Differences in the neurochemical and behavioural profiles of lisdexamfetamine methylphenidate and modafinil revealed by simultaneous dual-probe microdialysis and locomotor activity measurements in freely-moving rats

Helen L Rowley¹, Rajiv S Kulkarni¹, Jane Gosden¹, Richard J Brammer¹, David Hackett² and David J Heal¹

Abstract
Lisdexamfetamine dimesylate is a novel prodrug approved in North America, Europe and Brazil for treating attention deficit hyperactivity disorder (ADHD). It undergoes rate-limited hydrolysis by red blood cells to yield d-amphetamine. Following our previous work comparing lisdexamfetamine with d-amphetamine, the neurochemical and behavioural profiles of lisdexamfetamine, methylphenidate and modafinil were compared by dual-probe microdialysis in the prefrontal cortex (PFC) and striatum of conscious rats with simultaneous locomotor activity measurement. We employed pharmacologically equivalent doses of all compounds and those that spanned the therapeutically relevant and psychostimulant range. Lisdexamfetamine (0.5, 1.5, 4.5 mg/kg d-amphetamine base, per os (po)), methylphenidate (3, 10, 30 mg/kg base, po) and modafinil (100, 300, 600 mg/kg base, po) increased efflux of dopamine and noradrenaline in PFC, and dopamine in striatum. Only lisdexamfetamine increased 5-hydroxytryptamine (5-HT) efflux in PFC and striatum. Lisdexamfetamine had larger and more sustained effects on catecholaminergic neurotransmission than methylphenidate or modafinil. Linear correlations were observed between striatal dopamine efflux and locomotor activity for lisdexamfetamine and methylphenidate, but not modafinil. Regression slopes revealed greater increases in extracellular dopamine could be elicited without producing locomotor activation by lisdexamfetamine than methylphenidate. These results are consistent with clinical findings showing that lisdexamfetamine is an effective ADHD medication with prolonged duration of action and good separation between its therapeutic actions and stimulant side-effects.

Keywords
Dopamine, lisdexamfetamine, locomotor activity, methylphenidate, microdialysis, modafinil, noradrenaline, prefrontal cortex, 5-hydroxytryptamine, striatum

Introduction
Lisdexamfetamine dimesylate is a novel prodrug for the treatment of attention deficit hyperactivity disorder (ADHD) that consists of d-amphetamine (dexamfetamine) covalently conjugated to L-lysine. It is pharmacologically inactive, but following absorption into the blood-stream, lisdexamfetamine undergoes rate-limited, enzymatic hydrolysis by red blood cells to yield its active metabolite, d-amphetamine, and the naturally occurring amino acid, L-lysine (Pennick, 2010). Lisdexamfetamine was approved as a treatment for ADHD in North America in 2007, Brazil in 2011 and in Europe in 2013. The major pharmacological action of lisdexamfetamine’s metabolite, d-amphetamine, is noradrenaline (norepinephrine) and dopamine release in the brain. d-Amphetamine competes with endogenous catecholamines for transfer into presynaptic terminals via the noradrenaline transporter (NET) and the dopamine transporter (DAT) where it displaces these catecholamines from both the cytosolic and vesicular storage pools (Hurd and Ungerstedt, 1989; see also Heal et al., 2013a). By competing for NET and DAT, it also delays neurotransmitter clearance from the synapse (Heal et al., 2013a). As a weak inhibitor of monoamine oxidase (MAO) (Mantle et al., 1976), d-amphetamine may also enhance monoaminergic neurotransmission by decreasing the catabolism of these neurotransmitters (see Heal et al., 2009, 2013a). Although d-amphetamine is highly effective as an ADHD treatment, the ability of this drug to produce rapid and profound increases in dopaminergic neurotransmission endows it with powerful stimulant properties making it liable to recreational abuse (Drevets et al., 2001; Heal et al., 2009, 2013a;
Laruelle et al., 1995). d-Amphetamine is a Schedule 2 Controlled Drug (C-II) in the USA, UK and most other European countries. As a d-amphetamine prodrug, lisdarhamfetamine has also been classified as a C-IV in the USA and several other European countries.

Other stimulant drugs that are efficacious in ADHD include dl-threo-methylphenidate (methylphenidate) (Barkey et al., 1991; Schachter et al., 2001) and modafinil (Biederman et al., 2005; Swanson et al., 2006), although modafinil does not have marketing authorisation for this indication. Methylphenidate is a moderately potent inhibitor of noradrenaline and dopamine reuptake in vitro (Andersen, 1989), but it does not inhibit 5-hydroxytryptamine (5-HT) reuptake (Richelson and Pfennig, 1984). At pharmacologically relevant concentrations, methylphenidate does not inhibit MAO in vitro (Szporny and Görög, 1961) or in vivo (Sharan, 1966). Although methylphenidate is often classified as a noradrenaline and dopamine reuptake inhibitor with stimulant properties, its pharmacological effects in vivo are at odds with this classification (Cheetham et al., 2010; Heal, 2008; Heal et al., 2012). Thus, methylphenidate produces much faster and larger increases in dopamine than conventional dopamine or noradrenaline reuptake inhibitors with no apparent dose-effect ceiling (Heal et al., 2009, 2012; Kuczenski and Segal, 1997; Nomikos et al., 1990), which is similar to the actions of the β-phenylethylamine catecholamine releasing agents (Heal et al., 2009, 2012; Kuczenski and Segal, 1997; Nomikos et al., 1990). This pharmacology explains not only why methylphenidate is rapidly and highly effective in ADHD, but also why it is psycho-stimulant and produces reinforcing effects in humans (Rush et al., 2001; Smith and Davis, 1977). Methylphenidate is a C-II Controlled Drug in the USA, UK and most other European countries.

