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Mapping the central effects of methylphenidate in the rat using pharmacological MRI BOLD contrast

Neil Easton [a], Fiona H. Marshall [b], Charles A. Marsden [a], Kevin C.F. Fone [a,*]

[a] School of Biomedical Sciences, Medical School, Institute of Neuroscience, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, UK
[b] Heptares Therapeutics Limited, Biopark, Broadwater Rd., Welwyn Garden City, Hertfordshire AL7 3AX, UK

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ABSTRACT

Methylphenidate (Ritalin®) is a selective dopamine reuptake inhibitor and an effective treatment for attention deficit hyperactivity disorder (ADHD) however the anatomical foci and neuronal circuits involved in these therapeutic benefits are unclear. This study determines the temporal pattern of brain regional activity change produced by systemic administration of a therapeutically relevant dose of methylphenidate in anaesthetised Sprague–Dawley rats using BOLD MRI and a 2.35T Bruker magnet. Following 60 min basal recording separate rats received saline ($n = 9$) or ± methylphenidate hydrochloride (2 mg/kg, i.p., $n = 9$) and BOLD changes were recorded for 90 min using statistical parametric maps. Methylphenidate produced significant positive random BOLD effects in the nucleus accumbens, substantia nigra, entorhinal cortex and medial orbital cortex. Negative random BOLD effects were more widespread and intense, occurring in the motor and somatosensory cortices, caudate putamen, lateral globus pallidus and bed nucleus of the stria terminalis, without accompanying changes in blood pressure or respiratory rate. Methylphenidate-induced negative BOLD in the striatum, and other dopamine terminal areas, may reflect post-synaptic changes produced by blockade of the neuronal dopamine reuptake transporter. While increased positive BOLD in the medial orbital cortex may reflect altered dopamine and/or noradrenaline release indirectly altering striatal activity. The overall pattern of BOLD changes is comparable to that seen in previous studies using guanfacine, amphetamine and atomoxetine, and suggests that although these compounds operate through distinct pharmacological mechanisms the BOLD changes may represent a 'fingerprint pattern' predictive of therapeutic benefit in ADHD.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is the most prevalent adolescent psychiatric disorder, which, although it is heterogenous, is characterised by attentional dysfunction, impulsivity, and excessive motor activity (Biederman, 2005; Kutcher et al., 2004; Swanson et al., 2007). Converging evidence demonstrates that ADHD symptoms arise from dysregulation in particular of dopamine-modulated prefrontal cortex (PFC) and striatal circuits (Swanson et al., 2007). Functional deficits in the PFC results in poor impulse control, distractibility, hyperactivity, forgetfulness and poor organisation and planning (Barkley et al., 1992). Functional magnetic resonance imaging (fMRI) has shown supporting evidence for hypofrontality in unmedicated children and adolescents with ADHD (Lee et al., 2005; Rubia et al., 1999; Vaidya et al., 1998; Zang et al., 2005). Furthermore, structural MRI has shown a reduction in basal ganglia volume in particular in boys (Qiu et al., 2009) and a thinner cortex in the right inferior parietal lobe, the dorsolateral PFC and anterior cingulate cortex thought to support attention and executive function (Makris et al., 2007). Low doses of psychostimulants, such as methylphenidate (MPH; Ritalin), are the most effective and widely used drugs of choice for ADHD (reviewed in Greenhill et al., 2006). Indeed, MPH (which was first licensed for the treatment of narcolepsy) is the recommended first line therapy for ADHD in children, adolescents and adults (NICE, 2008; Nutt et al., 2007). Previous neuroimaging studies have shown that MPH improves PFC functional effciency in both ADHD patients (Vaidya et al., 1998) and control subjects (Mehta et al., 2000). Interestingly, MPH appears to increase striatal activation in ADHD but reduce it in healthy children (Vaidya et al., 1998) during response inhibition tasks. Furthermore, MPH exerts opposing effects on intracortical neuronal excitability measured by transcranial magnetic stimulation in healthy subjects and ADHD children (Moll et al., 2003, 2000). There is evidence of reduced dopamine storage in the PFC of ADHD patients (Ernst et al., 1998) and positron emission tomography (PET) shows that oral administration of therapeutic doses of

* Corresponding author. Tel.: +44 (0)115 82 30131; fax: +44 (0)115 82 30142.
E-mail address: kevin.fone@nottingham.ac.uk (K.C.F. Fone).

