Chapter 11

Functional MRI: Applications in Cognitive Neuroscience

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Abstract

Neuroimaging, in many respects, revolutionized the study of cognitive neuroscience, the discipline that attempts to determine the neural mechanisms underlying cognitive processes. Early studies of brain–behavior relationships relied on a precise neurological exam as the basis for hypothesizing the site of brain damage that was responsible for a given behavioral syndrome. The advent of structural brain imaging, first with computerized tomography and later with magnetic resonance imaging, paved the way for more precise anatomical localization of the cognitive deficits that manifest after brain injury. Functional neuroimaging, broadly defined as techniques that provide measures of brain activity, further increased our ability to study the neural basis of behavior. Functional MRI (fMRI), in particular, is an extremely powerful technique that affords excellent spatial and temporal resolution. This chapter focuses on the principles underlying fMRI as a cognitive neuroscience tool for exploring brain–behavior relationships.

Key words Functional MRI, Cognitive neuroscience, Experimental design, Statistics

1 Introduction

Cognitive neuroscience is a discipline that attempts to determine the neural mechanisms underlying cognitive processes. Specifically, cognitive neuroscientists test hypotheses about brain–behavior relationships that can be organized along two conceptual domains: *functional specialization*—the idea that functional modules exist within the brain, that is, areas of the cerebral cortex that are specialized for a specific cognitive process, and *functional integration*—the idea that a cognitive process can be an emergent property of interactions among a network of brain regions, which suggests that a brain region can play a different role across many functions.

Early investigations of brain–behavior relationships consisted of careful observation of individuals with neurological injury resulting in focal brain damage. The idea of functional specialization evolved from hypotheses that damage to a particular brain region was responsible for a given behavioral syndrome that was characterized by a precise neurological examination. For instance,

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the association of aphasia with right-sided limb weakness implicated the left hemisphere as the site of language abilities. Moreover, upon the death of a patient with a neurological disorder, clinicopathological correlations provided confirmatory information about the site of damage causing a specific neurobehavioral syndrome such as aphasia. For example, in 1861 Paul Broca's observations of nonfluent aphasia in the setting of a damaged left inferior frontal gyrus (IFG) cemented the belief that this brain region was critical for speech output [1]. The introduction of structural brain imaging more than 100 years after Broca's observations, first with computerized tomography (CT) and later with magnetic resonance imaging (MRI), paved the way for more precise anatomical localization in the living patient of the cognitive deficits that develop after brain injury. The superb spatial resolution of structural neuroimaging has reduced the reliance on the infrequently obtained autopsy for making brain–behavior correlations.

Functional neuroimaging, broadly defined as techniques that measure brain activity, expanded our ability to study the neural basis of cognitive processes. One such method, fMRI is as an extremely powerful technique that affords excellent spatial and temporal resolution. Measuring regional brain activity in healthy subjects while they perform cognitive tasks links localized brain activity with specific behaviors. For example, functional neuroimaging studies have demonstrated that the left IFG is consistently activated during the performance of speech production tasks in healthy individuals [2]. Such findings from functional neuroimaging are complementary to findings derived from observations of patients with focal brain damage. This chapter focuses on the principles underlying fMRI as a cognitive neuroscience tool for exploring brain–behavior relationships.

2 Inference in Functional Neuroimaging Studies of Cognitive Processes

Insight regarding the link between brain and behavior can be gained through a variety of approaches. It is unlikely that any single neuroscience method is sufficient to fully investigate any particular question regarding the mechanisms underlying cognitive function. From a methodological point of view, each method will offer different temporal and spatial resolution. From a conceptual point of view, each method will provide data that will support different types of inferences that can be drawn from it. Thus, data obtained addressing a single question but derived from multiple methods can provide more comprehensive and inferentially sound conclusions.

Functional neuroimaging studies support inferences about the association of a particular brain system with a cognitive process. However, it is difficult to prove in such a study that the observed activity is necessary for an isolated cognitive process because perfect

control over a subject's cognitive processes during a functional neuroimaging experiment is never possible. Even if the task performed by a subject is well designed, it is difficult to demonstrate conclusively that he or she is differentially engaging a single, identified cognitive process. The subject may engage in unwanted cognitive processes that either have no overt, measurable effects or are perfectly confounded with the process of interest. Consequently, the neural activity measured by the functional neuroimaging technique may result from some confounding neural computation that is itself not necessary for executing the cognitive process seemingly under study. In other words, functional neuroimaging is an observational, correlative method [3]. It is important to note that the inferences that can be drawn from functional neuroimaging studies such as fMRI apply to all methods of physiological measurement (e.g., electroencephalography, EEG, or magnetoencephalography, MEG).

The inference of necessity cannot be made without showing that a focal brain lesion disrupts the cognitive process in question. However, unlike precise surgical or neurotoxic lesions in animal models, lesions in patients are often extensive, damaging local neurons and "fibers of passage." For example, damage to prominent white matter tracts can cause cognitive deficits similar to those produced by cortical lesions, such as the amnesia resulting from lesions of the fornix, the main white matter pathway projecting from the hippocampus [4]. In addition, connections from region "A" may support the continued metabolic function of region "B," but region A may not be computationally involved in certain processes undertaken by region B. Thus, damage to region A could impair the function of region B via two possible mechanisms: (1) diaschisis [5, 6] and (2) retrograde trans-synaptic degeneration. Consequently, studies of patients with focal lesions cannot conclusively demonstrate that the neurons within a specific region are themselves critical to the computational support of an impaired cognitive process.

Empirical studies using lesion and electrophysiological methods demonstrate these issues regarding the types of inferences that can be logically drawn from them. For example, in monkeys, single-unit recording reveals neurons in the lateral prefrontal cortex (PFC) that increase their firing during the delay between the presentation of information to be remembered and a few seconds later when that information must be recalled [7, 8]. These studies are taken as evidence that persistent neural activity in the PFC is involved in temporary storage of information, a cognitive process known as working memory. The necessity of PFC for working memory was demonstrated in other monkey studies showing that PFC lesions impair performance on working memory tasks, but not on tasks that do not require temporarily holding information in memory [9]. Persistent neural activity during working memory tasks is also found in the hippocampus [10, 11]. Hippocampal lesions, however, do not impair performance on most working

memory tasks [12], which suggests that the hippocampus is *involved* in maintaining information over short periods of time, but is not *necessary* for this cognitive operation. Observations in humans support this notion. For example, the well-studied patient H.M., with complete bilateral hippocampal damage and the severe inability to learn new information, could nevertheless perform normally on working memory tasks such as digit span [13]. The hippocampus is implicated in long-term memory especially when relations between multiple items and multiple features of a complex, novel item must be retained. Thus, the hippocampus may only be engaged during working memory tasks that require someone to subsequently remember novel information [14].

When the results from lesion and functional neuroimaging studies are combined, a stronger level of inference emerges [15]. As in the examples of Broca's aphasia or working memory, a lesion of a specific brain region causes impairment of a given cognitive process and when engaged by an intact individual, that cognitive process evokes neural activity in the same brain region. Given these findings, the inference that this brain region is computationally necessary for the cognitive process is stronger than the data derived from each study performed in isolation. Thus, lesion and functional neuroimaging studies are complementary, each providing inferential support that the other lacks.

Other types of inferential failure can occur in the interpretation of functional neuroimaging studies when other common assumptions do not hold true. First, it is assumed that if a cognitive process activates a particular brain region (evoked by a particular task), the neural activity in that brain region must depend on engaging that particular cognitive process. For example, a brain region showing greater activation during the presentation of faces than to other types of stimuli, such as photographs of cars or buildings, is considered to engage face perception processes. However, this region may also support other higher-level cognitive processes such as memory processes, in addition to lower level perceptual processes [16]. See ref. [17] for a further discussion of this issue.

The opposite type of inference is made when it is assumed that if a particular brain region is activated during the performance of a cognitive task, the subject must have engaged the cognitive process supported by that region during the task (referred to as a "reverse inference"). For example, when activation of the frontal lobes was observed during a mental rotation task, it was proposed that subjects engaged working memory processes to recall the identity of the rotated target [18]. (They derived this assumption from other imaging studies showing activation of the frontal lobes during working memory tasks.) However, in this example, because some other cognitive process supported by the frontal lobes could have activated this region [19], one cannot be sure that working memory was engaged

leading to the activation of the frontal lobes. Unfortunately, this potentially faulty logic is a fairly common practice in fMRI studies. See ref. [20] for a further discussion of this issue.

In summary, interpretation of the results of functional neuroimaging studies attempting to link brain and behavior rests on numerous assumptions. Familiarity with the types of inferences that can and cannot be drawn from these studies is helpful for assessing the validity of the findings reported by such studies.

3 Functional MRI as a Cognitive Neuroscience Tool

Functional MRI has become the predominant functional neuroimaging method for studying the neural basis of cognitive processes in humans. Compared to its predecessor, positron emission tomography (PET) scanning, fMRI offers many advantages. For example, MRI scanners are much more widely available, and imaging costs are less expensive since MRI does not require a cyclotron to produce radioisotopes. MRI is also a noninvasive procedure since there is no requirement for injection of a radioisotope into the bloodstream. Also, given the half-life of available radioisotopes, PET scanning is unable to provide comparable temporal resolution to that of fMRI which can provide images of behavioral events occurring on the order of seconds rather than the summation of many behavioral events over tens of seconds.

