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Human pharmacological MRI

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During the past 5 years, functional magnetic resonance imaging (MRI) has been used increasingly to map the modulatory effects of psychopharmacological agents on cognitive activation of large-scale networks in the human brain. Such pharmacological MRI (phMRI) studies can be informative about pharmacodynamics, specific neurotransmitter mechanisms that underlie the adaptivity of neurocognitive systems to variation in task difficulty and familiarity, and changes in neurophysiological drug effects associated with genetic variation, neuropsychiatric disorders and normal aging. In this article, we review >50 relevant published articles from this rapidly growing literature and highlight some emerging themes, including: the importance of individual differences in genotype and cognitive phenotype as conditioners of drug effects on brain activation; the formulation of inverted-U models to describe similar effects of agonist and antagonist drugs acting on the same receptors; and the potential utility of this technique for testing transmitter models of disorders, predicting treatment response, and supporting the development of novel compounds in neuropsychiatry.

A previous *TiPS* review [1] of the application of functional magnetic resonance imaging (fMRI) to psychopharmacology [known as pharmacological MRI (phMRI)] noted that few laboratories had extended the use of this technique from animal to human investigations. The intervening four years has witnessed a proliferation of >50 reports in this field (Tables 1–3), confirming the prediction by Leslie and James [1] that a non-invasive *in vivo* pharmacodynamic assay would provide a powerful research tool for mapping the effects of centrally active compounds in humans and non-human primates [2]. In this article, we review these developments, and assess the extent to which phMRI has impacted on our understanding of the involvement of the major neurotransmitter systems in neurocognitive function, and the pathophysiology and treatment of neuropsychiatric disorders.

The blood oxygen level-dependent (BOLD) signal measured by fMRI is determined by local changes in the ratio of oxygenated to deoxygenated haemoglobin, resulting from increased blood flow in response to neural activity that is not matched proportionately by locally increased oxygen consumption. phMRI involves measuring drug-related changes in the BOLD signal. In humans, fMRI measurements are usually made while the subject is performing a cognitive task. Therefore, the results of phMRI potentially provide information about the pharmacodynamics of the drug (so-called 'neurocognitive profiling') and the transmitter mechanisms that normally underpin the coherent and adaptive activation of large-scale neurocognitive systems activated by task performance. An alternative approach, more commonly used in anaesthetised animals in which cognitive responses are precluded, is to identify BOLD signal changes in response to a bolus of the drug by, for example, identifying brain regions that show a change in BOLD signal that is correlated with the rapidly changing arterial concentrations of the drug following its intravenous administration (see section on substance addiction). However, no new technique should be regarded uncritically and there are methodological caveats concerning the interpretation of phMRI signals (Box 1).

Box 1. Methodological considerations

The blood oxygen level-dependent (BOLD) signal is generated by local changes in oxygenated blood flow triggered by neuronal activity. Therefore, drug effects on the BOLD signal could be mediated by effects on cerebral vasoactivity rather than direct effects on neurons. To date, pharmacological magnetic resonance imaging (phMRI) studies have relied largely on indirect evidence to mitigate this interpretation. For example, the observation that a region-specific pharmacological effect occurs in the absence of an effect in a related cognitive paradigm is difficult to reconcile with a neurovascular interpretation because a vascular, nonspecific effect would be expected to change the BOLD response to a similar degree in any region regardless of the cognitive task. More specific evidence is emerging by combining direct electrophysiological measurements of neural activity with haemodynamic measures such as functional MRI (fMRI). Arthurs *et al.* demonstrated that sulpiride has similar effects on the power law relationship observed between the objective intensity of somatosensory stimulation and both BOLD and evoked-potential responses, indicating that fMRI and electrophysiological data provide convergent indicators of drug effects on stimulus-response parameters [35].

A related issue is how to interpret drug-related changes in the BOLD response in the absence of behavioural changes. A nihilistic view might be that drug effects on the BOLD signal in the absence of behavioural correlates are cerebrovascular in origin or otherwise epiphenomenal. However, there are strong grounds for suggesting that fMRI might be more informative than behaviour as an indicator of underlying neurocognitive function [59], indicating the superior sensitivity of fMRI as a pharmacodynamic assay. For example, BOLD changes might be related to cognitive changes, such as adaptations of strategy formation or cognitive effort, which are not manifest in standard behavioural measures such as response accuracy or latency.

Human studies that cross-validate fMRI measures of drug action with behavioural, electrophysiological and perfusion-weighted MRI measures, and non-human primate studies that compare drug effects on the BOLD signal to local field potentials, axonal firing rates and other direct indices of neuronal activity, will be invaluable in resolving these important issues.