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MPH displaces striatal binding of the D_2 dopamine receptor ligand, raclopride (Volkow et al., 2001; Wang et al., 1999) consistent with the proposal that clinically effective doses of MPH elevate synaptic dopamine levels in man (Volkow et al., 2005). In agreement with this proposal, low systemic doses of MPH increase extracellular dopamine and noradrenaline release measured by microdialysis in the rat PFC and hippocampus (Berridge et al., 2006; Kuczenski and Segal, 2001). Although ADHD is highly heritable, it is a heterogeneous disorder involving multiple genes each with little to moderate effect. A recent comprehensive meta-analysis (Bobb et al., 2005) concluded that the four genes with the strongest association were the dopamine D4 and D5 receptors, and the dopamine and serotonin transporters, providing further indirect evidence for the involvement of dopamine dysfunction in ADHD. However, most genetic association findings have been inconsistent and relatively few replicated, as reviewed in a recent meta-analysis (Yang et al., 2007). Interestingly both the dopamine reuptake transporter (DAT) and D_2 dopamine receptors are present at very low levels in the PFC (Coulter et al., 1995; Meador-Woodruff et al., 1994) compared to those in the striatum and it has been proposed that MPH may block the noradrenaline transporter (NET) as well as DAT in the PFC (Gatley et al., 1996) and dopamine reuptake may occur by NET in this brain area. It is therefore possible that enhanced noradrenergic neurotransmission in the PFC may contribute to the *in vivo* effects of MPH (Fone and Nutt, 2005). However, no high affinity selective NET inhibitors are currently available to visualise NET sites by single positron emission computed tomography (SPECT) or PET, so the evidence for an effect of MPH on PFC NET is indirect.

The development of small animal pharmacological (ph) MRI allows the brain regional effects of drugs used to treat ADHD to be examined in rodents (Easton et al., 2007a,b, 2006; Shah and Marsden, 2004). An advantage of *in vivo* phMRI is the ability to simultaneously collect temporal data of drug effects in multiple brain regions during a single experiment and compile a global CNS profile of activity. We have previously characterised the acute effects of several other drugs used to treat ADHD (guanfacine, atomoxetine and amphetamine isomers) using phMRI BOLD (Easton et al., 2007a,b, 2006) and compared this to their *in vitro* pharmacological profile on monoamine uptake and release (Easton et al., 2007c). The impact of acute MPH has been previously examined on regional cerebral blood flow with functional imaging using paramagnetic contrast agents in drug niave rats (Mandeville et al., 2004) and following 15 day MPH administration to juvenile rats (Andersen et al., 2008) rather than with pharmacological MRI, and in a potential rodent model of ADHD (Hewitt et al., 2005). A further recent study (Canese et al., 2009) compared the impact of a relatively high dose of MPH (4 mg/kg i.p.) on MRI changes in selected forebrain regions (PFC, nucleus accumbens and hippocampus) in adolescent (PND 34–43) and adult (PND $>$ 60) rats. However, the current study determines the phMRI blood oxygenation level dependent (BOLD) response in the whole brain following acute administration of MPH hydrochloride to drug naive rats and attempts to identify whether there is a common pattern of brain activity changes associated with drugs that are used to treat ADHD, independent of their divergent pharmacological mechanisms of action.

2. Methods

2.1. Animals

Male Sprague Dawley rats (Charles River, UK) were maintained on a 12:12 h normal light/dark schedule (lights on 07.00 h) in groups of three to four per cage and all procedures were carried out in accordance with the local ethical committee, U.K. Animals (Scientific Procedures) Act, 1986 and the principles of laboratory animal care. Food and water were provided *ad libitum* and room temperature (22 $\pm$ 2 °C) and humidity (40–60%) were regulated.

2.2. Animal preparation

Rats (200–250 g; $n = 9$ for saline 1 ml/kg, $n = 9$ for MPH 2 mg/kg i.p.) were anaesthetised with isoflurane (3% induction and then reduced to 1.75–2.0% for maintenance of anaesthesia during surgery and scanning) administered in a mixture of nitrous oxide (0.4–0.6 l/min), oxygen (0.2–0.3 l/min). The right femoral artery was cannulated and blood pressure monitored using a transducer (AD Instruments-model: MLT0380/D) and a saline infusion line placed into the right femoral vein to maintain body fluid homeostasis. To facilitate administration of saline/drug during scanning an intraperitoneal cannula line was inserted into the rat abdomen and fastened to the rat with cyranoacrylate. The anaesthetised rat was transferred to an 'in-house' cradle, designed to fit inside the probe of a Bruker 2.35T Biospec Avance MR system (Bruker Karlsruhe, Germany). Body temperature was maintained at 37 $\pm$ 1 °C via a thermostatically controlled heated water bed fitted into the cradle and monitored using a rectal probe.

