr.: The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat. Biotechnol. 24, 1151–1161 (2006a)

Sibille, E., Arango, V., Galfalvy, H. C., Pavlidis, P., Erraji-Benchekroun, L., Ellis, S. P., John, M. J.: Gene expression profiling of depression and suicide in human prefrontal cortex. Neuropsychopharmacology 29, 351–361 (2004)

Smagin, G. N., Harris, R. B., Ryan, D. H.: Corticotropin-releasing factor receptor antagonist infused into the locus coeruleus attenuates immobilization stress-induced defensive withdrawal in rats. Neurosci Lett. 220, 167–170 (1996)

Souery, D., Oswald, P., Massat, I., Bailer, U., Bollen, J., Demyttenaere, K., Kasper, S., Lecrubier, Y., Montgomery, S., Serretti, A., Zohar, J., Mendlewicz, J.: Clinical factors associated with treatment resistance in major depressive disorder: results from a European multicenter study. J. Clin. Psychiatry 68, 1062–1070 (2007)

Sousa, N., Cerqueira, J. J., Almeida, O. F.: Corticosteroid receptors and neuroplasticity. Brain Res Rev (2007)

Stafford, P., Brun, M.: Three methods for optimization of cross-laboratory and cross-platform microarray expression data. Nucleic. Acids Res 35, e72 (2007)

Stahl, S. M., Woo, D. J., Mefford, I. N., Berger, P. A., Ciaranello, R. D.: Hyperserotonemia and platelet serotonin uptake and release in schizophrenia and affective disorders. Am. J. Psychiatry 140, 26–30 (1983)

Stewart, J. W., McGrath, P. J., Quitkin, F. M., Rabkin, J. G., Harrison, W., Wager, S., Nunes, E., Ocepek-Welikson, K., Tricamo, E.: Chronic depression: response to placebo, imipramine, and phenelzine. J. Clin. Psychopharmacol. 13, 391–396 (1993)

Sullivan, P. F., Fan, C., Perou, C. M.: Evaluating the comparability of gene expression in blood and brain. Am. J. Med. Genet. B Neuropsychiatr. Genet. 141, 261–268 (2006)

Sun, W., Park, K. W., Choe, J., Rhyu, I. J., Kim, I. H., Park, S. K., Choi, B., Choi, S. H., Park, S. H., Kim, H.: Identification of novel electroconvulsive shock-induced and activity-dependent genes in the rat brain. Biochem Biophys. Res Commun. 327, 848–856 (2005)

Suri, C., Fung, B. P., Tischler, A. S., Chikaraishi, D. M.: Catecholaminergic cell lines from the brain and adrenal glands of tyrosine hydroxylase-SV40 T antigen transgenic mice. J Neurosci 13, 1280–1291 (1993)

Suzuki, Y., Sawamura, K., Someya, T.: Polymorphisms in the 5-hydroxytryptamine 2A receptor and CytochromeP4502D6 genes synergistically predict fluvoxamine-induced side effects in japanese depressed patients. Neuropsychopharmacology 31, 825–831 (2006)

Swanson, C. J., Perry, K. W., Schoepp, D. D.: The mGlu2/3 receptor agonist, LY354740, blocks immobilization-induced increases in noradrenaline and dopamine release in the rat medial prefrontal cortex. J Neurochem 88, 194–202 (2004)

Swanson, C. J., Blackburn, T. P., Zhang, X., Zheng, K., Xu, Z. Q., Hokfelt, T., Wolinsky, T. D., Konkel, M. J., Chen, H., Zhong, H., Walker, M. W., Craig, D. A., Gerald, C. P., Branchek, T. A.: Anxiolytic- and antidepressant-like profiles of the galanin-3 receptor (Gal3) antagonists SNAP 37889 and SNAP 398299. Proc Natl Acad Sci U S A 102, 17489–17494 (2005a)

Swanson, C. J., Bures, M., Johnson, M. P., Linden, A. M., Monn, J. A., Schoepp, D. D.: Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nat. Rev Drug Discov. 4, 131–144 (2005b)