In selected circumstances, however, PET scanning can provide an advantage over fMRI for studying certain questions concerning the neural basis of cognition. For example, a particular advantage of PET scanning in the study of cognition that can nicely complement fMRI studies is its ability to assess neurochemical (neurotransmitter and neuromodulator) systems. Radioactively labeled ligands may be used to directly measure density and distribution of particular receptors and even receptor subtypes, distribution of presynaptic terminals or enzymes involved in the production or breakdown of particular neurochemicals [21]. For example, one study measured dopamine synthesis capacity in the striatum with PET and used fMRI to measure brain activity during a working memory task. It was found that activity in frontal cortex during the working memory task was related to caudate dopamine levels as well as task accuracy. Thus, combining PET and fMRI data in this unique way allowed the investigators to test a question regarding the neurochemical basis of cognition [22].

The MRI scanner, compared to a behavioral testing room, is less than ideal for performing most cognitive neuroscience experiments. Experiments are performed in the awkward position of lying on one's back, often requiring subjects to visualize the presentation of stimuli through a mirror, in an acoustically noisy environment. Moreover, most individuals develop some degree of claustrophobia

due to the small bore of the MRI scanner and find it difficult to remain completely motionless for a long duration of time that is required for most experiments (e.g., usually 60–90 min). These constraints of the MRI scanner make it especially difficult to scan children or certain patient populations (e.g., Parkinson's disease patients), which has resulted in many fewer fMRI studies involving children than adults and neurological patients in general. However, mock scanners have been built in many imaging centers, with motion devices that acclimate children to the scanner environment before they participate in an fMRI study. This approach has led to an increasing number of fMRI studies of children being reported in the literature that are providing tremendous insight regarding the mechanisms underlying the developing brain (*for review*, *see* [23]).

All sensory systems have been investigated with fMRI including the visual, auditory, somatosensory, olfactory, and gustatory systems. Each system requires different technologies for successful presentation of relevant stimuli within an MRI environment. At the time of this writing, there are now many off-the-shelf commercial products that exist that are MRI-compatible. Acquiring ancillary electrophysiological data such as electromyographic recordings to measure muscle contraction or electrodermal responses to measure autonomic activity enhances many cognitive neuroscience experiments. Devices have been developed that are MR compatible for these types of measurements as well as other physiological measures such as heart rate, electrocardiography, oxygen saturation, and respiratory rate. The recording of eye movements is commonplace in MRI scanners predominantly with the use of infrared video cameras equipped with long range optics. Video images of the pupil–corneal reflection can be sampled at 500–1000 Hz allowing for the accurate (<0.5°) localization of gaze within 50 horizontal and 40 vertical degrees of visual angle.

EEG recordings have also been successfully performed during MRI scanning [24, 25]. Both measures of event-related potentials (ERPs) and spectral EEG power in specific frequency bands and have been successfully recorded and related to variations in underlying BOLD activity and behavior [26–29]. However, the recording of low amplitude EEG events, such as ERPs and transient changes in spectral EEG power, can be more difficult in a magnetic field due to large artifacts induced by gradient switching and head movement and voltage changes from cardiac pulsation. The optimization of data acquisition methods and post-processing algorithms to remove artifacts have allowed for reliable measurements of ERPs and transient EEG events during fMRI scanning [30–33]. In summary, most challenges facing cognitive experiments and the study of spontaneous activity within the MRI environment have been overcome, creating an environment that is comparable to standard psychophysical testing labs outside of a scanner. Recent work has focused on minimizing exacerbated EEG

artifacts present during high-field MRI scanning [34]. Although individual laboratories have achieved most of these advancements, MRI scanners originally designed for clinical use by manufacturers are now being designed with consideration of many of these research-related issues.

Another promising technique is the delivery of transcranial magnetic stimulation (TMS) during MRI scanning [35, 36]. TMS induces depolarization of neurons under the coil and, when combined with functional MRI, can be used to reveal patterns of remote connectivity, such as between the frontal eye field (FEF) and early visual cortex [36], the lateral prefrontal cortex and face- and house-selective regions in temporal cortex [35], and within and between large-scale brain networks [37]. There are many challenges in combining TMS and MRI such as the need for a large MRI head coil to accommodate the presence of the TMS coil, the difficulty of precise localization [38], and the increased subject discomfort. However, perhaps the largest challenge of delivering TMS in a manner that does not lead to artifacts in the MRI signal has been largely overcome by new commercially available TMS coils

3.1 Temporal Resolution

Two types of temporal resolution need to be considered for cognitive neuroscience experiments. First, what is the briefest neural event that can be detected as an fMRI signal? Second, how close together can two neural events occur and be resolved as separable fMRI signals?

The time scale on which neural changes occur is quite rapid. For example, neural activity in the lateral intraparietal area of monkeys increases within 100 ms of the visual presentation of a saccade target [39]. In contrast, the fMRI signal gradually increases to its peak magnitude within 4–6 s after an experimentally induced brief (<1 s) change in neural activity, and then decays back to baseline after several more seconds [40–42]. This slow time course of fMRI signal change in response to such a brief increase in neural activity is informally referred to as the blood oxygen level-dependent (BOLD) fMRI hemodynamic response or simply, the hemodynamic response (Fig. 1). Thus, neural dynamics and neurally evoked hemodynamics, as measured with fMRI, are on quite different time scales.

The sluggishness of the hemodynamic response limits the temporal resolution of the fMRI signal to hundreds of milliseconds to seconds as opposed to the millisecond temporal resolution of electrophysiological recordings of neural activity, such as from single-unit recording in monkeys and EEG or MEG in humans. However, it has been clearly demonstrated that brief changes in neural activity can be detected with reasonable statistical power using fMRI. For example, appreciable fMRI signal can be observed in sensorimotor cortex in association with single finger movements [43] and in visual cortex during very briefly presented (34 ms) visual stimuli [44]. In contrast, the temporal resolution of fMRI

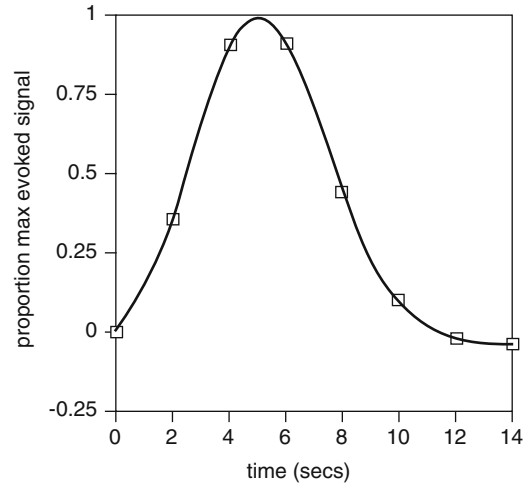

Fig. 1 A typical hemodynamic response (i.e., fMRI signal change in response to a brief increase of neural activity) from the primary sensorimotor cortex. The fMRI signal peaked approximately 5 s after the onset of the motor response (at time zero)

limits the detection of sequential changes in neural activity that occur rapidly with respect to the hemodynamic response. That is, the ability to resolve the changes in the fMRI signal associated with two neural events often requires the separation of those events by a relatively long period of time compared with the width of the hemodynamic response. This is because two neural events closely spaced in time will produce a hemodynamic response that reflects the accumulation from both neural events, making it difficult to estimate the contribution of each individual neural event. In general, evoked fMRI responses to discrete neural events separated by at least 4 s appear to be within the range of resolution [45]. However, provided that the stimuli are presented randomly, significant differential functional responses between two events (e.g., flashing visual stimuli) spaced as closely as 500 ms apart can be detected [46–48]. The effect of fixed and randomized intertrial intervals on the BOLD signal is illustrated in Fig. 2.

In some tasks, the order of individual trial events cannot be randomized. For example, in certain types of working memory tasks, the presentation of the information to be remembered during the delay period, and the period when the subject must recall the information, are individual trial events whose order cannot be randomized. In these types of tasks, short time scales (<4 s) cannot be temporally resolved. These temporal resolution issues in fMRI have been extensively considered regarding their impact on experimental design [49, 50].