Modafinil is approved for the medical management of narcolepsy. Although it has stimulant-like effects in humans and animals, it has a complex pharmacology which is clearly different from those of the catecholaminergic stimulants like d-amphetamine and methylphenidate. Although modafinil has only low millimolar affinity for DAT (Madras et al., 2006; Zolkowska et al., 2009) and no affinity for NET or 5-hydroxytryptamine transporter (SERT) (Madras et al., 2006; Minzenberg and Carter, 2008), positron emission tomography experiments in humans have paradoxically revealed that therapeutic doses of modafinil occupy a substantial proportion of striatal DAT sites in human subjects and increase synaptic dopamine concentrations (Volkow et al., 2009). Modafinil produced dose-dependent reports of ‘drug liking’ and ‘high’ in polydrug users (Jasinski, 2000) and a significant level of cocaine-appropriate responding in individuals trained to distinguish cocaine from placebo (Rush et al., 2002). Modafinil is a Schedule 4 Controlled Drug (C-IV) in the USA, but is not classified as a Controlled Drug in most European countries.

The pharmacology that mediates the therapeutic actions of these stimulants is also responsible for their many of their side-effects (see Heal and Pierce, 2006; Heal et al., 2012, 2013a). The aim of this study was, therefore, to compare the neurochemical and behavioural effects of various doses of lisdarhamfetamine, methylphenidate and modafinil to obtain a greater insight into the balance between efficacy and side-effects. This objective was achieved by combining dual-probe microdialysis sampling of the extracellular concentrations of the monoamine neurotransmitters, noradrenaline, dopamine and 5-HT, and their major metabolites in the brains of freely-moving rats with simultaneous locomotor activity measurements. The brain regions selected were the PFC and striatum because both areas are believed to be important for the therapeutic actions of ADHD drugs (Amstern and Dudley, 2005; Heal et al., 2009, 2012). Since profound enhancement of dopaminergic neurotransmission in the striatum is associated with the psychostimulant effects of stimulant drugs in animals (Bradberry et al., 2000; Kuczenski and Segal, 1989; Rowlow et al., 2012; Sharp et al., 1987) and humans (Drevets et al., 2001; Martinez et al., 2007; Volkow et al., 1999), changes in the efflux of this neurotransmitter supplemented by locomotor activity measurements were used as a surrogate of the potential to cause psychostimulant adverse events.

**Methods and materials**

**Animals and environment**

Experiments were carried out in male Sprague-Dawley rats (250–350 g; Charles River UK). Animals were housed in groups of four on a 12 hour/12 hour light/dark cycle (lights on at 07:00) at an ambient temperature of 21±2°C and 55±20% humidity. Food and water were available ad libitum. Animals were allowed to acclimatise to these conditions for at least five days prior to the study.

All experiments were performed in strict accordance with Home Office Guidelines and licensed under the Animals (Scientific Procedures) Act 1986.

**Surgery**

Rats were anaesthetised with isoflurane (5% to induce, 2% to maintain) in O₂ (1 L/min) delivered via an anaesthetic unit (Burtons Medical Equipment Ltd, UK). A dual-probe study was performed whereby two concentric microdialysis probes with an exposed polyarylethersulphone (PAES) membrane tip (CMA, Sweden) were stereotaxically implanted bilaterally into (a) the prefrontal cortex (2 mm tip, coordinates: AP: +3.2 mm; L: +/-2.5 mm relative to bregma; V: –4.0 mm relative to the skull surface) and (b) the striatum (4 mm tip, coordinates: AP: +0.2 mm; L: +/-3.0 mm relative to bregma; V: –7.8 mm relative to the skull surface). Coordinates were taken from the stereotaxic atlas of Paxinos and Watson (1986). The upper incisor bar was set at 3.3 mm below the interaural line so that the skull surface between bregma and lambda was horizontal. Additional burr holes were made for skull screws (stainless steel) and the probes were secured using dental cement.

Following surgery, animals were individually housed in the dialysis bowl (245 mm internal diameter at base of bowl, 360 mm wall height) Culex Bambino (BAS, Inc, USA) apparatus with the microdialysis probes connected to swivels and a counter-balanced arm to allow unrestricted movement. Rats were allowed a recovery period of at least 16 h with food and water available ad libitum. During this time, the probes were continuously perfused at a flow rate of 1.2 µL/min with an artificial cerebrospinal fluid (aCSF; Harvard Apparatus, UK) of the following electrolyte composition (in mM): sodium 150; potassium 3.0; magnesium 0.8; calcium 1.4; phosphate 1.0; chloride 155.0, pH 7.2.
Microdialysis, locomotor activity measurement and administration of drugs

Dialysate samples were collected from freely-moving rats at 15 min intervals from 60 min before drug administration until 5 h after drug administration (four basal samples and 20 post-drug samples). Samples were collected into Eppendorf vials (300 µL) contained within a refrigerated fraction collector. Vials contained 5.0 µL of 0.1 M perchloric acid to prevent oxidation of monoamines. After completion of the experiment, all samples were stored at −80°C until analysis, which was conducted over the remainder of the working week. Samples collected from the PFC were assayed for noradrenaline, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT and 5-hydroxyindolacteric acid (5-HIAA), whilst samples from the striatum were assayed for dopamine, DOPAC, HVA, 5-HT and 5-HIAA. Noradrenaline was not determined in the striatum because the levels were the below the limit of detection.

The Ratum (BAS, Inc, USA) system that is part of the Culex Bambino apparatus automatically measures the locomotor activity of rats as the total time spent that they spent moving around the dialysis bowl during each 15 min period throughout the 5h experiment.

Drugs and selection of doses

Lisdexamfetamine dimesylate was supplied by Shire Pharmaceuticals (SPD489; Lot No 040900118): vehicle (deionised water; pH 7.7–7.8) was added to the vial containing lisdexamfetamine, the vial was shaken to produce a clear solution that was suitable for dosing (pH 0.5 mg/kg solution 6.5–7.0; 1.5 mg/kg solution 6.4–6.5; 4.5 mg/kg solution 5.7–5.9).