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Table 1. Pharmacological MRI studies of neurotransmitter systems[a]

Aims and hypotheses	Main findings and conclusions	Refs
Dopamine		
To examine physiological correlates of the effects of dextroamphetamine (which increases the concentration of dopamine in the synaptic cleft) on a graded-load working-memory task	Behavioural and neurophysiological effects of dextroamphetamine are not homogeneous, and might be explained by genetic variations that interact with the effects of dextroamphetamine	[3]
To determine the effects of methylphenidate (which increases the concentration of dopamine in the synaptic cleft) on the BOLD response during a motor task	No changes in performance or fMRI signals were observed, indicating that methylphenidate does not alter BOLD neural-haemodynamic coupling	[7]
To examine task-specific and general changes in cortical activity associated with bromocriptine (a dopamine D2 receptor agonist)	Bromocriptine shows both task-specific modulation and task-generic inhibition of neural activity as a result of dopamine-mediated neurotransmission; effects are influenced by baseline variation in performance	[32]
To test the hypothesis that the anxiogenic effect of amphetamine reflects an effect on amygdala activity during affect processing	Dextroamphetamine potentiated the response of the amygdala during the perceptual processing of angry and fearful facial expressions, providing a possible neural substrate for its anxiogenic effects	[33]
To investigate the acute effects of 5 mg haloperidol (a dopamine receptor antagonist) on the BOLD response to sensory processing	Haloperidol reduced the activation of the middle occipital gyrus and increased the activation of the fusiform gyrus; the activity in both areas normalised by 24 h following drug administration	[34]
To anatomically dissociate the adaptive response to task practice and difficulty and establish the effects of pharmacological modulation	Findings indicate that practice and difficulty evoke anatomically distinct systems and are differentially modulated by pharmacological manipulations	[8]
To test the hypothesis that opposing dopamine-related manipulations will have opposing effects of nigrostriatal connectivity	Sulpiride (a selective dopamine D2 receptor antagonist) increased and methylphenidate decreased connectivity from the ventral tegmental area to the caudate, indicating that dopamine modulates effective connections within the basal ganglia	[22]
To test the hypothesis that opposing effects of amphetamine on PFC function would be observed in subjects with differing cortical efficiency, relating to a functional polymorphism (Val108/158Met) in the *COMT* gene	Subjects homozygous for the *val* allele (encoding the more efficient COMT enzyme) showed improved PFC efficiency whereas subjects homozygous for the *met* allele showed reduced PFC efficiency during working memory following treatment with amphetamine, supporting an inverted-U function of the dopamine-mediated PFC response	[6]
To test the hypothesis that the intensity of peripheral stimulation and its pharmacological modulation is related to the magnitude of cortical response by a power law relationship in both SEP and fMRI measures	A power law relationship was observed between the intensity of stimulation and cortical response measured by both modalities; sulpiride attenuated the gain, but not the power law exponent, and fMRI provided a more sensitive indicator than SEP of this effect	[35]
Acetylcholine		
To examine the mechanism by which enhancement of the acetylcholine system improves non-verbal working memory	Enhancement of the acetylcholine system improves memory performance by focusing perceptual processing during encoding, simplifying processing demands during memory maintenance, and reducing the need for prefrontal participation	[9]
To investigate acetylcholine-mediated and GABA-mediated modulation of repetition priming in a word-stem completion paradigm	Scopolamine (a selective muscarinic acetylcholine receptor antagonist) and lorazepam (a benzodiazepine) abolished cortical 'repetition suppression' effects, suggesting that GABA and acetylcholine systems influence neuronal plasticity necessary for repetition priming	[12]
To examine alterations in brain activation associated with pharmacologically induced memory impairment	Performance deficits were observed following administration of scopolamine and lorazepam, which correlated with reduced activation in the hippocampus and fusiform gyrus	[36]
To investigate acetylcholine- and GABA-mediated modulation of repetition priming in a face recognition paradigm	Scopolamine, but not lorazepam, impairs repetition priming, via disruption of acetylcholine-related mechanisms associated with acquisition processes	[13]
To investigate acetylcholine-mediated modulation of experience-dependent plasticity in the human auditory cortex	Scopolamine disrupted conditioning-specific activation in the auditory cortex, evident during placebo treatment, demonstrating acetylcholine-mediated modulation of experience-dependent plasticity	[37]
To investigate the effect of enhancement of the acetylcholine system on experience-dependent cortical responses	Conditioning-related activation by the conditioned stimulus was not evident after physostigmine (a cholinesterase inhibitor) as a result of enhanced responses to behaviourally irrelevant stimuli CS−; stimulation of the acetylcholine system might cause increased processing of behaviourally irrelevant stimuli	[10]
To examine whether behavioural and neural effects of repeating faces are modulated by selective attention, emotion and acetylcholine function	Physostigmine enhanced repetition decreases in the inferior occipital cortex selectively for attended faces, and reversed the emotional interaction with repetition in the lateral orbitofrontal cortex	[38]

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Table 1 (*continued*)