Tanna, V. L., Wilson, A. F., Winokur, G., Elston, R. C.: Linkage analysis of pure depressive disease. J Psychiatr. Res 23, 99–107 (1989)

Tanner, M. A., Berk, L. S., Felten, D. L., Blidy, A. D., Bit, S. L., Ruff, D. W.: Substantial changes in gene expression level due to the storage temperature and storage duration of human whole blood. Clin Lab. Haematol. 24, 337–341 (2002)

Taylor, D. J., Lichstein, K. L., Durrence, H. H., Reidel, B. W., Bush, A. J.: Epidemiology of insomnia, depression, and anxiety. Sleep 28, 1457–1464 (2005)

Taylor, M. A., Fink, M.: Restoring melancholia in the classification of mood disorders. J. Affect. Disord. (2007)

Thiel, G., Cibelli, G.: Corticotropin-releasing factor and vasoactive intestinal polypeptide activate gene transcription through the cAMP signaling pathway in a catecholaminergic immortalized neuron. Neurochem Int 34, 183–191 (1999)

Toro, C. T., Deakin, J. F.: Adult neurogenesis and schizophrenia: a window on abnormal early brain development? Schizophr. Res 90, 1–14 (2007)

Tsao, C. W., Lin, Y. S., Chen, C. C., Bai, C. H., Wu, S. R.: Cytokines and serotonin transporter in patients with major depression. Prog. Neuropsychopharmacol. Biol Psychiatry 30, 899–905 (2006)

Tsuang, M. T., Nossova, N., Yager, T., Tsuang, M. M., Guo, S. C., Shyu, K. G., Glatt, S. J., Liew, C. C.: Assessing the validity of blood-based gene expression profiles for the classification of schizophrenia and bipolar disorder: a preliminary report. Am. J Med Genet B Neuropsychiatr. Genet 133, 1–5 (2005)

Ungerstedt, U.: Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol Scand. Suppl. 367, 1–48 (1971)

Vandergriff, J., Rasmussen, K.: The selective mGlu2/3 receptor agonist LY354740 attenuates morphine-withdrawal-induced activation of locus coeruleus neurons and behavioral signs of morphine withdrawal. Neuropharmacology 38, 217–222 (1999)

Vandesompele, J., De, P. K., Pattyn, F., Poppe, B., Van, R. N., De, P. A., Speleman, F.: Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome. Biol 3, RESEARCH0034 (2002)

Van Praag, H. M.: On what evidence should education concerning depression be based? Int Clin Psychopharmacol. 14, Suppl 3, S35–S39 (1999)

Vawter, M. P., Ferran, E., Galke, B., Cooper, K., Bunney, W. E., Byerley, W.: Microarray screening of lymphocyte gene expression differences in a multiplex schizophrenia pedigree. Schizophr. Res 67, 41–52 (2004a)

Vawter, M. P., Shannon, W. C., Ferran, E., Matsumoto, M., Overman, K., Hyde, T. M., Weinberger, D. R., Bunney, W. E., Kleinman, J. E.: Gene expression of metabolic enzymes and a protease inhibitor in the prefrontal cortex are decreased in schizophrenia. Neurochem Res 29, 1245–1255 (2004b)

Velculescu, V. E., Zhang, L., Vogelstein, B., Kinzler, K. W.: Serial analysis of gene expression. Science 270, 484–487 (1995)

Vermetten, E., Vythilingam, M., Southwick, S. M., Charney, D. S., Bremner, J. D.: Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. Biol. Psychiatry 54, 693–702 (2003)

Vgontzas, A. N., Bixler, E. O., Chrousos, G. P.: Obesity-related sleepiness and fatigue: the role of the stress system and cytokines. Ann. N. Y. Acad. Sci. 1083, 329–344 (2006)

Vogel, M., Pfeifer, S., Schaub, R. T., Grabe, H. J., Barnow, S., Freyberger, H. J., Cascorbi, I.: Decreased levels of dopamine D3 receptor mRNA in schizophrenic and bipolar patients. Neuropsychobiology 50, 305–310 (2004)