Methylphenidate hydrochloride was supplied by Macfarlan Smith Ltd (Edinburgh, UK): vehicle (1% methylcellulose in deionised water; pH 7.1–7.7) was added to the vial containing methylphenidate; the vial was shaken and sonicated for 1 min to produce a clear solution that was suitable for dosing (pH 3 mg/kg solution 6.7–7.2; 10 mg/kg solution 6.9–7.5; 30 mg/kg solution 6.4–6.9).

Modafinil free base was supplied by Tocris (Batch No 2A/113911): vehicle (1% methylcellulose in deionised water; pH 7.1–7.7). The compound was ground using a pestle and mortar and vehicle was added gradually to make a white opaque suspension (pH 6.0) suitable for oral dosing.

Compounds and vehicles were dosed orally (per os (po)) with dosage route by gavage at 2 mL/kg.

Doses of lisdexamfetamine are reported as mg/kg d-amphetamine base using the relevant factor of 3.37. The doses used equate to 1.69, 5.05 and 15.17 mg/kg, po of lisdexamfetamine dimesylate or 0.95, 2.84 and 8.54 mg/kg, po of lisdexamfetamine base. Doses of methylphenidate are expressed as mg/kg base using the relevant factor of 3.37. The doses used equate to rat dopamine (0.5 mg/kg, ip) from saline (Heal et al., 2013b). The low and high doses of these compounds were set at 0.5 log units above and below this value, i.e. 0.5, 1.5 and 4.5 mg/kg, po for lisdexamfetamine and 3, 10 and 30 mg/kg, po for methylphenidate (Heal et al., 2013b).

We adopted a similar approach for selecting the oral doses of modafinil, but because this drug was only tested in the d-amphetamine-cued drug discrimination model by the intraperitoneal (ip) route (Heal et al., 2013b), absolute pharmacological equivalence cannot be claimed for the comparison between lisdexamfetamine and modafinil. In rats, modafinil showed high level partial generalisation to d-amphetamine at doses of 150 and 200 mg/kg, ip (Heal et al., 2013b). Ishizuka et al. (2008) reported that the increase in rats' locomotor activity induced by modafinil (150 mg/kg, ip) was not significantly different from that caused by methylphenidate (3 mg/kg, ip), and in our hands, methylphenidate (3 mg/kg, ip) also generalised fully to d-amphetamine in rat drug-discrimination testing (Heal et al., 2013b). In this investigation, we also observed that the potency of stimulants increased approximately three-fold when dosing was switched from the oral to the ip route. Using these data as a guide, we selected 300 mg/kg, po as the intermediate dose of modafinil (Heal et al., 2013b). The 0.5 log unit lower dose of modafinil, i.e. 100 mg/kg, po, was also tested. However, a pilot experiment revealed that the 0.5 log unit higher dose of modafinil of 900 mg/kg, po was not suitable for testing as in rats it produced intense stereotypy, precluding measurement of locomotor activation. For this reason, 600 mg/kg, po was selected as the highest dose of modafinil suitable for evaluation.

Clinically effective doses of lisdexamfetamine, methylphenidate and modafinil in ADHD are approximately 1.0–1.3 mg/kg, po, 0.3–0.5 mg/kg, po and 10–15 mg/kg, po (Biederman et al., 2005), respectively. When a 10-fold factor is applied to take into account the allometric and pharmacokinetic differences between humans and rats (Caldwell et al., 2004), these doses would translate to oral doses in rats of 10–13 mg/kg for lisdexamfetamine dimesylate, 3.0–5.0 mg/kg for methylphenidate hydrochloride and 100–150 mg/kg for modafinil, which are similar to those employed in this experimental study.

High-performance liquid chromatography (HPLC) analysis

Detection and subsequent quantification of noradrenaline, dopamine, DOPAC, HVA, 5-HT and 5-HIAA in the dialysis samples was achieved by reverse-phase, ion-pair HPLC coupled with electrochemical detection and involved the use of an ALEXYS monoamine analyser (Antec Leyden, The Netherlands). The system consisted of two separate analytical columns (ALF-115, 150 mm × 1 mm internal diameter) that shared a dual-loop autosampler, allowing for one sample to be simultaneously analysed by two systems optimised for different neurotransmitters. One column separated noradrenaline, DOPAC, HVA and 5-HIAA, while the other separated dopamine and 5-HT. Two solvent delivery pumps (LC 110) were used to circulate the respective mobile phases (noradrenaline, DOPAC, HVA and 5-HIAA: 50 mM citric acid, 50 mM phosphoric acid, 8 mM NaCl, 0.1 mM EDTA, 3 mM l-octane sulphonic acid, 10% methanol, pH 3.25; DA/5-HT: 50 mM phosphoric acid, 8 mM NaCl, 0.1 mM EDTA, 2.5 mM l-octane sulphonic acid, 20% methanol, pH 6.0) at a flow rate of
50 µL/min. An Antec in-line degassing unit was used to remove air. Samples (10 µl) were injected onto the columns via an autosampler (AS 110) with a cooling tray set at 4°C. Antec DECADE II electrochemical detectors were used comprising Antec micro VT 03 cells employing a high-density, glassy carbon working electrode (+0.59 V for NA, DOPAC, HVA and 5-HIAA; +0.3 V for dopamine and 5-HT) combined with an Antec ISAAC reference electrode.

The electrode signal was integrated using Antec’s CLARITY data acquisition system. Individual stock solutions of noradrenaline, dopamine, DOPAC, HVA, 5-HT and 5-HIAA (1.0 mM) were prepared by their dissolution in a mixture of equal quantities of deionised water and 0.1 M perchloric acid and stored at 4°C. A working solution containing all the transmitters and metabolites was prepared daily by dilution in aCSF.