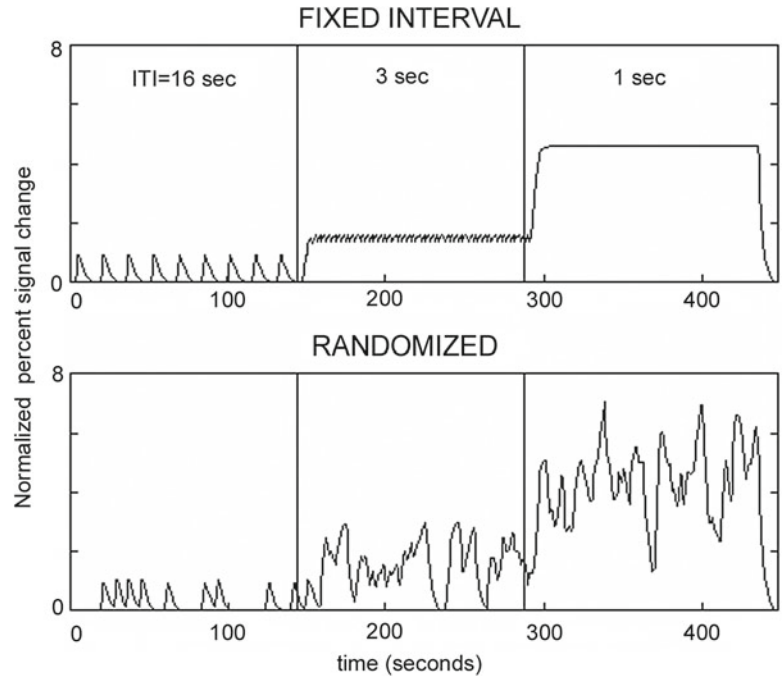

Fig. 2 Effect of fixed vs. randomized intertrial intervals on the blood oxygen level-dependent (BOLD) fMRI signal [46]

3.2 Spatial Resolution

As approaches are sought that maximize both BOLD signal strength and in-plane resolution, fMRI studies in humans have recently been extended to higher magnetic field strengths (7.0 T and 9.4 T) [51–53]. Such studies have the power to potentially evaluate much finer cortical details, such as the representation of individual fingertips within primary somatosensory cortex [51]. However, as the field strength increases, factors that are less consequential at 3.0 T—including magnetic field inhomogeneities [52] and the contribution of macrovascular structures to the typical gradient-echo signal [53]—become significantly more problematic, requiring further innovations in pulse sequence development. Single-echo gradient-echo sequences using echo times (TE) that exceed the repetition time (TR), for example, take advantage of reduced distortion relative to single-shot gradient-echo sequences, while also avoiding the prolonged acquisition times of typical single-echo sequences. During functional MRI of a simple finger tapping task at 9.4 T using such a sequence, researchers were able to obtain 0.4×0.4 mm in-plane resolution within presumptive primary motor cortex [53]. Similarly, spin-echo sequences, which have a reduced signal-to-noise ratio relative to gradient-echo sequences but greater spatial specificity, become feasible for use at 9.4 T. Within a finger-tapping paradigm, a study taking this approach reduced the influence of macrovascular contributions to

the BOLD signal relative to a gradient-echo sequence, while obtaining 1 mm isotropic resolution. As such techniques are validated and extended, they may someday allow for imaging of thousands of neurons per voxel, as opposed to the hundreds of thousands of neurons per voxel currently more typical for a human cognitive neuroscience fMRI experiment.

Virtually all fMRI studies model the large BOLD signal increase, which is due to a local low-deoxyhemoglobin state, in order to detect changes correlating with a behavioral task. However, optical imaging studies have demonstrated that preceding this large positive response there is an initial negative response reflecting a localized increase in oxygen consumption causing a high-deoxyhemoglobin state [54]. This early hemodynamic response is called the "initial dip" and is thought to be more tightly coupled to the actual site of neural activity evoking the BOLD signal as compared to the later positive portion of the BOLD response. For example, Kim et al., scanning cats in a high field scanner, demonstrated that the early negative BOLD response (e.g., initial dip) produced activation maps that were consistent with orientation columns within visual cortex. This finding is quite remarkable given that the average spacing between two adjacent orientation columns in cortex is approximately 1 mm. In contrast, the activation maps produced by the delayed positive BOLD response appeared more diffuse and cortical columnar organization could not be identified [55]. Thus, empirical evidence suggests that deriving activation maps by correlating behavioral responses with the initial dip may markedly improve spatial resolution.

Another unique method for improving spatial resolution has been called functional magnetic resonance-adaptation (fMR-A), which could provide a means for identifying and assessing the functional attributes of sharply defined neuronal populations within a given region of the brain [56]. Even if the spatial resolution of fMRI evolves to the point of being able to resolve a population of a few hundred neurons within a voxel, it is still likely that this small population will contain neurons with very different functional properties that will be averaged together. The adaptation method is based on several basic principles. First, repeated presentation of the same type of stimuli (i.e., a picture of the one object) causes neurons to adapt to those stimuli (i.e., neuronal firing is reduced). Second, if these neurons are then exposed to a different type of stimulus (i.e., a picture of another object) or a change in some property of the stimulus (i.e., the same object in a different orientation), then recovery from adaptation can be assessed (i.e., whether or not the BOLD signal returns to its original state). If the signal remains adapted it implies that the neurons are invariant to the attribute that was changed or if the signal recovers from the adapted state it would imply that the neurons are sensitive to that attribute. For example, Grill-Spector et al. demonstrated that an area of lateral occipital cortex thought to

be important for object recognition was less sensitive to changes in object size and position as compared to changes in illumination and viewpoint [57]. Thus, with this method it is possible to investigate the functional properties of neuronal populations with a level of spatial resolution that is beyond that obtained from conventional fMRI data analysis methods.

Considering all the neuroscientific methods available today for studying human brain–behavior relationships, fMRI provides an excellent balance of temporal and spatial resolution. Improvements on both fronts will clearly add to type of basic and clinical neuroscientific questions that can be addressed with this method.

4 Issues in Functional MRI Experimental Design

Numerous options exist for designing experiments using fMRI. The prototypical fMRI experimental design consists of two behavioral tasks presented in blocks of trials alternating over the course of a scanning session, and the fMRI signal between the two tasks is compared. This is known as a blocked design. For example, a given block might present a series of faces to be viewed passively, which evokes a particular cognitive process, such as face perception. The "experimental" block alternates with a "control" block that is designed to evoke all of the cognitive processes present in the experimental block except for the cognitive process of interest. In this experiment the control block may comprise a series of objects. In this way, the stimuli used in experimental and control tasks have similar visual attributes, but differ in the attribute of interest (i.e., faces). The inferential framework of "cognitive subtraction" [58] attributes differences in neural activity between the two tasks to the specific cognitive process (i.e., face perception). Cognitive subtraction was originally conceived by Donders in the late 1800s for studying the chronometric substrates of cognitive processes [59] and was a major innovation in imaging [58, 60].

The assumptions required for cognitive subtraction may not always hold and could produce erroneous interpretation of functional neuroimaging data [45]. Cognitive subtraction relies on two assumptions: "pure insertion" and linearity. Pure insertion implies that a cognitive process can be added to a preexisting set of cognitive processes without affecting them. This assumption is difficult to prove because one needs an independent measure of the preexisting processes in the absence and presence of the new process [59]. If pure insertion fails as an assumption, a difference in the neuroimaging signal between the two tasks might be observed, not because a specific cognitive process was engaged in one task and not the other, but because the added cognitive process and the preexisting cognitive processes interact.

An example of this point is illustrated in working memory studies using delayed-response tasks [61]. These tasks [62] typically present information that the subject must remember (engaging an *encoding* process), followed by a delay period during which the subject must hold the information in memory over a short period of time (engaging a *memory* process), followed by a probe that requires the subject to make a decision based on the stored information (engaging a *retrieval* process). The brain regions engaged by evoking the *memory* process theoretically are revealed by subtracting the BOLD signal measured by fMRI during a block of trials that the subject performs that do not have a delay period (only engaging the *encoding* and *retrieval* processes) from a block of trials with a delay period (engaging the *encoding*, *memory*, and *retrieval* processes). In this example, if the addition or "insertion" of a delay period between the *encoding* and *retrieval* processes affects these other behavioral processes in the task, the result is failure to meet the assumptions of cognitive subtraction. That is, these "nonmemory" processes may differ in delay trials and no-delay trials, resulting in a failure to cancel each other out in the two types of trials that are being compared.

Empirical evidence of such failure exists [63]. For example, Figure 3 demonstrates BOLD signal derived from the PFC from a subject performing a delayed-response task similar to the tasks

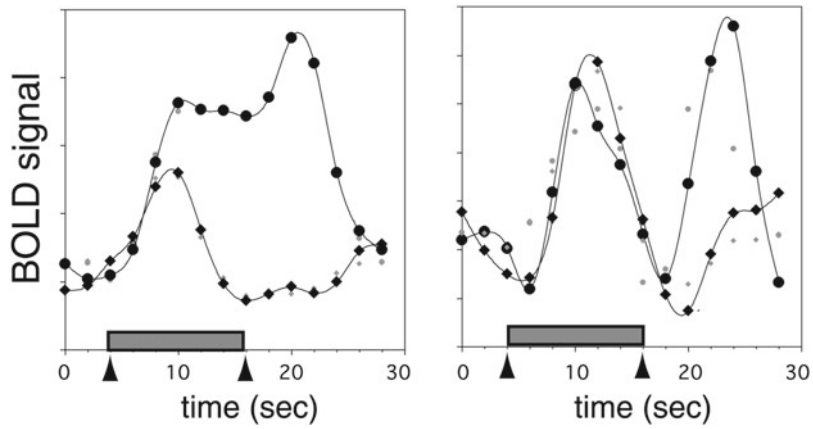

Fig. 3 Data derived from the performance of a normal subject on a spatial delayed-response task [64]. This task comprised both delay trials (*circles*) as well as trials without a delay period (no-delay trials; *diamonds*). (**a**) Trial averaged fMRI signal from prefrontal cortex that displayed delay-correlated activity. The gray bar along the *x*-axis denotes the 12 s delay period during delay trials. The delay trials display a level of fMRI signal greater than baseline throughout the period of time corresponding to the retention delay (taking into account the delay and dispersion of the fMRI signal). The peaks seen in the signal correspond to the encoding and retrieval periods. (**b**) Trial averaged fMRI signal from a region in prefrontal cortex that did not display the characteristics of delay-correlated activity. This region displays a significant functional change associated with the no-delay trials, and a significant functional change associated with the encoding and retrieval periods of the delay trials, but not one associated with the retention delay of delay trials

described above. The left side of the figure illustrates BOLD signal consistent with delay period activity whereas the right side of the figure illustrates BOLD signal from another region of PFC that did not display sustained activity during the delay yet showed greater activity in the delay trials as compared to the trials without a delay. In any blocked functional neuroimaging study that compares delay vs. no-delay trials with subtraction, such a region would be detected and likely assumed to be a "memory" region. Thus, this result provides empirical grounds for adopting a healthy doubt regarding the inferences drawn from imaging studies that rely exclusively on cognitive subtraction.