Aims and hypotheses	Main findings and conclusions	Refs
To examine the effects of enhancement of the acetylcholine system on regions associated with attention and emotion	Acetylcholine modulates attention and emotion via independent mechanisms that act via the occipital cortex	[11]
GABA		
To investigate the influence of GABA-mediated mechanisms on prefrontal activation during affect processing	Lorazepam (a benzodiazepine) reversed the pattern of orbitofrontal function observed using fMRI and MEG, providing evidence of GABA involvement in affect processing	[39]
To investigate the role of active inhibition by GABA on brain adaptivity to task repetition and task difficulty	Lorazepam and flumazenil (an antagonist at the benzodiazepine binding site of the $GABA_A$ receptor) both disrupted attenuation of the response by occipito-temporal regions to task repetition observed under placebo, but did not significantly modulate fronto-striatal adaptivity to task difficulty	[14]
Glutamate		
To test the hypothesis that ketamine (a non-competitive NMDA receptor antagonist) would alter limbic activation as a model of depersonalization disorder and schizophrenia	Ketamine disrupted activity in response to both fearful and neutral facial expressions, suggesting that emotional blunting might be associated with reduced limbic responses to emotional stimuli	[40]
5-HT		
To investigate whether paroxetine (a SSRI) modulates motor-related activity, as demonstrated with other 5-HT-related compounds	Increased cortical activation and decreased subcortical activation was observed with paroxetine treatment, demonstrating reorganized motor processing following an acute dose of an SSRI	[41]
Noradrenaline		
To examine whether noradrenaline-mediated disruption of the alerting effect results from an effect on temporal orienting	The anatomical dissociation of the effects of clonidine (α_2-adrenoceptor agonist) suggest that its disruption of the alerting response is not related to its effect on temporal orienting	[16]

[a]Abbreviations: BOLD, blood oxygen level-dependent; *COMT*, gene encoding catechol *O*-methyl transferase; fMRI, functional magnetic resonance imaging; MEG, magnetoencephalography; MRI, magnetic resonance imaging; PFC, prefrontal cortex; SEP, somatosensory evoked potential; SSRI, 5-HT-selective reuptake inhibitor.

Neurotransmitter systems

Dopamine

The dopamine system has been the focus of several phMRI studies (Table 1), most probably as a result of its innervation of key cortical and sub-cortical regions implicated in motor and neurocognitive functions, in addition to its implication in a range of psychiatric and neurological conditions. The first research group to report the use of fMRI to explore the neuromodulatory effects of an acute dopamine-related pharmacological challenge in humans provided a benchmark, not simply because this broke new methodological ground but because, by exploiting the capacity of fMRI to examine inter-subject variability in brain activation, Mattay and colleagues were able to link drug-related changes in brain activation to individual variation in cognitive capacity [3]. They demonstrated region-specific prefrontal activation during a working memory task that was greatest in subjects whose capacity for task performance was greatest at baseline. Prefrontal activation in these high-performing individuals tended to deteriorate following administration of D-amphetamine (which increases the concentration of dopamine in the synaptic cleft), whereas individuals with lower baseline capacity showed improved post-drug performance and increased prefrontal activation following administration of D-amphetamine. These results were consistent with an 'inverted-U' relationship between dopamine tone and both cognitive performance and BOLD activation (Figure 1), and were analogous to relationships demonstrated previously between dopamine tone and single unit activity recorded from prefrontal neurons in non-human primates [4]. A common polymorphism (Val108/158Met) in the gene encoding catechol *O*-methyltransferase (COMT), which is important for the metabolism of synaptically released dopamine in the prefrontal cortex, was subsequently related to cognitive performance and prefrontal cortical efficiency [5]. In a recent seminal phMRI study, performance-dependent effects of amphetamine on prefrontal function reported previously were related to *COMT* gene polymorphisms (i.e. individuals who possessed the allele encoding the more efficient form of the enzyme demonstrated relatively poor baseline working memory performance and more salient effects of D-amphetamine on the

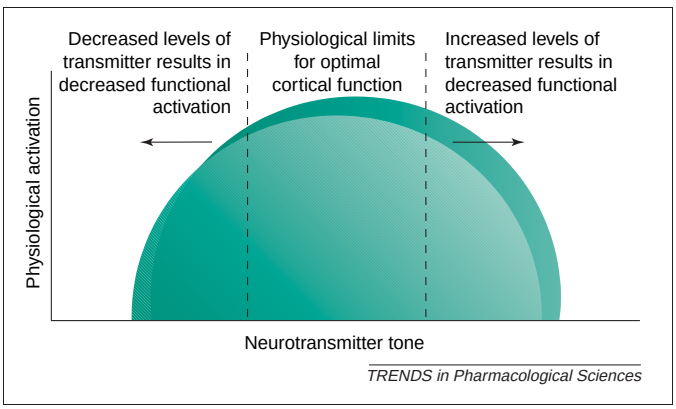

Figure 1. An illustration of the 'inverted-U' relationship between cortical activation and neurotransmitter availability. The narrow optimal range of dopamine tone in relation to task-related neuronal response was originally demonstrated in non-human primates by single unit recordings. These observations have since been echoed in pharmacological magnetic resonance imaging (phMRI) studies of both the dopamine and GABA transmitter systems, evidenced by similar effects on cortical activation of both agonist and antagonist drugs acting at the same receptors.