At the end of the experiment, the rats were killed; their brains were removed rapidly and stored in a 10% v/v formal saline solution for a minimum of five days. Probe placements were visualised and localised with reference to a stereotaxic atlas (Paxinos and Watson, 1986). Data are reported only from animals where probe membranes were correctly positioned.

Reagents

All reagents used in HPLC analysis were of HPLC grade. Phosphoric acid, EDTA, methanol, l-octane sulphonic acid, perchloric acid, citric acid, sodium chloride and 10% formal saline solution were obtained from Fisher Scientific (UK). (-) Noradrenaline (Batch No. 088K1147), dopamine hydrochloride (Batch No. 1381581), DOPAC (Batch No 1364209), HVA (Batch No 107K5002), 5-hydroxytryptamine creatinine sulphate (Batch No. BCBB9681) and 5-HIAA (Batch No 046K1520) were purchased from Sigma-Aldrich (UK).

Behavioural assessment

General observations of the behaviour of the rats were made throughout the 5h microdialysis sampling period. These behaviours included stereotypy, increased vigilance, exploratory behaviour, resting, sleeping, sniffing, rearing, chewing and circling.

Statistical analysis

All microdialysis data were log transformed. Baseline was defined as the geometric mean of the four pre-drug administration samples. Both raw data and percent of baseline values were analysed. Analysis of covariance (ANCOVA) with log(baseline) as a covariate was employed. Each dose of drug was compared to vehicle values by Williams’ test.

Locomotor activity was recorded as the time spent active during 15 min time-bins. Data were square root transformed. Baseline was defined as the back-transformed mean of the four pre-drug activity scores. Analysis was by analysis of covariance with √(baseline) as a covariate.

Each time-point was analysed separately for both the microdialysis samples and locomotor activity. A p value of <0.05 was considered statistically significant.

Linear relationships were plotted between increases in extracellular concentrations of striatal dopamine and locomotor activity. Data for the highest doses of lisdexamfetamine (4.5 mg/kg, po), methylphenidate (30 mg/kg, po) or modafinil (600 mg/kg,) were plotted as the mean activity (min moving/15 min time-bin) against extracellular dopamine concentrations (percentage of baseline). Activity data was analysed by ANCOVA with √(baseline) as covariate. dopamine data was analysed by ANCOVA with log(baseline) as a covariate.

Results

Baseline concentration of extracellular monoamines and their metabolites

Table 1 reports the basal efflux of noradrenaline, dopamine and 5-HT, and their metabolites, DOPAC, HVA and 5-HIAA in the PFC and striatum for the groups of rats receiving lisdexamfetamine, methylphenidate, modafinil and water (vehicle). One-way analysis of variance of the log-transformed data revealed there were no statistically significant differences between the basal values of these monoamines and their metabolites across the treatment groups with the exception of the basal dopamine efflux in the modafinil group. Basal efflux of dopamine in the PFC and striatum of the modafinil group was significantly lower than that of the corresponding vehicle controls.

Lisdexamfetamine

Effects of lisdexamfetamine on extracellular concentrations of monoamines and metabolites in the prefrontal cortex. Lisdexamfetamine (0.5, 1.5 and 4.5 mg/kg, po) significantly increased the extracellular concentration of noradrenaline in the PFC (Figure 1(a); Table 2). There was no difference between the magnitude or duration of effect following administration of 0.5 and 1.5 mg/kg, po. At the highest dose, the extracellular concentration of noradrenaline was significantly increased 45 min after administration and it remained elevated for the duration of the experiment (Figure 1(a)).

Lisdexamfetamine (0.5, 1.5 and 4.5 mg/kg, po) increased dopamine efflux in the PFC between 60–300 min after administration (Figure 2(a); Table 2). None of the doses of lisdexamfetamine produced any significant effect on extracellular levels of DOPAC or HVA (Table 3).

Although much smaller and less sustained than the enhancement of noradrenaline and dopamine, lisdexamfetamine (0.5 and 1.5 mg/kg, po) nonetheless produced sporadic significant increases in 5-HT efflux in the PFC (Figure 3(a)). The highest dose of lisdexamfetamine (4.5 mg/kg, po) resulted in a transient increase in cortical 5-HT efflux in the initial 60 min after dosing (Figure 3(a)). Lisdexamfetamine had no significant effect on extracellular 5-HIAA levels in PFC (Table 3).