2.3. Anaesthetic consideration

Isoflurane was used because it produces stable anaesthesia over a prolonged period with minimal physiological complications. The effect of isoflurane on excitatory neurotransmission is unclear, but even though neuronal coupling is reduced compared to that in the conscious animal it is still clearly detectable with 2% isoflurane (Sicard et al., 2003). Furthermore, oxygen consumption and BOLD have been recorded after forepaw stimulation in isoflurane anaesthetised rats (Liu et al., 2004; Sicard and Duong, 2005) and we have used a similar protocol to record the fMRI effects of other drugs used to treat ADHD (Easton et al., 2007a,b, 2006). Moreover, inhalation anaesthetics have been used by several laboratories to evaluate BOLD changes following pharmacological stimulation in rats (Cash et al., 2002; Jones et al., 2005). The current experimental design includes a separate saline group to eliminate potential confounding effects of isoflurane on the phMRI data obtained. Sequential administration of saline and MPH was not utilised so as to avoid long experimental scan times. While some groups have used awake rats in short duration MRI studies (Febo et al., 2005; Skoubis et al., 2006), the current requirement of scanning for 3–4 h makes this unsuitable for examination of the effect of psychostimulants.

2.4. Drug administration

A repetitive phMRI scanning protocol was used to study MPH (2 mg/kg; i.p. $\pm$ methylphenidate hydrochloride Sigma–Aldrich, Poole, UK dissolved in 0.9% w/v sterile saline) evoked changes in brain signal intensity, measured with the T2-weighted BOLD contrast method in isoflurane anaesthetised rats. BOLD effects were measured at 4 min 40 s intervals, over a 60 min period, at all brain levels for basal effects (prior to drug administration) and a 90 min period post saline or drug administration. The relevance of the current preclinical findings to the therapeutic effect of MPH in man is clearly critically dependent on the legitimacy of the dose of MPH selected. In man therapeutic administration of MPH produces plasma levels of 8–40 ng/mL (Swanson and Volkow, 2002; Teicher et al., 2006) while injection of rats with 0.5 mg/kg i.p. results in plasma levels, of 36 ng/mL in within 5 min (Berridge et al., 2006) which is within this therapeutic range. Behavioural studies in rats show that MPH at doses of 1–2 mg/kg i.p. improves performance in a sustained attention task (Shumsky et al., 2006). Furthermore, by using microdialysis in the conscious rat, doses of MPH between 1 and 5 mg/kg i.p. selectively increase extracellular dopamine and noradrenaline (but not serotonin) both in the rat frontal cortex (Bymaster et al., 2002) and the nucleus accumbens and hippocampus (Kuczenski and Segal, 1997), noradrenaline in the primary sensory cortex (Drouin et al., 2006) and dopamine in the striatum (Schiffer et al., 2006). Furthermore, the dose was selected on the basis of a previous fMRI study (4 mg/kg i.p. MPH) which reported changes in BOLD in a small selective number of brain regions without any accompanying change in heart rate or mean arterial blood pressure, albeit the latter was measured in a separate set of isoflurane anaesthetised rats, not used for MRI (Canese et al., 2009).

2.5. Magnetic Resonance (MR) methods

Radiofrequency (RF) pulses were transmitted using a 72 mm (internal) diameter birdcage coil. An electronically decoupled receive-only coil (4 cm long, 4 cm wide and 1.5 cm high) was placed on the dorsal surface of the rat's head. MR images were acquired using the rapid acquisition relaxation enhanced (RARE) sequence with a field view of 50 mm. An initial anatomical volume data set (flip angle 90°, TE 62.7 ms, TR 5112.5 ms, matrix dimensions 256 $\times$ 256, slice width 1 mm, slice orientation coronal, number of slices 30 with an echo train length of 8 echoes, echo spacing of 15 ms and refocusing of 180°) was acquired to confirm optimal positioning of the rat within the magnet bore. This volume set was also used as an anatomical reference for subsequent functional images. The imaging parameters