Table 2. Pharmacological MRI studies of psychiatric and neurological conditions[a]

Aims and hypotheses	Main findings and conclusions	Refs
Schizophrenia		
To test the prediction that atypical anti-psychotic treatment would enhance prefrontal function as a result of increased levels of prefrontal dopamine	The use of the atypical anti-psychotic risperidone instead of typical antipsychotics increased functional activation in the right PFC, SMA and posterior parietal cortex, which might relate to increased clinical and cognitive efficacy of atypical anti-psychotics	[17]
To test the hypothesis that the cognitive mechanism of neuroleptics is to alter the functional connectivity of the cerebellum	Cerebellar connectivity was normalized by the neuroleptic olanzapine in the right, but not the left, hemisphere, indicating that olanzapine affects critical components of the 'cognitive dysmetria' circuit	[20]
To investigate cortical and subcortical motor organization in treated and untreated patients with schizophrenia	Cortical and subcortical brain activation was significantly lower in patients than in healthy controls; untreated patients showed a significant relative over-activation, indicating that anti-psychotic treatment is associated with decreased activation of the motor system	[42]
To test the hypothesis that addition of a cognition-enhancing drug to current anti-psychotic therapy might modulate fronto-temporal interaction, possibly mediated via the cingulate cortex	Donepezil (a cholinesterase inhibitor) increased activation in the left frontal lobe and cingulate activity compared with placebo and baseline scans, supporting the role of the cingulate in modulating integrative fronto-temporal function	[21]
Parkinson's Disease (PD)		
To investigate the modulatory effects of dopamine-related therapy on neural systems subserving working memory and motor function in patients with PD	The study indicates the differential effect of treatment on the nigrostriatal and mesocortical dopamine systems in PD, and also the complex relationship between prefrontal dopamine and cortical efficiency	[23]
To explore the neural basis of abnormal emotional behaviour in PD and the physiological effects of dopamine-related therapy on the response of the amygdala	Patients failed to show amygdala activation, which was partially restored by dopamimetic treatment, consistent with involvement of the amygdala in emotional blunting in PD	[43]
To determine the effects of dopamine in the nigrostriatal pathway in PD on M1 (primary motor cortex) activation and its modulation by L-dopa	Increased activation of M1 contralateral to the affected hand and SMA following administration of L-dopa, which correlated with improved motor performance and restored hypoactivity of these regions (which was evident before treatment)	[44]
To study the effect of dopamine-like stimulation on motor function in PD	The dopamine receptor agonist apomorphine reduced activation of the contralateral precentral gyrus, suggesting a presynaptic effect of the drug that resulted in inhibition of dopamine release	[45]
Depression		
To identify the neural circuitry underlying emotional processing in control and depressed subjects, and the effects of medication	Activation of secondary visual cortex to positive images was enhanced after treatment; results indicate that fMRI can be used as a tool to investigate mechanisms of treatment efficacy in depressed subjects	[24]
To test the prediction that depressed subjects would show an abnormal amygdala response to masked fearful faces, which would resolve with anti-depressant treatment	Increased activation of the left amygdala to masked fearful faces was observed in patients; bilateral amygdala activation was reduced following treatment; the study indicates that patients have an abnormal response even to images that are outside conscious awareness	[46]
To explore the effects of venlafaxine (a 5-HT and noradrenaline reuptake inhibitor) on brain regions associated with positive and negative affect in patients with depression	Increased activation of the left insula (2 weeks) and left anterior cingulate cortex (8 weeks) following treatment; increased anterior cingulate activation to negative stimuli at baseline predicted a positive response to treatment at 8 weeks	[25]
Attention deficit hyperactivity disorder (ADHD)		
To investigate the involvement of the fronto-striatal system in ADHD, and its modulation by methylphenidate (which increases the concentration of dopamine in the synaptic cleft)	Methylphenidate improved response inhibition; frontal increases were observed in patients and controls, but opposing effects were observed in the striatum	[47]
To test the effects of methylphenidate on steady-state blood volume in the midline vermis of the cerebellum in ADHD	Moderate and high doses of methylphenidate increased activation in a symptom severity-related manner: activation was increased in the most active children but reduced in subjects with ADHD who were not objectively hyperactive	[48]
Neurological conditions		
To determine the influence of the SSRI fluoxetine on the motor activation of lacunar stroke patients in the early phase of recovery	Improved motor skill and hyperactivation in the ipsi-lesional primary motor cortex was observed during the active task following fluoxetine treatment, indicating post-stroke reorganization	[49]
To investigate the influence of carbamazepine (an anti-epileptic drug) on memory induced activation of the mesial temporal lobes in patients with symptomatic TLE	Medial temporal activation was negatively correlated with carbamazepine serum level; activation of the medial temporal lobe contralateral to the seizure onset was reduced compared with controls	[50]
Investigation of the dynamic pharmacological modulation of pain-related brain activity	Pain-related activity in the insular cortex contralateral to the stimulus was significantly modulated by remifentanil (opioid), indicating that localization of pharmacological modulation of pain response is possible using fMRI	[51]