Effects of lisdexamfetamine on extracellular concentrations of monoamines and metabolites in the striatum. Lisdexamfetamine (0.5, 1.5 and 4.5 mg/kg, po) dose-dependently increased dopamine efflux in the striatum (Figure 4(a), Table 2). The increases were gradual in onset reaching a maximum 75–90 min
Table 1. Baseline extracellular concentrations of monoamines and metabolites in the prefrontal cortex and striatum.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Basal extracellular concentration of monoamine (fmol/5 μL/15 min)</th>
<th>Vehicle (Water, po)</th>
<th>Lisdexamfetamine (0.5–4.5 mg/kg, po)</th>
<th>Vehicle (Water, po)</th>
<th>Methylphenidate (3–30 mg/kg, po)</th>
<th>Modafinil (100–600 mg/kg, po)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prefrontal cortex</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>2.98±0.99</td>
<td>2.38±0.44</td>
<td>0.96±0.37</td>
<td>1.65±0.44</td>
<td>0.94±0.30</td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>0.717±0.154</td>
<td>0.772±0.131</td>
<td>0.702±0.097</td>
<td>0.668±0.190</td>
<td>0.214±0.025</td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>0.056±0.008</td>
<td>0.072±0.012</td>
<td>0.050±0.014</td>
<td>0.047±0.008</td>
<td>0.031±0.003</td>
<td></td>
</tr>
<tr>
<td>DOPAC</td>
<td>162±30</td>
<td>155±28</td>
<td>53±15</td>
<td>65±12</td>
<td>56±9</td>
<td></td>
</tr>
<tr>
<td>HVA</td>
<td>380±56</td>
<td>370±34</td>
<td>171±41</td>
<td>205±20</td>
<td>156±14</td>
<td></td>
</tr>
<tr>
<td>5-HIAA</td>
<td>491±49</td>
<td>323±42</td>
<td>237±30</td>
<td>170±24</td>
<td>153±22</td>
<td></td>
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<tr>
<td><strong>Striatum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dopamine</td>
<td>43.7±5.0</td>
<td>40.6±4.0</td>
<td>16.9±1.2</td>
<td>16.5±2.0</td>
<td>9.2±0.9</td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>0.212±0.033</td>
<td>0.104±0.033</td>
<td>0.017±0.002</td>
<td>0.022±0.002</td>
<td>0.024±0.004</td>
<td></td>
</tr>
<tr>
<td>DOPAC</td>
<td>11,740±1150</td>
<td>8450±1100</td>
<td>7090±1080</td>
<td>6880±830</td>
<td>7630±560</td>
<td></td>
</tr>
<tr>
<td>HVA</td>
<td>6520±490</td>
<td>6390±660</td>
<td>5430±690</td>
<td>5180±310</td>
<td>5190±390</td>
<td></td>
</tr>
<tr>
<td>5-HIAA</td>
<td>1700±110</td>
<td>1280±190</td>
<td>830±160</td>
<td>730±77</td>
<td>690±79</td>
<td></td>
</tr>
</tbody>
</table>

5-HIAA: 5-hydroxyindolacetic acid; 5-HT: 5-hydroxytryptamine; DOPAC: 3,4-dihydroxyphenylacetic acid; HVA: homovanillic acid; po: per os.

Data are means ± standard error of the mean (SEM). Means are back-transformed and adjusted for differences between treatment groups at baseline. SEMs are calculated from the residuals of the statistical model. F tests are for one-way analysis of variance (ANOVA) of the log-transformed data.

Doses of lisdexamfetamine are expressed as mg/kg of d-amphetamine base equivalents, while those of methylphenidate and modafinil are expressed as base.

after dosing and sustained with significant increases observed ≤150 min at the lowest dose and ≤210 min at the two higher ones (Figure 4(a)). The two lower doses of lisdexamfetamine produced trends for decreases in extracellular DOPAC levels that just failed to reach statistical significance (p=0.065 and p=0.05 in the 0–5.0 h time-bin, respectively; Table 3). Lisdexamfetamine (4.5 mg/kg, po) produced a gradual, sustained reduction in extracellular DOPAC with a 75±10% maximal decrease at 300 min (p<0.05) (Table 3). Extracellular HVA levels were also significantly reduced following administration of lisdexamfetamine 0.5, 1.5 and 4.5 mg/kg, po) between 75–300 min (Table 3). Maximal reductions from baseline were 28±6% (p<0.001), 28±6% (p<0.001) and 47±5% (p<0.001) at 0.5, 1.5 and 4.5 mg/kg, po, respectively.

Lisdexamfetamine (0.5 mg/kg, po) did not significantly alter the extracellular concentration of 5-HT in the striatum (Figure 5(a), Table 2). At the intermediate dose of 1.5 mg/kg, po, striatal 5-HT efflux was slightly elevated above control levels for ~180 min post-administration; an effect that reached statistical significance at 90 min (Figure 5). At 4.5 mg/kg, po, striatal 5-HT efflux was significantly increased from 60–135 min with a maximal increase at 75 min (Figure 5(a)). Lisdexamfetamine (0.5, 1.5 and 4.5 mg/kg, po) had no significant effect on the extracellular level of 5-HIAA (Table 3).

Effects of lisdexamfetamine on locomotor activity and behaviour. Lisdexamfetamine (0.5 and 1.5 mg/kg, po) did not significantly increase locomotor activity of the rats at any time-point (Figure 6(a)). At the highest dose, lisdexamfetamine (4.5 mg/kg, po), increased rat locomotor activity between 45–300 min. The level of activity was relatively low (~2–3 min activation during each 15 min time-bin), maximal at 60 min, and it remained relatively stable throughout the experiment.

Behavioural changes induced by lisdexamfetamine (0.5, 1.5 and 4.5 mg/kg, po) were dose-dependent. Rats administered lisdexamfetamine (0.5 mg/kg, po) exhibited a behavioural profile that was very similar to that of the vehicle-treated animals with only a small increase in exploratory behaviour immediately after dosing that lasted ~5–10 min. The animals tended to rest or sleep for the remainder of the experiment. Rats administered 1.5 mg/kg, po of lisdexamfetamine exhibited a noticeable increase in overall exploratory behaviour. The onset of activity varied between 15–60 min from the time of dosing. This exploratory behaviour manifested itself as an increase in sniffing, rearing,
Figure 1. Effect of lisdexamfetamine, methylphenidate and modafinil on extracellular noradrenaline (NA) levels in prefrontal cortex. Data are means ± standard error of the mean (SEM); n=5–8. Means are back-transformed and adjusted for differences between treatment groups at baseline. SEMs are calculated from the residuals of the statistical model. For clarity, the SEMs are not shown on the figures. SEM values as percentages of the mean were: Vehicle=18–48; Lisdexamfetamine=19–38 (0.5 mg/kg, per os (po)), 18–48 (4.5 mg/kg, po); Methylphenidate=25–70 (3 mg/kg, po), 25–41 (30 mg/kg); Modafinil=27–54 (100 mg/kg, po), 26–65 (300 mg/kg), 27–70 (600 mg/kg). Doses of lisdexamfetamine are expressed as d-amphetamine base equivalents, and methylphenidate and modafinil doses are as base. Time of administration is indicated by the vertical arrows. Data were analysed by analysis of covariance (ANCOVA) with each dose of drug compared to the vehicle-treated control group by Williams’ test. Significant differences are denoted by the open symbols.