In functional neuroimaging, the transform between the neural signal and the hemodynamic response (measured by fMRI) must also be linear for the cognitive subtractive method to yield valid results. In other words, it is assumed that the BOLD signal being measured is approximately proportional to the local neural activity that evokes it. Surprisingly, although thousands of empirical studies using fMRI to study brain–behavior relationships have been published, only a handful exist that have explored the neurophysiological basis of the BOLD signal (for reviews *see* refs. [64, 65]). In several studies it has been demonstrated that linearity does not strictly hold for the BOLD fMRI system but a linear transform model is reasonably consistent with the data. For example, Boynton et al. tested whether the BOLD signal in response to long duration stimuli can be predicted by summing the responses to shorter duration stimuli [42]. Using pulses of flickering checkerboard patterns and measuring within human primary visual cortex, these investigators found that the BOLD signal response to various durations of stimulus presentation (6, 12, or 24 s) could be predicted from the responses they obtained from shorter stimulus presentations. For example, the BOLD signal response to a 6 s pulse could be predicted from the summation of the BOLD signal response to the 3 s pulse with a copy of the same response delayed by 3 s. However, temporal summation did not always hold, and there are clearly nonlinear effects in the transform of neural activity to a hemodynamic response that must be considered [66–69]. If these nonlinearities lead to saturation of the BOLD effect at a certain stimulus intensity, erroneous interpretation of particular results of fMRI experiments may occur.

Another class of experimental designs, called event-related fMRI, attempts to detect changes associated with individual trials, as opposed to the larger unit of time comprising a block of trials [70, 71]. Each individual trial may be composed of one behavioral "event," such as the presentation of a single stimulus (e.g., a face or object to be perceived) or several behavioral events such as in the delayed-response task described above (e.g., an item to be remembered, a delay period, and a motor response in a delayed-response task). For example, with an event-related design, activity

within the PFC has consistently been shown to correlate with the delay period, supporting the role of the PFC in temporarily maintaining information [63]. This finding is consistent with single-neuron recording studies in the PFC of monkeys [7]. An event-related design offers numerous advantages. For example, it allows for stimulus or trial randomization avoiding the behavioral confounds of blocked trials. It also permits the separate analysis of functional responses that are identified only in retrospect (i.e., trials on which the subject made a correct or incorrect response). Of course, an experiment does not have to be limited to either a block or event-related designs—a mixed-type (both event-related and blocked) design where particular trial types are randomized within a block is perfectly feasible. In this type of design, both item-related processes (e.g., transient responses to stimuli) as well as state-related processes (processes sustained throughout a block of trials or a task) are perfectly feasible [72, 73].

Overall, much flexibility exists in the type of experimental design that can be utilized in fMRI experiments and continued innovation in this area will greatly expand the types of neuroscientific questions that can be addressed.

5 Issues in Interpretation of fMRI Data

5.1 Statistics

Many statistical techniques are used for analyzing fMRI data, but no single method has emerged as the ideal or "gold standard." The analysis of any fMRI experiment designed to contradict the null hypothesis (i.e., there is no difference between experimental conditions) requires inferential statistics. If the difference between two experimental conditions is too large to be reasonably due to chance, then the null hypothesis is rejected in favor of the alternative hypothesis, which typically is the experimenter's hypothesis (e.g., the fusiform gyrus is activated to a greater extent by viewing faces than objects). Unfortunately, since errors can occur in any statistical test, experimenters will never know when an error is committed and can only try to minimize them [74]. Knowledge of several basic statistical issues provides a solid foundation for the correct interpretation of the data derived from fMRI studies.

Two types of statistical errors can occur. A type I error is committed when the null hypothesis is falsely rejected, that is, a difference between experimental conditions is found but a difference does not truly exist. This type of error is also called a false-positive error. In an fMRI study, a false-positive error would be finding a brain region activated during a cognitive task, when actually it is not. A type II error is committed when the null hypothesis is accepted when it is false, that is, no difference between experimental conditions exists when a difference does exist. This type of error is also called a false-negative error. A false-negative error in an fMRI study would be

failing to find a brain region activated during the performance of a cognitive task when actually it is. The concept of type II error is closely related to the idea of statistical power. If the false-negative rate for a given study design is 20%, for instance, then the "power" of that design to detect an activation is 100–20% or 80%.

In cognitive neuroscience studies, much emphasis has been placed on avoiding type I errors. The negative effects of incorrectly identifying a brain region as task-active include the expenditures of time, money, and effort spent in replicating and/or expanding upon a false positive result. Type II error, on the other hand, is seen as less damning; failure to detect brain activity in a research study has fewer implications for future research, provided that one is careful to interpret so-called null results correctly. For example, cognitive neuroscience studies (due to factors such as the expense and the difficulty of finding research participants, for example) tend to employ a small number of subjects—15 would typical—and therefore frequently lack power to detect significant brain activations. One must consequently be careful to avoid interpreting a lack of activation in one part of the brain as true inactivity during the task.

In a clinical research study, on the contrary, the emphasis may be different, especially when fMRI studies are being used diagnostically in individual patients. A type II error—failing to detect active brain regions related to movement or language in the vicinity of a brain tumor, for example—may lead to a larger surgical resection that leaves the patient with avoidable residual deficits. On the contrary, a type I error—for example, identifying motor activity adjacent to a tumor when in fact none exists—may erroneously lead to a more cautious surgical resection, or to use of a different treatment modality. Which error is deemed more tolerable may depend on the clinical situation.

In fMRI experiments, like all experiments, a tolerable probability for type I error, typically less than 5%, is chosen for adequate control of specificity, that is, control of false-positive rates. Two features of fMRI data can cause unacceptable false-positive rates, even with traditional parametric statistical tests. First, there is the problem of multiple comparisons. For the typical resolution of images acquired during fMRI scans, the full extent of the human brain could comprise as many as 15,000 voxels. Thus, with any given statistical comparison of two experimental conditions, there are actually 15,000 statistical comparisons being performed. With such a large number of statistical tests, the probability of finding a false-positive activation, that is, committing a type I error, somewhere in the brain increases. Several methods exist to deal with this problem. One method, a Bonferroni correction, assumes that each statistical test is independent and calculates the probability of type I error by dividing the chosen probability ($p=0.05$) by the number of statistical tests performed. Another method is based on Gaussian field theory [75], and calculates the probability of type I error when imaging

data are spatially smoothed. Many other methods for determining thresholds of statistical maps are proposed and utilized [76, 77] but unfortunately, no single method has been universally accepted. Nevertheless, all fMRI studies must apply some type of correction for multiple comparisons to control the false-positive rate.

The second feature that might increase the false-positive rate is the "noise" in fMRI data. Data from BOLD fMRI are temporally autocorrelated, with more noise at some frequencies than at others. The shape of this noise distribution is characterized by a 1/frequency function with increasing noise at lower frequencies [78]. Traditional parametric and nonparametric statistical tests assume that the noise is not temporally autocorrelated, that is, each observation is independent. Therefore, any statistical test used in fMRI studies must account for the noise structure of fMRI data. If not, the false-positive rates will inflate [78, 79].

Type II error is rarely considered in functional neuroimaging studies. When a brain map from an fMRI experiment is presented, several areas of activation are typically attributed to some experimental manipulation. The focus of most fMRI studies is on brain activation whereas it is often implicitly assumed that all of the other areas (typically most of the brain) were not activated during the experiment. Power as a statistical concept refers to the probability of correctly rejecting the null hypothesis [74]. As the power of an fMRI study to detect changes in brain activity increases, the false-negative rate decreases. Unfortunately, power calculations for particular fMRI experiments are rarely performed, although methods exist to address this issue [80–82]. Reports that specific brain areas were not active during an experimental manipulation should provide an estimate of the power required for detection of a change in the region. All experiments should be designed to maximize power. Relatively simple strategies can increase power in an fMRI experiment in certain circumstances, such as increasing the amount of imaging data collected or increasing the number of subjects studied. It is also important to note that task designs can affect sensitivity [83]. For example, since BOLD fMRI data are temporally autocorrelated, experiments with fundamental frequencies in the lower range (e.g., a boxcar design with 60 s epochs) will have reduced sensitivity, due to the presence of greater noise at these lower frequencies. Finally, in a study that simultaneously measured neural signal via intracortical recording and BOLD signal in a monkey, it was observed that the SNR of the neural signal was on average at least one order of magnitude higher than that of the BOLD signal. The investigators of this study concluded that "the statistical and thresholding methods applied to the hemodynamic responses probably underestimate a great deal of actual neural activity related to a stimulus or task" [84]. Thus, the magnitude of type II error in BOLD fMRI may currently be underestimated and warrants further consideration in the interpretation of almost any cognitive neuroscience experiment.