Wagner, J. A., Williams, S. A., Webster, C. J.: Biomarkers and surrogate end points for fit-for-purpose development and regulatory evaluation of new drugs. Clin. Pharmacol. Ther. 81, 104–107 (2007)

Walker, E. F., Walder, D. J., Reynolds, F.: Developmental changes in cortisol secretion in normal and at-risk youth. Dev. Psychopathol. 13, 721–732 (2001)

Walsh, B. T., Seidman, S. N., Sysko, R., Gould, M.: Placebo response in studies of major depression: variable, substantial, and growing. JAMA 287, 1840–1847 (2002)

Walsh, J. K., Deacon, S., Dijk, D. J., Lundahl, J.: The selective extrasynaptic GABAA agonist, gaboxadol, improves traditional hypnotic efficacy measures and enhances slow wave activity in a model of transient insomnia. Sleep 30, 593–602 (2007)

Wang, H. D., Dunnavant, F. D., Jarman, T., Deutch, A. Y.: Effects of antipsychotic drugs on neurogenesis in the forebrain of the adult rat. Neuropsychopharmacology 29, 1230–1238 (2004)

Wang, Y., Barbacioru, C., Hyland, F., Xiao, W., Hunkapiller, K. L., Blake, J., Chan, F., Gonzalez, C., Zhang, L., Samaha, R. R.: Large scale real-time PCR validation on gene expression measurements from two commercial long-oligonucleotide microarrays. BMC Genomics 7, 59 (2006)

Warner-Schmidt, J. L., Duman, R. S.: VEGF is an essential mediator of the neurogenic and behavioral actions of antidepressants. Proc Natl Acad Sci U S A 104, 4647–4652 (2007)

Weintraub, D., Stern, M. B.: Psychiatric complications in Parkinson disease. Am. J. Geriatr. Psychiatry 13, 844–851 (2005)

West, A. P.: Neurobehavioral studies of forced swimming: the role of learning and memory in the forced swim test. Prog. Neuropsychopharmacol. Biol Psychiatry 14, 863–877 (1990)

Wichers, M. C., Koek, G. H., Robaeys, G., Verkerk, R., Scharpe, S., Maes, M.: IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. Mol. Psychiatry 10, 538–544 (2005)

Willner, P.: Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology. (Berl.) 134, 319–329 (1997)

Wong, D. L., Tank, A. W.: Stress-induced catecholaminergic function: transcriptional and post-transcriptional control. Stress 10, 121–130 (2007)

Xu, J. F., Chen, X. Q., Du, J. Z., Wang, T. Y.: CRF receptor type 1 mediates continual hypoxia-induced CRF peptide and CRF mRNA expression increase in hypothalamic PVN of rats. Peptides. 26, 639–646 (2005)

Zetzsche, T., Frodl, T., Preuss, U. W., Schmitt, G., Seifert, D., Leinsinger, G., Born, C., Reiser, M., Moller, H. J., Meisenzahl, E. M.: Amygdala volume and depressive symptoms in patients with borderline personality disorder. Biol. Psychiatry 60, 302–310 (2006)

Zhu, C. B., Blakely, R. D., Hewlett, W. A.: The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. Neuropsychopharmacology 31, 2121–2131 (2006)

Zieker, J., Zieker, D., Jatzko, A., Dietzsch, J., Nieselt, K., Schmitt, A., Bertsch, T., Fassbender, K., Spanagel, R., Northoff, H., Gebicke-Haerter, P. J.: Differential gene expression in peripheral blood of patients suffering from post-traumatic stress disorder. Mol Psychiatry 12, 116–118 (2007)

Zimmerman, M., Chelminski, I., Posternak, M. A.: Generalizability of antidepressant efficacy trials: differences between depressed psychiatric outpatients who would or would not qualify for an efficacy trial. Am. J. Psychiatry 162, 1370–1372 (2005)

Zusso, M., Debetto, P., Guidolin, D., Giusti, P.: Cerebellar granular cell cultures as an *in vitro* model for antidepressant drug-induced neurogenesis. Crit. Rev Neurobiol 16, 59–65 (2004)