Table 2. Effect of lisdexamfetamine (LDX), methylphenidate and modafinil on extracellular noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) levels in the prefrontal cortex and striatum.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Prefrontal cortex</th>
<th>Striatum</th>
<th></th>
<th></th>
<th></th>
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po: per os.

Results represent ↑ an increase or ± no change in the extracellular concentrations of the noradrenaline, dopamine, and 5-HT relative to the appropriate vehicle control. Data are based on statistical comparisons of the AUC over at least a single one-hour time-bin after drug administration, i.e. 0–1 hr, 2–3 hr, 3–4 h or 4–5 h. Doses of lisdexamfetamine are expressed as mg/kg of d-amphetamine base, while those of methylphenidate and modafinil are expressed as base.
Figure 2. Effect of lisdexamfetamine, methylphenidate and modafinil on extracellular dopamine (DA) levels in prefrontal cortex.

Data are means ± standard error of the mean (SEM); n=5–8. Means are back-transformed and adjusted for differences between treatment groups at baseline. SEMs are calculated from the residuals of the statistical model. For clarity, the SEMs are not shown on the figures. SEM values as percentages of the mean were: Vehicle=14–44; Lisdexamfetamine=16–31 (0.5 mg/kg, per os (po)), 15–23 (1.5 mg/kg, po), 15–22 (4.5 mg/kg, po); Methylphenidate=22–44 (3 mg/kg, po), 23–44 (10 mg/kg, po), 22–43 (30 mg/kg, po); Modafinil=16–46 (100 mg/kg, po), 23–50 (300 mg/kg, po), 18–48 (600 mg/kg, po). Doses of lisdexamfetamine are expressed as d-amphetamine base equivalents, and methylphenidate and modafinil doses are as base. Time of administration is indicated by the vertical arrows. Data were analysed by analysis of covariance (ANCOVA) with each dose of drug compared to the vehicle-treated control group by Williams’ test. Significant differences are denoted by the open symbols.

chewing and locomotor activity around the circular bowls that lasted 1–2 h. Rats given the highest dose of lisdexamfetamine (4.5 mg/kg, po) showed increased sniffing, rearing, chewing and circling. The onset of this increase was again variable (15–60 min after dosing), and in some cases, the increase in activation was sustained for the duration of the experiment. In animals where increased locomotor activity around the circular bowls was not as apparent, signs of behavioural activation were always evident. Large increases in sniffing and rearing were also observed in all the rats in this treatment group.

**Methylphenidate**

**Effects of methylphenidate on extracellular concentrations of monoamines and metabolites in the PFC.** Methylphenidate (3, 10 and 30 mg/kg, po) did not significantly increase noradrenaline efflux in the PFC at the lowest dose (Figure 1(b), Table 2). The two higher doses both produced significant increases in noradrenaline efflux of approximately equal magnitude and duration (Figure 1(b)). Compared with lisdexamfetamine, the time of peak effect of methylphenidate was earlier at 60 min, the effect size was smaller and there was a clear dose-ceiling on the maximum increase of noradrenaline efflux.

The effects of methylphenidate (3, 10 and 30 mg/kg, po) on extracellular dopamine levels in the PFC were subtly different from its effects on noradrenaline with significant increases observed at all doses (Figure 2(b), Table 2). The lowest dose of 3 mg/kg, po, methylphenidate produced a maximal increase of 202% at 60 min (p<0.05), but this effect was of short duration, i.e. 60–90 min. The two higher doses of methylphenidate (10 and 30 mg/kg, po) produced maximal increases of 217% (p<0.01) and 343% (p<0.01), respectively, that were sustained at the highest dose. Once again, the peak effects were observed 45–60 min after dosing, but in this case, there was no apparent ceiling on the effect of methylphenidate (Figure 2(b)). Methylphenidate (3, 10 and 30 mg/kg, po) produced gradual and sustained decreases in extracellular DOPAC. Although the decreases were not dose-related, they were maintained at the two lower doses, but returned to vehicle control values after ~3.0 h at the highest dose (Table 3). The maximum decreases were 41% of baseline at 165 min.
(p<0.05), 35% at 210 min (p<0.05) and 52% at 180 min (p<0.05) for 3, 10 and 30 mg/kg, po, respectively. The only significant changes in extracellular HVA were observed after the 30 mg/kg, po dose with maximal ~90% (p<0.05) decreases at 105 min and 120 min (Table 3).

Cortical 5-HT efflux was not significantly changed after administration of methylphenidate (3, 10 and 30 mg/kg, po) (Figure 3(b), Table 2). The only significant effect of methylphenidate on extracellular concentration of 5-HIAA was a decrease to 61% (p<0.01) of baseline at 60 min in rats receiving methylphenidate (30 mg/kg, po) (Table 3).

Effects of methylphenidate on extracellular concentrations of monoamines and metabolites in the striatum. Methylphenidate (3, 10 and 30 mg/kg, po) had no effect on extracellular dopamine in the striatum at the lowest dose and it induced only a small increase at the higher doses of 10 mg/kg, po; maximal increase of 131% of baseline at 60 min (p<0.01) (Figure 4(b), Table 2). It was only at the highest dose of 30 mg/kg, po that methylphenidate produced a moderate and sustained increase in striatal dopamine efflux. The peak increase of 243% (p<0.001) of baseline occurred 45 min after drug administration (Figure 4(b)). Methylphenidate (3, 10 and 30 mg/kg, po) did not significantly alter extracellular levels of DOPAC (Table 3). Extracellular HVA levels declined gradually following administration of vehicle and compared with control values, methylphenidate (3, 10 and 30 mg/kg, po) evoked significant increases in extracellular HVA that were not dose-dependent (Table 3). HVA was most consistently elevated 165–300 min after dosing. The maximum increases in extracellular HVA expressed as percentage of above saline control levels were 24% at 240 min (p<0.01), 20% at 210 min (p<0.05) and 18% at 210 min (p<0.05), respectively, at methylphenidate doses of 3, 10 and 30 mg/kg, po.