5.2 Altered Hemodynamic Response

When comparing changes in fMRI BOLD signal levels within the brain of an individual subject across different cognitive tasks and making conclusions regarding changes in neural activity and the pattern of activity, numerous assumptions are made regarding the steps comprising neurovascular coupling (stimulus → neural activity → hemodynamic response → BOLD signal) and the regional variability of the metabolic and vascular parameters influencing the BOLD signal. It should be obvious that fMRI studies of cognition of individuals with local vascular compromise or diffuse vascular disease (e.g., patients with strokes or normal elderly) are potentially problematic. For example, many fMRI studies have sought to identify age-related changes in the neural substrates of cognitive processes. Those studies that directly compare changes in fMRI BOLD signal intensity across age groups rely upon the assumption of age-equivalent coupling of neural activity to BOLD signal. However, there is empirical evidence that suggests that this general assumption may not hold true. Extensive research on the aging neurovascular system has revealed that it undergoes significant changes in multiple domains in a continuum throughout the human lifespan, probably as early as the fourth decade (for review *see* ref. [85]). These changes affect the vascular ultrastructure [86], the resting cerebral blood flow [87, 88], the vascular responsiveness of the vessels [89], and the cerebral metabolic rate of oxygen consumption [90, 91]. Aging is also frequently associated with comorbidities such as diabetes, hypertension, and hyperlipidemia, all of which may affect the fMRI BOLD signal by affecting cerebral blood flow and neurovascular coupling [92]. Any one of these age-related differences in the vascular system could conceivably produce age-related differences in BOLD fMRI signal responsiveness, greatly affecting the interpretation of results from such studies.

Our laboratory compared the hemodynamic response function (HRF) characteristics in the sensorimotor cortex of young and older subjects in response to a simple motor reaction-time task [70]. The provisional assumption was made that there was identical neural activity between the two populations based on physiological findings of equivalent movement-related electrical potentials in subjects under similar conditions [93]. Thus, we presumed that any changes that we observed in BOLD fMRI signal between young and older individuals in motor cortex would be due to vascular, and not neural activity changes in normal aging. Several important similarities and differences were observed between age groups. Although there was no significant difference in the shape of the hemodynamic response curve or peak amplitude of the signal, we found a significantly decreased SNR in the fMRI BOLD signal in older individuals as compared to young individuals. This was attributed to a greater level of noise in the older individuals. We also observed a decrease in the spatial extent of the BOLD signal in older individuals compared to younger individuals in

sensorimotor cortex (i.e., the median number of suprathreshold voxels). Similar results have been replicated by other laboratories (**e.g.**, [94, 95]). These findings suggest that there is some property of the coupling between neural activity and fMRI BOLD signal that changes with age.

The notion that vascular differences among individuals may affect BOLD signal is especially a concern when considering studies of patient populations with known vascular changes such as stroke. For example, in a fMRI study of patients with an isolated subcortical lacunar stroke compared to a group of age-matched controls, a decrease in the rate of rise and the maximal fMRI BOLD HRF to a finger- or hand-tapping task in both the sensorimotor cortex of the hemisphere affected by the stroke and the unaffected hemisphere was found [96]. These investigators proposed that given the widespread changes of these fMRI BOLD signal differences, the change was unlikely a direct consequence of the subcortical lacunar stroke, but rather a manifestation of preexisting diffuse vascular pathology.

In summary, comparing BOLD signal in two different groups of individuals that may differ in their vascular system should be done with caution [97]. For example, in one scenario, a comparison of activation of young and elderly individuals during a cognitive task may show less activation by elderly (as compared to young subjects) in some brain regions, but greater activation in other regions. In this scenario, it is unlikely that regional variations in the hemodynamic coupling of neural activity to fMRI signal would account for such age-related differences in patterns of activation. In another scenario, a comparison of young and elderly subjects may show less activation by elderly (as compared to young subjects) in some brain regions, but no evidence of greater activation in any other region. In this case, it is possible that the observed age-related differences are not due to differences in intensity of neural activity, but rather to other nonneuronal contributions to the imaging signal, i.e., neurovascular coupling.

In summary, fMRI BOLD contrast methods yield signal changes that result from a complex mix of vascular effects and provide only relative, rather than absolute, measures. One approach to accounting for the influence of purely vascular effects is to directly measure regional and individual variability in vascular reactivity via a breathholding task, which increases carbon dioxide concentration in the blood and leads to vascular dilatation [98]. The task-related BOLD signal in each subject can then be corrected for particular region- and subject-specific vascular effects. One alternative functional neuroimaging approach, based on more direct measurements of cerebral blood flow to active brain areas, is known as arterial spin labeling (ASL). In the various ASL techniques, the MRI scanner selectively magnetizes blood flow with a particular range of locations and/or velocities, then waits for the appearance of the magnetic "tag" in downstream vessels. It thus becomes possible to

obtain absolute measures of cerebral perfusion [99], thereby opening up the possibility of more quantitatively distinguishing between the differential influence of a disease on blood flow, and its effect on brain activity [100]. Additionally, relative to BOLD contrast these absolute measurements appear to be more stable over long experiments because of better signal-to-noise at very low frequencies [101], to show less between-subject and between-session variability [102], and to produce decreased susceptibility artifact in areas such as medial temporal lobe [103]. A significant limitation is temporal resolution: one must both wait for the generation of sufficient magnetic label, and also acquire two scans, a reference scan and a post-labeling scan, to produce a single data point. However, a recently a new MRI acquisition method has been developed that allows for more slices for measuring perfusion in a larger region of the brain than currently possible with previous methods [104]. Another potential disadvantage somewhat related to the temporal issues is the lower SNR of ASL relative to BOLD, but this decline may be compensated in group studies by the observation that ASL methods appear to be less variable across subjects [99].

6 Types of Hypotheses Tested Using fMRI

Functional neuroimaging experiments test hypotheses regarding the anatomical specificity for cognitive processes (functional specialization) or direct or indirect interactions among brain regions (functional integration). The experimental design and statistical analyses chosen will determine the types of questions that can be addressed. Ultimately, the most powerful approach for the testing of theories on brain–behavior relationships is the analysis of converging data from multiple methods.

6.1 Functional Specialization

The major focus of fMRI studies of cognition is testing theories on functional specialization. The concept of functional specialization is based on the premise that functional modules exist within the brain, that is, areas of the cerebral cortex are specialized for a specific cognitive process. For example, facial recognition is a critical primary function likely served by a functional module. Prosopagnosia is the selective inability to recognize faces. Patients with prosopagnosia, however, can recognize familiar faces, such as those of relatives, by other means, such as the voice, dress, or shape. Other types of visual recognition, such as identifying common objects, are normal. Prosopagnosia arises from lesions of the inferomedial temporo-occipital lobe, which are usually due to a stroke within the posterior cerebral artery circulation. No lesion studies have precisely localized the area crucial for facial perception. However, they provide strong evidence that a brain area is specialized for processing faces. Functional imaging studies have

provided anatomical specificity for such a module. For example, Kanwisher et al. [105] used fMRI to test a group of healthy individuals and found that the fusiform gyrus was significantly more active when the subjects viewed faces than when they viewed assorted common objects. The specificity of a "fusiform face area" was further demonstrated by the finding that this area also responded significantly more strongly to passive viewing of faces than to scrambled two-tone faces, front-view photographs of houses, and photographs of human hands. These elegant experiments allowed the investigators to reject alternative functions of the face area, such as visual attention, subordinate-level classification, or general processing of any animate or human forms, demonstrating that this region selectively perceives faces.

Of course, the existence of brain areas specialized for certain functions does not exclude the strong possibility that those areas show either finer, voxel-level structure or are part of larger networks. Recent neuroimaging work has focused on pattern classification methods—that is, on techniques to explore whether a distributed spatial pattern of brain activity, both within a single region and across larger brain areas, corresponds to object (or more abstract) representations. This area of research draws on results from physics, computer science, and statistics, among other disciplines, to search for more broadly distributed structure in neuroimaging data. As such, the techniques themselves differ. For example, to distinguish between voxel activity patterns across experimental conditions, various reports have used correlations between the set of activations in visual responses to faces and other objects [106]; neural network classifiers to identify particular patterns correlated with particular memories [107]; and variants of a matrix algebra transformation known as singular value decomposition to look for distributed spatial correlates of memory storage and search [108]. By establishing sophisticated models of the relationships between brain activity and visual stimuli in visual cortex, representations of natural images may even be successfully decoded [109]. A large number of other techniques—too large to be reviewed here—are also continually being developed [110, 111]. As such research demonstrates that task-relevant brain activity can be detected even in the absence of classic univariate activity changes. However, it will remain important to control for potential confounds in brain activity data, with validation via comparison with behavioral responses, in order to ensure that these patterns are not epiphenomenal or a result of confounds such as reaction time [112]. At a higher tier of analysis, information decoding techniques are being used to examine mechanisms by which higher order cognition can modulate information representations. A step beyond simply detecting the existence of a particular representational code, one can now ask, for example, to what extent goal-direction (attention) might change the tuning of neural network codes to better represent information related to a goal [113, 114].