[a]Abbreviations: fMRI, functional magnetic resonance imaging; L-dopa, L-dihydroxyphenylalanine; MRI, magnetic resonance imaging; PFC, prefrontal cortex; SMA, supplementary motor area; SSRI, 5-HT-selective reuptake inhibitor; TLE, temporal lobe epilepsy.

Table 3. Pharmacological MRI studies of recreational and/or addictive drugs[a]

Aims and hypotheses	Main findings and conclusions	Refs
Heroin		
To determine the acute effects of heroin administration on functional activation during visual stimulation	Decreased visual activation of the occipital cortex was observed in all subjects after heroin administration	[52]
Cocaine		
To investigate brain circuitry mediating cocaine (an inhibitor of monoamine reuptake)-induced euphoria and craving	Early, short-duration signals correlated with 'rush' ratings; early, sustained signals correlated with cravings; thus, fMRI could be used to map cocaine-induced euphoria and craving	[26]
To determine whether acute i.v. cocaine use would change global CBF or visual stimulation-induced functional activation	Cortical grey matter CBF decreased after cocaine infusion; visual stimulation resulted in comparable occipital BOLD signal increases after drug compared with placebo, indicating reliable BOLD measures despite changes in global blood flow	[30]
To observe the effects of cocaine administration on the function of the visual and motor cortices	Reduced spatial correlations were observed within the visual and motor cortices after cocaine infusion compared with a resting condition	[53]
Nicotine		
To explore the neuropharmacology and sites of action of nicotine in the human brain	Dose-dependent increase in feelings of 'rush', 'high' and drug liking, and increased limbic activation, consistent with the effect of nicotine on arousal, reinforcement and mood-elevation	[27]
To test for evidence of alteration of BOLD response of the occipital cortex to photic stimulation during i.v. infusion of nicotine	Nicotine did not produce a change in BOLD response to photic stimulation, suggesting that nicotine does not cause neurovascular de-coupling in the visual cortex	[31]
To determine the neural substrates of the effects of nicotine on sustained attention	Activation in the parietal cortex and caudate was reduced in mildly abstinent smokers; transdermal nicotine replacement improved task performance in smokers and increased activation	[54]
Alcohol		
To assess the potential of fMRI for studying the effect of alcohol on brain function	Visual cortical activation was progressively reduced following alcohol administration during the session, and on the right hemispheric baseline activation, consistent with visuospatial effects	[55]
To study the effects of ethanol on an acoustically stimulated BOLD signal	Spatial extent of activation in primary auditory cortex reduced by 40% after ethanol exposure, estimated to be 7-10% greater than the reduction as a result of the vasodilatory effects of ethanol	[56]
To investigate neural correlates of craving in alcoholic patients	Alcoholic patients exhibited craving-related activation in the subcortical-limbic region of the right amygdala, hippocampal area and in the cerebellum, which resolved after treatment	[29]
To develop new standardized alcohol-associated cues and assess their effects on brain activation	Alcohol-related stimuli activated regions associated with visual emotional processes (fusiform gyrus), reward (basal ganglia and orbitofrontal gyrus) and attention (frontal and parietal cortices)	[28]
Caffeine		
To test the hypothesis that caffeine has differential effects on the BOLD signal in high- and low-caffeine users	Increased activation of visual cortex was observed in high users compared with low users in the presence of caffeine, and was correlated with caffeine consumption	[57]
To investigate the effects of caffeine on the BOLD signal and global CBF	Caffeine has the potential to be used as a contrast enhancer for fMRI experiments because the vasculature responds from below-normal baseline levels, whereas the increase in blood flow and volume is unperturbed, with an overall increase in the BOLD contrast	[58]

[a]Abbreviations: BOLD, blood oxygen level-dependent; CBF, cerebral blood flow; fMRI, functional magnetic resonance imaging; i.v., intravenous; MRI, magnetic resonance imaging.

prefrontal BOLD signal) [6]. These studies are collectively important in establishing links between cognitive and fMRI measures of dopamine-related drug effects, and in linking both behavioural and physiological phenotypes to underlying allelic variation. There is clearly the potential for phMRI to be used more widely in the emerging field of pharmacogenomics.