Methylphenidate (3, 10 and 30 mg/kg, po) produced only sporadic and inconsistent changes in extracellular 5-HT levels in the striatum (Figure 5(b), Table 2). It induced a significant increase in 5-HT efflux at only one time-point, i.e. 319% of baseline (p<0.01) 45 min after administration of 30 mg/kg, po. This was

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**Figure 3.** Effect of lisdexamfetamine, methylphenidate and modafinil on extracellular 5-hydroxytryptamine (5-HT) levels in prefrontal cortex. Data are means ± standard error of the mean (SEM); n=5–8. Means are back-transformed and adjusted for differences between treatment groups at baseline. SEMs are calculated from the residuals of the statistical model. For clarity, the SEMs are not shown on the figures. SEM values as percentages of the mean were: Vehicle=15–82; Lisdexamfetamine=15–42 (0.5 mg/kg, per os (po)), 18–91 (1.5 mg/kg, po), 19–53 (4.5 mg/kg, po); Methylphenidate=20–65 (3 mg/kg, po), 24–48 (10 mg/kg, po), 17–55 (30 mg/kg, po); Modafinil=15–52 (100 mg/kg, po), 16–53 (300 mg/kg, po), 21–59 (600 mg/kg, po). Doses of lisdexamfetamine are expressed as d-amphetamine base equivalents, and methylphenidate and modafinil doses are as base. Time of administration is indicated by the vertical arrows. Data were analysed by analysis of covariance (ANCOVA) with each dose of drug compared to the vehicle-treated control group by Williams’ test. Significant differences are denoted by the open symbols.
Table 3. Effect of lisdexamfetamine (LDX), methylphenidate and modafinil on extracellular 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA) levels in the prefrontal cortex and striatum.

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po: per os.

Results represent an increase, a decrease or no change in the extracellular concentrations of the dopamine metabolites, DOPAC and HVA, and the 5-HT metabolite, 5-HIAA, relative to the appropriate vehicle control. Data are based on statistical comparisons of the area under the curve (AUC) over at least a single one-hour time-bin after drug administration, i.e. 0–1 hr, 2–3 hr, 3–4 hr or 4–5 hr. Doses of lisdexamfetamine are expressed as mg/kg of d-amphetamine base equivalents, while those of methylphenidate and modafinil are expressed as base.

Figure 4. Effect of lisdexamfetamine, methylphenidate and modafinil on extracellular dopamine (DA) levels in striatum.

Data are means ± standard error of the mean (SEM); n=5–8. Means are back-transformed and adjusted for differences between treatment groups at baseline. SEMs are calculated from the residuals of the statistical model. For clarity, the SEMs are not shown on the figures. SEM values as percentages of the mean were: Vehicle=8–12; Lisdexamfetamine=8–10 (0.5 mg/kg, po), 8–12 (1.5 mg/kg, po), 8–18 (4.5 mg/kg, po); Methylphenidate=9–17 (3 mg/kg, po), 9–21 (10 mg/kg, po), 9–15 (30 mg/kg, po); Modafinil=10–33 (100 mg/kg, po), 10–18 (300 mg/kg, po), 10–15 (600 mg/kg, po). Doses of lisdexamfetamine are expressed as d-amphetamine base equivalents, and methylphenidate and modafinil doses are as base. Time of administration is indicated by the vertical arrows. Data were analysed by analysis of covariance (ANCOVA) with each dose of drug compared to the vehicle-treated control group by Williams’ test. Significant differences are denoted by the open symbols.
followed by a decrease to 59% baseline at 240 min ($p < 0.01$; Figure 5(b)). At the lower doses of 3 and 10 mg/kg, po, methylphenidate significantly decreased 5-HT efflux to 60–83% of baseline at 240 min post-administration ($p < 0.01$, $p < 0.001$, respectively; Figure 5(b)). An area under the curve (AUC) analysis revealed that compared to vehicle control, all of the doses of methylphenidate significantly decreased 5-HT efflux in 3.0–4.0 h time-bin, but evoked no changes in any of the others.

Methylphenidate (3, 10 and 30 mg/kg, po) exerted a similar mixed pattern of effects on extracellular 5-HIAA levels (Table 3). At the lowest and highest doses, methylphenidate induced significant increases 210–300 min and 150–300 min, respectively (Table 3). The maximum increases at 3 and 30 mg/kg, po were 184% at 270 min ($p < 0.05$) and 129% at 225 min ($p < 0.01$), respectively. In contrast, methylphenidate (10 mg/kg, po) decreased extracellular 5-HIAA by 50–60% ($p < 0.05$) over the same period (Table 3).

Effects of methylphenidate on locomotor activity and behaviour. Methylphenidate (3 mg/kg, po) had no effect on locomotor activity with only a single significant ($p < 0.05$) increase at the 60 min time-point (Figure 6(b)). The two higher doses of methylphenidate (10 and 30 mg/kg, po) caused dose-related increases in rat locomotor activity from 45–60 min and 15 – 255 min, respectively. The level of activation at the highest dose peaked at 45 min with 4.74 min activity/15 min time-bin ($p < 0.001$), and it then declined gradually thereafter.