6.2 Functional Integration

Functional neuroimaging experiments can also test hypotheses about interactions between brain regions by focusing on covariances of activation levels between regions [115, 116]. These covariances reflect "functional connectivity," a concept that was originally developed in reference to temporal interactions among individual neurons [117].

In addition to providing information about the specialization of various brain regions, functional neuroimaging can also address the interactions between brain regions that underlie cognitive processing. Understanding the various techniques that permit these types of analysis comprises a very active area of current research [118]. However, most, if not all, of the techniques used to test for regional interactions are ultimately based on the covariance of activation levels in different brain regions across time—in other words, on the way in which activity levels in different areas of the brain rise or fall in relation to each other. Such statistical techniques are commonly known as "multivariate," both because they rely on interactions between two or more brain areas, and to distinguish them from the "univariate" methods applied in most tests of functional specialization.

The universe of multivariate techniques is further subdivided into two types, determined by whether the method in question is designed to assess connectivity in a model-free ("functional connectivity") or model-based ("effective connectivity") fashion. The former refers simply to methods that measure the temporal covariance in activity between brain areas without a priori notions about which brain areas are relevant or how they should interact. Examples of model-free techniques would include correlation and its frequency-based analogue, coherence, which can be applied irrespective of hypotheses about the neural events that produced them. On the contrary, model-based, or effective connectivity, approaches begin with hypotheses about the interactions between different brain regions, and attempt to support/refute them by evaluating the presence/absence of specific activity covariance patterns. Examples of these techniques would include structural equation modeling and dynamic causal modeling, both of which start by postulating the existence of influences (potentially complex, potentially time-varying) between specific brain regions. Both types of statistical techniques have value, of course; their use is determined by the problem at hand. Model-free approaches are more general, and more easily deployed in exploratory analyses. However, they are not as powerful as model-based methods that address specific hypotheses about how regions interact—but which fail if the model is misspecified. Model-free methods, for example, may be more useful when attempting to determine which networks of brain areas might be involved in a task, whereas model-based methods may be most appropriate when the nodes of the network are known, and specific notions about how they interact need to be tested.

In our own laboratory, we have developed and used functional connectivity techniques to understand how brain interactions change under different task conditions, and over time [119, 120]. For example, we have shown that functional connectivity changes as subjects learn a complex finger tapping task [121]. In the early phases of learning, the data show that subjects not only activate wide areas of primary sensorimotor cortex, premotor cortex, and the supplementary motor area, but also that the coherence between these areas is increased relative to later stages. Such changes were not observed when subjects performed an already learned motor skill; and more importantly, they were not found in the univariate responses, whose means were unchanged despite the changes in the subjects' facility at the task. Similarly, in a working memory task for faces [122], we have found an interesting dissociation between their univariate and multivariate analyses in the networks that support so-called delay period activity (see below). In our protocol, subjects encoded a cue face, maintained the image across a delay of several seconds, and then decided whether a subsequently presented probe face matched the initial one. Interestingly, we found that despite a general decrease in the univariate activity from the cue to the delay period, there was a robust increase in the correlation between activity in the right fusiform face area (a brain region known to be sensitive to face stimuli) and a diffuse set of brain regions including the frontal and parietal cortices as well as the basal ganglia.

In such known networks, effective connectivity techniques can be employed to more specifically evaluate the influence of the nodes of the network on each other. McIntosh et al., for example, were able to exploit their own functional neuroimaging research on working memory networks to formulate a hypothesis about the interactions of the PFC, cingulate cortex, and other brain regions during task performance [116]. Using structural equation modeling, the authors found shifting prefrontal and limbic interactions in a working memory task for faces as the retention delay increased (Fig. 4). The different interactions between brain regions at short and long delays were interpreted as a functional change. For example, strong corticolimbic interactions were found at short delays, but at longer delays, when the image of the face was more difficult to maintain, strong fronto-cingulate-occipital interactions were found. The investigators postulated that the former finding was due to maintaining an iconic facial representation, and the latter due to an expanded encoding strategy, resulting in more resilient memory. As in our own previous studies, information that was not seen in the univariate analysis was captured by an approach sensitive to regional interactions. In addition to structural equation modeling, other approaches have been applied to fMRI datasets to capture information regarding the relative timing of activation across brain regions such as Granger causality, information analysis, and coherence (*see* [119, 120, 123]).

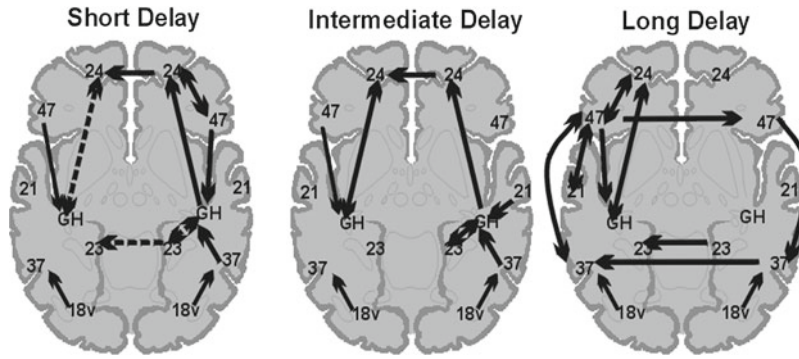

Fig. 4 Network analysis of fMRI data using structural equation modeling during performance of a working memory task across three different delay periods [111]. Areas of correlated increases in activation (*solid lines*) and areas of correlated decreases in activation (*dotted lines*) are shown. Note the different pattern of interactions among brain regions at short and long delays

Mathematical tools based on graph theory have recently emerged as a method to quantify large-scale network properties of the brain as well as to identify the role of individual brain regions within these large-scale networks. These tools, developed for analyzing a wide variety of networks (e.g., social networks, the internet, protein associations), allow one to make quantitative measurements of brain network structure. Typically, these methods are used to analyze the spontaneous coherent fluctuations in BOLD signal measured by fMRI at rest, which consistently identifies stable intrinsic functional networks, that, in a short fMRI recording session, recapitulate a number of sub-networks normally engaged by a variety of different tasks (see Fig. 5).

6.3 Cognitive Theory

Experiments using fMRI can also test theories of the underlying mechanisms of cognition. For example, an fMRI study [124] attempted to answer the question, "To what extent does perception depend on attention?" One hypothesis is that unattended stimuli in the environment receive very little processing [125], but another hypothesis is that the processing load in a relevant task determines the extent to which irrelevant stimuli are processed [126]. These alternative hypotheses were tested by asking normal individuals to perform linguistic tasks of low or high load while ignoring irrelevant visual motion in the periphery of a display. Visual motion was used as the distracting stimulus, because it activates a distinct region of the brain (cortical area MT or V5, another functional module in the visual system). Activation of area MT would indicate that irrelevant visual motion was processed. Although task and irrelevant stimuli were unrelated, fMRI of motion-related activity in MT showed a reduction in motion processing during the high-processing load condition in the linguistic task. These findings supported the hypothesis that perception of irrelevant environmental

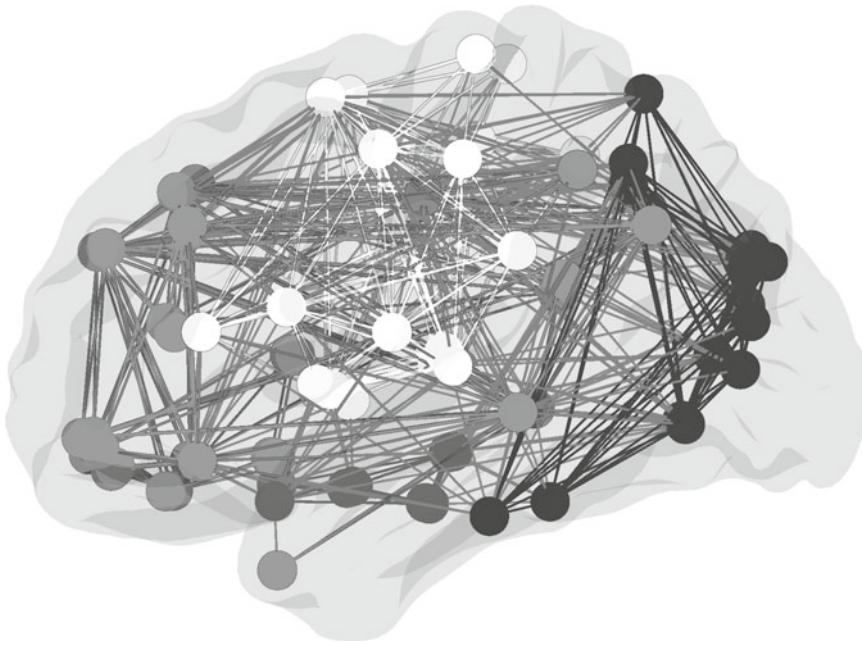

Fig. 5 A brain graph derived from resting state fMRI data collected from healthy young subjects illustrating identified modules, represented as different *shades of color*. There are four distinct modules identified in this graph

information depends on the information processing load that is currently relevant and being attended to. Thus, by the finding that perception depends on attention, this fMRI experiment provides insight regarding underlying cognitive mechanism.