Further studies indicate that the effects of dopamine manipulation in the lateral prefrontal cortex could be process and region specific. For example, methylphenidate (which, like amphetamine, increases the concentration of dopamine in the synaptic cleft) had no effect on the functional activation of the motor system [7] but attenuated the adaptive response of a fronto-striatal network to cognitive load in a graded object-location learning task [8]. In the latter experiment, sulpiride (a selective dopamine D2 receptor antagonist) also attenuated fronto-striatal load response, suggesting that drugs such as methylphenidate and sulpiride, which have opposing effects on dopamine transmission at a synaptic level, might have

similar effects on dopamine-related function of large-scale brain systems. This is another observation that is consistent with the inverted-U model already described for the relationship between dopamine tone and prefrontal activation.

Acetylcholine

The acetylcholine system has been studied extensively in psychopharmacological studies of memory, particularly following the proposal of a 'cholinergic model' of Alzheimer's disease. The use of the selective muscarinic acetylcholine receptor antagonist scopolamine has been proposed as a pharmacological model of Alzheimer's disease and its effects can be reversed by compounds that increase the levels of acetylcholine, such as physostigmine, an inhibitor of cholinesterase (which metabolises acetylcholine and thus reduces levels of acetylcholine). phMRI studies are beginning to provide mechanistic accounts of the effects of manipulations of the acetylcholine system on memory processes (Table 1).

The first phMRI study to investigate acetylcholine-mediated modulation of memory systems exploited the temporal resolution of event-related fMRI, in the order of seconds, which allowed the investigators to distinguish drug effects on component processes engaged by task performance (which would be technically intractable with other functional neuroimaging techniques) [9]. Furey *et al*. reported that physostigmine increased the activation of extrastriate cortex during a working memory paradigm, particularly during the early, stimulus-encoding phase of each trial, with reduced activation of the prefrontal cortex [9]. These neurophysiological effects were also associated with improvements in memory performance. The authors interpreted such enhancement of the acetylcholine system as augmenting perceptual 'bottom-up' processing of task-relevant stimuli during encoding, resulting in a reduced requirement for 'top-down' prefrontal involvement in the task. Given this 'tuning' effect of enhancement of the acetylcholine system, Thiel *et al*. predicted a facilitatory role of physostigmine in an operant conditioning paradigm, enhancing activation to behaviourally relevant stimuli (CS+), and decreasing the response to behaviourally irrelevant stimuli (CS−) [10]. However, conditioning-related activations were absent in the presence of physostigmine as a result of augmented responses to the CS− trials; this discrepancy might reflect a region-specific sensitivity to the effects of manipulation of the acetylcholine system. This was supported in a subsequent study in which physostigmine was shown to enhance activity in the anterior fusiform gyrus during conscious attention to facial stimuli compared with consciously unattended faces but suppressed the differential response in the posterolateral occipital cortex for conscious attention to images of houses compared with consciously unattended houses [11]. Indeed, the same pattern was observed for null trials, when subjects were cued but no stimulus appeared. The study therefore demonstrated that the effect of enhancement of the acetylcholine system on selective attention is region specific within the extrastriate cortex and serves to modify general responsiveness.

Further studies have examined the effects of disruption of the acetylcholine system by the muscarinic receptor antagonist scopolamine. Repeated presentation of stimuli results in priming, a reaction time advantage for response to the second stimulus, and 'repetition suppression' of brain activation. Scopolamine has been shown to disrupt both priming of response latency and repetition suppression [12,13]. Intriguingly, in a group of elderly subjects with a mean age of 72 years, no effect of scopolamine on repetition adaptivity was observed, suggesting that functional changes in neurotransmitter systems associated with normal aging might disrupt the contribution of the acetylcholine system to learning and memory [8].

Other neurotransmitters

Benzodiazepines also have amnestic effects behaviourally and can attenuate priming. GABA-mediated mechanisms have been implicated in the adaptivity of brain systems to repeated presentation of the same task or stimulus. Thiel *et al*. used an event-related design to demonstrate attenuation by the benzodiazepine lorazepam of item-specific repetition suppression in frontal and extrastriate cortex [12]. Stephenson *et al*. reported the similar observation that lorazepam abolished repetition adaptivity over a longer time period (minutes) in the context of a blocked periodic working memory experiment. This study also reported a comparable effect of flumazenil, an antagonist at the benzodiazepine binding site of the $GABA_A$ receptor, leading these authors to propose an inverted-U model for the relationship between GABA tone and repetition adaptivity [14]. The hypothesis is that repetition-adaptivity, both attenuation and enhancement of signal with repeated task presentation, and over a range of time scales, might be dependent on dynamic changes in GABA tone. Therefore, drugs that act tonically to either increase or decrease GABA tone outside the physiologically optimal range are expected to impair systems adaptivity to repeated task presentation (Figure 1).