7 Integration of Multiple Methods

The most powerful approach toward understanding brain–behavior relationships comes from analyzing converging data from multiple methods. There are several ways in which different methods can provide complementary data. For example, one method can provide superior spatial resolution (e.g., fMRI) whereas the other can provide superior temporal resolution (e.g., ERP). Also, the data from one method may allow for different conclusions to be drawn from it such as whether a particular brain region is necessary to implement a cognitive process (i.e., lesion methods) or whether it is only involved during its implementation (i.e., physiological methods). The following sections describe examples of such approaches.

7.1 Combined fMRI/ Lesion Studies

The combined use of functional neuroimaging and lesions studies can be illustrated with studies of the neural basis of semantic memory, the cognitive system that represents our knowledge of the

world. Early studies of patients with focal lesions supported the notion that the temporal lobes mediate the retrieval of semantic knowledge [127]. For example, patients with temporal lobe lesions may show a disproportionate impairment in the knowledge of living things (e.g., animals) compared with nonliving things. Other patients have a disproportionate deficit in the knowledge of nonliving things [128]. These observations led to the notion that the semantic memory system is subdivided into different sensorimotor modalities, that is, living things, compared with nonliving things, are represented by their visual and other sensory attributes (e.g., a banana is yellow), while nonliving things are represented by their function (e.g., a hammer is a tool but comes in many different visual forms). The small number of patients with these deficits, and often large lesions, limits precise anatomical-behavioral relationships. However, functional neuroimaging studies in normal subjects can provide spatial resolution that the lesion method lacks [129].

These original observations regarding the neural basis of semantic memory conflicted with functional neuroimaging studies consistently showing activation of the left IFG during the retrieval of semantic knowledge. For example, an early cognitive activation PET study revealed IFG activation during a verb generation task compared with a simple word repetition task [60]. A subsequent fMRI study [130] offered a fundamentally different interpretation of the apparent conflict between lesion and functional neuroimaging studies of semantic knowledge: left IFG activity is associated with the need to select some relevant feature of semantic knowledge from competing alternatives, not retrieval of semantic knowledge per se. This interpretation was supported by an fMRI experiment in normal individuals in which selection, but not retrieval, demands were varied across three semantic tasks. In a verb generation task, in a high selection condition, subjects generated verbs to nouns with many appropriate associated responses without any clearly dominant response (e.g., "wheel"), but in a low selection condition nouns with few associated responses or with a clear dominant response (e.g., "scissors") were used. In this way, all tasks required semantic retrieval, and differed only in the amount of selection required. The fMRI signal within the left IFG increased as the selection demands increased (Fig. 6). When the degree of semantic processing varied independently of selection demands, there was no difference in left IFG activity, suggesting that selection, not retrieval, of semantic knowledge drives activity in the left IFG.

To determine if left IFG activity was correlated with but not necessary for selecting information from semantic memory, the same task used during the fMRI study was used to examine the ability of patients with focal frontal lesions to generate verbs [131]. Supporting the earlier claim regarding left IFG function derived from an fMRI study [130], the overlap of the lesions in patients with deficits on this task corresponded to the site of maximum

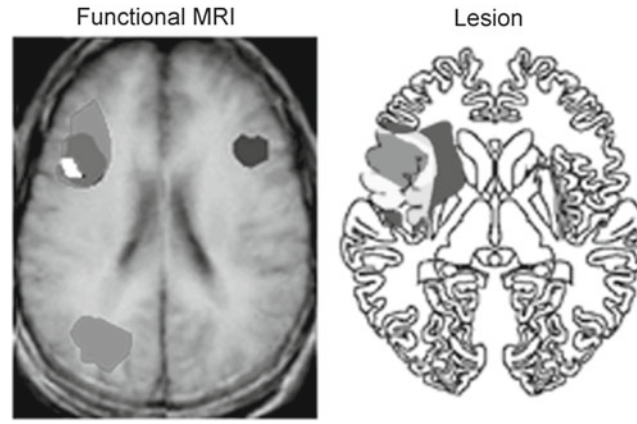

Fig. 6 Regions of overlap of fMRI activity in healthy human subjects (*left side of figure*) during the performance of three semantic memory tasks, with the convergence of activity within the left inferior frontal gyrus (*white region*) [125]. Regions of overlap of lesion location in patients with selection-related deficits on a verb generation task (*right side of figure*) with maximal overlap within the left inferior frontal gyrus [126]

fMRI activation in healthy young subjects during the verb generation task (Fig. 6). In this example, the approach of using converging evidence from lesion and fMRI studies differs in a subtle but important way from the study described earlier that isolated the face processing module. Patients with left IFG lesions do not present with an identifiable neurobehavioral syndrome reflecting the nature of the processing in this region. Guided by the fMRI results from healthy young subjects, the investigators studied patients with left IFG lesions to test a hypothesis regarding the necessity of this region in a specific cognitive process. Coupled with the well-established finding that lesions of the left temporal lobe impair semantic knowledge, these studies further our understanding of the neural network mediating semantic memory.

7.2 Combined fMRI/ Transcranial Magnetic Stimulation Studies

Transcranial magnetic stimulation (TMS) is a noninvasive method that can induce a reversible "virtual" lesion of the cerebral cortex in a normal human subject [132]. Using both fMRI and TMS provides another means of combining brain activation data with data derived from the lesion method. There are several advantages for using TMS as a lesion method. First, brain injury likely results in brain reorganization after the injury and studies of patients with lesions assume that the nonlesioned brain areas have not been affected, whereas TMS is performed on the normal brain. Another advantage for using TMS is that it has excellent spatial resolution and can target specific locations in the brain whereas lesions in patients with brain injury are markedly variable in location and size across individuals. Such an approach can be illustrated in an investigation of the role of the

medial frontal cortex in task switching [133]. In this study, subjects first performed an fMRI study that identified the regions that were active when they stayed on the current task vs. when they switched to a new task. It was found that medial frontal cortex is activated when switching between tasks. In order to determine if the medial frontal cortex was necessary for the processes involved in task switching, the same paradigm was utilized during inactivation of the medial frontal cortex with TMS. Guided by the locations of activation observed in the fMRI study, and using an MRI guided frameless stereotaxic procedure, it was found that applying a TMS pulse over the medial frontal cortex disrupted performance only during trials during which the subject was required to switch between tasks. TMS over adjacent brain regions did not show this effect. Also, the excellent temporal resolution of TMS allowed the investigators to stimulate during precise periods of the task, determining that the observed effect was during the time when the subjects were presented a cue indicating they must switch tasks prior to the actual performance of the new task. Thus, combining the results from both fMRI and TMS, it was concluded that medial PFC was essential for allowing individuals to intentionally switch to a new task.

It is possible to perform TMS studies not only as an adjunct to, but also concurrently with, fMRI. The advantage of this approach is clear: applying TMS at various times *during* (rather than after) fMRI scans permits it to be causally linked with functional changes in the brain, even independently of behavior. In an early study employing this technique, Ruff, Driver and colleagues [36, 134] examined the influence on early visual cortex of a parietal region (the anterior intraparietal sulcus, or aIPS) implicated in the generation of both covert spatial attention and eye movements. They chose a range of TMS)stimulus intensities, all of which were thought to be in an effectively stimulatory rather than inhibitory range, and applied them to the aIPS while subjects fixated the center of a viewing screen. On some trials, a randomly moving visual stimulus was present; subjects had no other task than to maintain fixation. Using this approach, the authors were able to demonstrate a parametric, so-called top-down effect from aIPS following TMS—an increase in the BOLD response in early visual cortex with increasing TMS intensity—that could be found only when visual stimuli were absent, and that did not vary with retinotopic eccentricity. In distinction, their previous work (extended here) had shown that TMS of the frontal eye field (FEF) led to a decrease in BOLD response in the central visual field but to an increase in BOLD response in the peripheral visual field, irrespective of the presence or absence of a visual stimulus. The authors were consequently able to conclude that the aIPS and the FEF have distinct top-down effects on visual cortex, a finding that would not have been possible without concurrent TMS.

7.3 Combined fMRI/Event-Related Potential Studies

The strength of combining these two methods is coupling the superb spatial resolution of fMRI with the superb temporal resolution of ERP recording. An example of such a study was reported by Dehaene et al. who asked the question "Does the human capacity for mathematical intuition depend on linguistic competence or on visuospatial representations?" [135]. In this study, subjects performed two addition tasks—one in which they were instructed to select the correct sum from two numerically close numbers (exact condition) and one in which they were instructed to estimate the result and select the closest number (approximate condition). During fMRI scanning greater bilateral parietal lobe activation was observed in the approximation condition as compared to the exact condition. Since this activation was outside the perisylvian language zone, it was taken as support that visuospatial processes were engaged during the cognitive operations involved in approximate calculation. Greater left lateralized frontal lobe activation was observed to be greater in the exact condition as compared to the approximate condition, which was taken as evidence for language dependent coding of exact addition facts. In order to consider an alternative explanation of the fMRI findings, the investigators also performed an ERP study. The alternative explanation was that in both the exact and approximate tasks, subjects would compute the exact result using the same representation for numbers but later processing, when they had to make a decision as to the correct choice, was what led to the differences in brain activation. Since fMRI does not offer adequate temporal resolution to resolve these two behavioral events on such a brief time scale, ERP was the appropriate method to test this hypothesis. In the ERP study it was demonstrated that the evoked neural response during exact and approximate trials already differed significantly during the first 400 ms of a trial before subjects had to make a decision.