phMRI investigations of glutamate, 5-HT and noradrenaline, in addition to studies of the effects of hormonal changes [15], have been more limited. However, several elegant studies have demonstrated that each of these transmitter systems is amenable to *in vivo* investigation using phMRI (Table 1). Of particular note is the study by Coull *et al*. [16], who examined the α_2-adrenoceptor agonists clonidine and guanfacine. Clonidine was shown to disrupt measures of alerting and temporal and spatial orienting, with concomitant attenuation of neurophysiological responses. However, no behavioural or neurophysiological effects were observed with guanfacine. Although replication over a range of doses will clearly be required, these preliminary findings suggest the intriguing possibility of a functional dissociation using phMRI at the level of (α_2-adrenoceptor) receptor subtypes.

Clinical psychopharmacology

The studies cited so far have incorporated an acute dosing regime, in which relatively selective compounds are used to target a particular transmitter system or receptor complex. In clinical research this experimental design is often not ethical or feasible, or indeed even desirable, given

that the clinical effects of most treatments emerge over a period of several weeks. The careful manipulation of treatment regimes has therefore provided a productive approach in clinical phMRI studies, to explore the mechanisms of pharmacotherapeutic agents and disease pathophysiology (Table 2).

Schizophrenia

The clinical management of patients with schizophrenia has evolved in recent years with the introduction of 'atypical' anti-psychotics. The neurobiological mechanisms that mediate the improved efficacy and reduced parkinsonism associated with these drugs remain unknown. phMRI offers the opportunity to study the effects of these compounds on neurocognitive systems in human patients *in vivo*, and holds considerable promise for future drug discovery and development.

The first phMRI study to investigate the effects of atypical anti-psychotics tested the hypothesis that improvement in negative and cognitive symptoms could reflect remediation of a 'hypodopamine' state in the frontal cortex [17]. Honey *et al*. hypothesized that substituting the atypical anti-psychotic risperidone for typical anti-psychotics would enhance prefrontal function during the performance of a working memory task, hypothetically via increased dopamine-mediated drive to the prefrontal cortex. This was predicted on the basis that patients with schizophrenia perform poorly on tests of working memory, and typically exhibit a hypofrontal response to such tasks, which can be reversed by administration of dopamine receptor agonists [18]. Furthermore, atypical anti-psychotics have been shown to increase prefrontal dopamine-mediated activity in animal models [19]. Patients treated with typical anti-psychotics were scanned during the performance of a working memory task, and then switched to risperidone for 6 weeks treatment, following which patients were re-scanned. In comparison to patients treated with typical anti-psychotics, treatment with risperidone was associated with increased activation of the prefrontal cortex, independent of symptomatic change, providing direct evidence of enhanced prefrontal function following atypical anti-psychotic treatment.

Schizophrenia is increasingly viewed as a disconnection syndrome, in which the inter-regional communication in the brain is compromised, as opposed to a localized deficit in any one region. Accordingly, the mechanism of action of anti-psychotic treatments presumably involves the modulation of inter-regional functional connectivity. Recent studies have begun to characterize the effects of pharmacological treatment on integrative brain function: functional connections between the cerebellum and prefrontal cortex have been shown to be significantly affected by treatment with the anti-psychotic olanzapine [20], and fronto-temporal dysconnectivity in patients receiving anti-psychotics was 'normalized' by adjunctive treatment with the cholinesterase inhibitor donepezil [21]. The integration of phMRI designs with multivariate data analysis of inter-regional connectivity is therefore likely to be of importance in future psychiatric drug development, and a recent study demonstrated that acute pharmacological effects on functional connectivity can be detected in healthy volunteers [22] (Figure 2).

Parkinson's disease

Dopamine-related pathology is an established feature of Parkinson's disease, with degeneration of the substantia nigra pars compacta resulting in disruption of cortico-striato-thalamic circuit function. Mattay *et al*. reported a phMRI study in which competing hypotheses of frontal cortical involvement in the cognitive deficits of Parkinson's disease patients were tested [23]. Activation by a working memory task was increased in the pre-treatment state of low dopamine levels, consistent with a primary mesocortical deficit underlying cognitive dysfunction. However, motor cortical regions were more active during the (post-treatment) dopamine-replete phase, indicating a separable effect of treatment on motor dysfunction mediated via nigrostriatal projections. These studies demonstrate that neurodegenerative changes in dopamine-containing nuclei affect multiple large-scale cortical-subcortical systems