7.4 Combined fMRI/Pharmacological Studies

Combining pharmacological challenges during the performance of cognitive tasks during fMRI scanning may yield significantly different information than either method alone. In isolation, fMRI cognitive task paradigms provide little information with respect to the underlying pharmacologic systems involved in cognition. On the contrary, drug administration without a brain measure cannot determine underlying neural mechanisms of the effects of neuromodulatory systems on cognition. Combining the two approaches allows the potential of probing the pharmacologic bases of behavior. One may measure the interactive effects of drug (compared to placebo, or a range of doses) with cognitive task-related modulation of brain activity. It is fair to infer that drug × task interactions reflect modulation of the underlying anatomical and chemical brain systems, and do not simply reflect nonspecific vascular effects. For example, dopaminergic agonists have been shown to have task-specific effects [136–138], and different component processes of working memory

are differentially affected by a dopaminergic drug, with effects that may differ between individuals depending on their baseline state [139]. This latter study demonstrated that a dopamine agonist improved the flexible updating (switching) of relevant information in working memory. However, the effect only occurred in individuals with low working memory capacity, but not in individuals with higher working memory capacity. This behavioral effect was accompanied by dissociable effects of the dopaminergic agonist on frontostriatal activity. The dopamine agonist modulated the striatum during switching but not during distraction from relevant information in working memory, while the lateral frontal cortex was modulated by the drug during distraction but not during switching.

8 Application of a Cognitive Neuroscience Approach Toward Clinical Studies

8.1 Use of Biomarkers Derived from Cognitive Neuroscience Studies

Cognitive neuroscience studies using fMRI may provide an important foundation for clinical studies. A biomarker is an indicator that reflects a process, event, or condition in a biological system. Biomarkers may be useful for providing a measure of exposure, effect, or susceptibility. Reliable biomarkers of a neural system could reliably quantify how such a neural system is affected by almost any input. The input may be the effects of a drug, the effects of cognitive therapy, or the effects of a disease process. For a measurement to be useful as a biomarker in clinical studies, it needs to have well-defined significance based on preclinical studies. That is, a change in an fMRI measurement would ideally reflect a change in a well-understood process, thus providing a clear a priori hypothesis and interpretation of the findings. Once the processes are established, fMRI biomarkers may then be useful for addressing a number of clinical questions. For any neurophysiologic measurement to be a *surrogate* marker, a stable, reliable relationship between the fMRI measurement and a defined clinical outcome needs to be defined. Only in that scenario would an fMRI measurement provide a suitable surrogate for other clinical outcomes. Cognitive neuroscience studies provide the foundation for fMRI biomarkers, but the studies necessary for defining fMRI surrogate markers are rarely done.

Questions regarding the mechanisms of brain function disrupted by pathologic states, processes affected by treatment interventions, or the nature of post-injury reorganization of function are examples of clinical questions that can be tested with fMRI. For example, attentional modulation of information processing-related activity in visual cortex is a well-established phenomenon in cognitive neuroscience studies, with effects measurable using fMRI. For example, it has been shown that activity in category-selective regions of inferior temporal cortex is modulated based on the target of attention, relatively up-modulated if the target is relevant to the region and down-modulated if not relevant [140, 141]. This

finding provides a biomarker of attentional control over visual processing, and as noted below, could serve as a useful biomarker for clinical interventions such as cognitive training in individuals with attentional deficits.

8.2 Functional MRI for Measuring the Effect of Clinical Interventions

Functional MRI may be useful not only in defining "static" brain–behavioral relationships, but also may be applied to defining the neural mechanisms that underlie learning, experience, or injury. Two general categories of questions may be investigated. First, fMRI can be used to examine factors that influence response to the perturbations of training (learning), experience or injury. Second, fMRI can be used to examine changes that underlie or are the result of these various perturbations.

Investigation of baseline factors that may influence response to training has particular clinical relevance. A better understanding of pre-training neural characteristics that influence response to rehabilitation training could have major clinical value in guiding treatment decisions. fMRI could provide a number of possible measurements that could mark an important neural process. For example, certain parameters of brain network organization may be particularly important in supporting the potential for learning and plasticity. For example, parameters of the functional organization of whole brain networks have been shown to predict response to training of attention regulation after injury [142]. In another example, a simple measurement of the quantity of activation in prefrontal cortex has been shown to predict response to training to use a verbal memory strategy [143]. Such approaches may help elucidate either personal factors or strategic approaches that underlie variations in learning or response to interventions.

Investigation of *changes* over time is particularly relevant for understanding neural mechanisms of post-injury rehabilitation. In order to assess changes with intervention, longitudinal or repeated measurements are required. Because fMRI involves no exposure-limiting factors such as radiation, it is suitable for repeated measurements. However, multi-session studies are also significantly more complicated to design, analyze, and interpret due to a number of issues discussed below.

There are at least two distinct approaches relevant to assessing changes within an individual. First, fMRI may be used for determining the after-effects of a learning intervention. Functional MRI measures pre- and post-intervention may be used to address this question. For example, after two pieces of information have been strongly associated over repetitive exposures, one may find reduced activation in response to presentation of that information, but increased functional connectivity between regions of the brain that process the two types of information [115]. Second, fMRI may be used for determining the processes that occur during an intervention, such as cognitive training. To do this one would need to

acquire fMRI data *during* the process of training. An alternative approach is to use a cross-sectional approach to examine differences across individuals rather than within individuals [144]. For example, brain activation differences between experts in a particular skill (e.g., long-term meditation practitioners, pianists) and novices may be used to infer the neural effects of training to achieve expertise. However, other confounding effects of differences between cohorts are difficult to exclude (e.g., self-selection in persevering to achieve expertise), and a stronger inference for causation requires longitudinal, prospective studies.

The use of fMRI to define changes over time requires consideration of certain additional methodological issues. Test–retest reliability needs to be considered. Estimates of reliability depend on what is being measured. For example, in statistical parametric mapping, the question may be whether particular brain regions are stably labeled as "active" or not in serial sessions. A handful of studies have addressed this question. For example, one group showed that with a classification learning task, scans 1 year apart resulted in highly concordant results with defined regions of interest [145]. Another group showed that maps obtained from a working memory task were similar across time [146], but with a motor task, there appeared to be significant variation over time in volume and spatial location of activation [147].

In longitudinal studies, sources of variability may be both physiologic and nonphysiologic (e.g., MRI hardware). In some cases, the magnitudes of activation in specific brain regions of interest are themselves an outcome of interest. In these instances the stability of BOLD signal measurements becomes an even more salient issue. It may be worthwhile to utilize within-session indices that effectively normalize parameters of interest. For example, rather than comparing estimates of the magnitudes of activation, it may be worthwhile utilizing an index of activity for one condition compared to a second controlled condition with each session. An additional statistical approach that could account for potential variability in SNR is to combine data sets across sessions and then "whiten" the noise, effectively normalizing noise contribution across sessions. Another promising future direction is the use of quantitative techniques such as arterial spin labeling (ASL), mentioned earlier in this chapter, to help reduce nonphysiologic sources of variability. This type of quantitative index may be particularly valuable in studies that attempt to examine brain functioning longitudinally.

Other factors that concurrently change over time can produce confounds to the interpretation of longitudinal studies. For example, performance may change, resulting in changes in reaction time or accuracy. All of these may alter measured responses making determination of the neural bases of the process of interest, such as a treatment intervention, more difficult. These and a number of other theoretical issues are discussed by Poldrack in consideration of learning-related (though not post-injury) changes [144].

Other analytic approaches may be taken that are less sensitive to nonphysiologic instabilities. For example, one could test for changes in the spatial *pattern* of activation, which is not necessarily affected by signal magnitude changes. For example, one could test whether the patterns of activity are identical to within a scaling factor [108]. Furthermore, one could examine the more fundamental measurement of the information coded within brain activity patterns. These measurements may provide more informative indices of particular neural process, while also being more robust for longitudinal studies.

9 Conclusions

Functional MRI is an extremely valuable tool for studying brain–behavior relationships, as it is widely available, noninvasive, and has superb temporal and spatial resolution. New approaches in fMRI experimental design and data analysis continue to appear at an almost exponential rate, leading to numerous options for testing hypotheses on brain–behavior relationships. Combined with information from complementary methods, such as the study of patients with focal lesions, healthy individuals with TMS, pharmacological interventions, or ERP, data from fMRI studies provide new insights regarding the organization of the cerebral cortex as well as the neural mechanisms underlying cognition. Moreover, cognitive neuroscience approaches that have been developed for fMRI provide an excellent foundation for its use as a clinical tool.

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