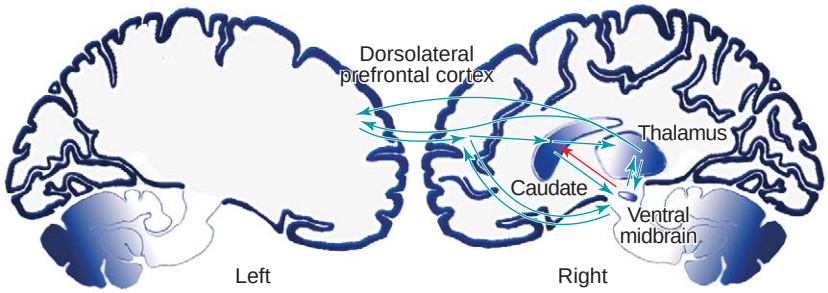

Figure 2. Pharmacological modulation of the dopamine projection between the ventral midbrain and striatum (caudate) in healthy volunteers. Honey *et al.* [23] reported a model of the cortico-striato-thalamic system based on the known anatomical projections between components of the system. Arrows indicate the directional inter-regional effective connections included in the model. The model fit was tested using path analysis in two independent samples of healthy, elderly volunteers ($n = 12$ in each group) and shown to provide an acceptable account of the inter-regional covariance matrix in both groups treated with placebo. Dopamine-mediated (sulpiride and methylphenidate) and non-dopamine-mediated (scopolamine and diazepam) modulation of the path coefficients was tested. Sulpiride was specifically associated with enhancement of effective connectivity in the nigrostriatal projection (highlighted in red). This study demonstrates that in addition to the regional localization of drug effects on brain function, it is also possible to use systems-level multivariate analyses to evaluate the *in vivo* effects of pharmacological manipulations on inter-regional connectivity in the human brain. Reproduced, with permission, from [22].

in the brain and might be partially restored following dopamine repletion.

Unipolar depression

phMRI has been used successfully to examine the effects of anti-depressants on brain function, and has demonstrated that enhanced responses to negative, affectively valent images (e.g. sad or fearful faces) in depressed subjects are normalised in some regions within 2 weeks of treatment, with further changes evident at 8 weeks [24,25]. These changes correspond well to the time-scale of clinical efficacy of anti-depressants and indicate that phMRI is an appropriate tool for evaluating treatment mechanisms. Moreover, phMRI might be used to predict treatment response: consistent with data from other imaging modalities, baseline level of functional activation in the anterior cingulate was related to treatment response at 8 weeks [25]. The prospect of tailoring therapy to patients based on an initial fMRI assessment will be of great clinical and economic benefit, and future studies are likely to place considerable emphasis on this possibility.

Substance addiction

Animal studies have established that the reinforcing properties of addictive substances are linked to the involvement of the dopamine projection from the ventral tegmental area to the nucleus accumbens. phMRI enables research into the neurobiological basis of substance addiction to progress beyond animal models, which are not amenable to measures of subjective experience, such as drug craving, 'rush' and 'high'. In contrast to the phMRI studies reviewed so far, studies investigating the neurobiological effects of drugs of abuse have typically used subjective ratings following a bolus dose as the regressor to identify regional BOLD effects related to drug administration (Table 3), rather than seeking to demonstrate drug modulation of cognitive task-related activation. Breiter *et al*. demonstrated that regions with early and short duration signal maxima, including the ventral tegmental area, pons, basal forebrain, caudate, cingulate and lateral prefrontal cortex, correlated with subjective ratings of rush following administration of cocaine [26]. Regions with early but sustained signal maxima were related to ratings of craving, including the nucleus accumbens, parahippocampal gyrus and amygdala. Similar activation of reward-related circuitry was also reported for the effects of nicotine [27] and alcohol [28]. Schneider *et al*. [29] demonstrated that craving-related activation of subcortical limbic regions in alcoholics in response to alcohol-related cues resolved after 3 weeks of a cognitive-behavioural therapy programme that incorporated controlled abstinence. These findings are unlikely to be related to drug-induced global vascular changes because reductions in cortical blood flow have been observed in the absence of effects of visual stimulation on the BOLD response following administration of cocaine [30] and nicotine [31].

Summary and future directions

In the short period of time that MRI has been used as a psychopharmacological tool of investigation, research has quickly capitalised on the advantages of this technique, exploiting the unparalleled spatio-temporal resolution for non-invasive, *in vivo* mapping of large-scale physiological or neurocognitive properties of centrally active compounds. This has led directly to greater insights into transmitter-related modulation of large-scale neurocognitive systems. The application of phMRI to clinical research has begun to reveal important principles regarding the interaction of cognitive, neurophysiological and genetic mechanisms in disease processes, and this is likely to be extended to an increasingly broad range of symptoms and disorders in future years. To date, studies have focused largely on regionally localized effects of pharmacological manipulations; however, future developments are likely to include an increasing emphasis on inter-regional integrative function in both basic [22] (Figure 2) and clinical [20,21] psychopharmacology. Based on the rapid progress that has been made in this field, phMRI is expected to have an increasingly strong influence on clinical practice and drug discovery.

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