The locomotor effects of methylphenidate (3, 10 and 30 mg/kg, po) were accompanied by dose-dependent behavioural changes. After the 3 mg/kg, po dose, methylphenidate produced very little overt activation and the rats spent much of the period after methylphenidate administration either resting or asleep. At the intermediate dose, some activation was seen with alerting behaviour, increased vigilance and locomotor activity that started 15–30 min after dosing and continued for the next 90 min. At 30 mg/kg, po, methylphenidate produced a range of typical stimulant-induced behaviours including exploratory activity, sniffing, rearing and walking around the circular dialysis chambers. The onset of these behaviours was rapid and reached a peak ~45 min after drug administration. There was marked variability in the responses of the rats to methylphenidate (30 mg/kg, po) with some rats showing profound activation throughout the

Figure 5. Effect of lisdexamfetamine, methylphenidate and modafinil on extracellular 5-hydroxytryptamine (5-HT) levels in striatum. Data are means ± standard error of the mean (SEM); n=5–8. Means are back-transformed and adjusted for differences between treatment groups at baseline. SEMs are calculated from the residuals of the statistical model. For clarity, the SEMs are not shown on the figures. SEM values as percentages of the mean were: Vehicle=11–47; Lisdexamfetamine=27–74 (0.5 mg/kg, per os (po)), 24–52 (1.5 mg/kg, po), 30–54 (4.5 mg/kg, po); Methylphenidate=13–39 (3 mg/kg, po), 10–40 (10 mg/kg, po), 18–63 (30 mg/kg, po); Modafinil=20–52 (100 mg/kg, po), 18–48 (300 mg/kg, po), 13–57 (600 mg/kg, po). Doses of lisdexamfetamine are expressed as d-amphetamine base equivalents, and methylphenidate and modafinil doses are as base. Time of administration is indicated by the vertical arrows. Data were analysed by analysis of covariance (ANCOVA) with each dose of drug compared to the vehicle-treated control group by Williams’ test. Significant differences are denoted by the open symbols.
microdialysis sampling period while others became quiet and settled in the later part of the experiment after an initial period of activation.

**Modafinil**

Effects of modafinil on extracellular concentrations of monoamines and metabolites in the prefrontal cortex. When modafinil (100, 300 and 600 mg/kg, po) was evaluated, it did not significantly change noradrenaline efflux in the prefrontal cortex at the lowest dose (Figure 1(c), Table 2). At 300 mg/kg, po, there was a tendency for a small increase the extracellular concentration of noradrenaline throughout the sampling period, but there was considerable inter-animal variability and the effect only achieved statistical significance (p<0.05) 90 min post-dosing (167% of basal) (Figure 1(c)). Modafinil (600 mg/kg, po) produced larger and more consistent increases in noradrenaline efflux that sporadically reached statistical significance throughout the 3.5 h sampling period (Figure 1(c)). A peak increase of 229% of baseline (p<0.01) occurred 90 min after administration of modafinil (600 mg/kg, po). An AUC analysis revealed that modafinil (600 mg/kg, po) significantly enhanced noradrenaline efflux compared to vehicle between 1.0–2.0 h (p=0.023) and 0–5.0 h (p=0.019).

Modafinil (100 mg/kg, po) did not alter the extracellular concentration of dopamine (Figure 2(c), Table 2). Modafinil (300 and 600 mg/kg, po) increased dopamine efflux, but this effect was most pronounced in the first 2.0 h after administration of the drug (Figure 2(c)). The peak increases induced by these two doses of modafinil were very similar in size, i.e. a maximal increase of 214% (p<0.05) at 300 mg/kg, po and 197% (p<0.05) 600 mg/kg, po both observed 30 min post-dosing. An AUC analysis confirmed significantly increased dopamine efflux between 0–1.0 h compared to vehicle controls with modafinil doses of 300 mg/kg, po (p=0.031) and 600 mg/kg, po (p=0.026). Modafinil (300 mg/kg, po) produced a gradual and sustained decrease in the extracellular concentration of DOPAC with a maximum decrease.
Amines and metabolites in the striatum. Effects of modafinil on extracellular concentrations of monoamines after modafinil (100 mg/kg) dose. However, there was also a non-significant trend for a decrease in extracellular 5-HIAA (Table 3). An AUC analysis confirmed that modafinil (300 and 600 mg/kg, po) significantly (p<0.01) increased the overall efflux of dopamine compared to vehicle by ~30% in the 0–1.0 h post-dosing time-bin. Modafinil (100, 300 and 600 mg/kg, po) did not significantly alter extracellular DOPAC or HVA levels in the striatum at any time-point (Table 3).

Modafinil (100, 300 and 600 mg/kg, po) induced sporadic changes in extracellular 5-HT levels in the striatum, but none of them was statistically significant (Figure 5(c), Table 2). The lack of effect of modafinil (100, 300 and 600 mg/kg, po) on 5-HT efflux was confirmed by various AUC comparisons. Modafinil (100 or 300 mg/kg, po) did not produce any statistically significant changes in the extracellular concentration of 5-HIAA (Table 3). At the highest dose of 600 mg/kg, po, modafinil induced significant increases at 15 and 45 min after dosing and also at 180–225 min. The maximum increase in extracellular 5-HIAA of 153% of baseline (p<0.05) occurred at 180 min. An AUC analysis confirmed that compared to vehicle, modafinil (600 mg/kg, po) significantly increased the extracellular concentration of 5-HIAA in the 0–1.0 h time-bin (Table 3)

Effects of modafinil on locomotor activity and behaviour. Modafinil dose-dependently increased the locomotor activity of rats (Figure 6(c)). Although modafinil (100 mg/kg, po) did not significantly increase locomotor activity above vehicle control values at any time-point, the two higher doses of modafinil produced statistically significant increases in activity at various time-points from 60–165 min after 300 mg/kg, po and 30–300 min after 600 mg/kg, po. The peak level of activation at 300 mg/kg, po was at 90 min (1.10 min activity/15 min time-bin (p<0.001)) and at 600 mg/kg, po, it occurred at 60 min (3.32 min activity/15 min time-bin (p<0.001)). AUC analyses revealed that relative to vehicle control, modafinil (300 mg/kg, po) significantly enhanced locomotor activity in the 0–1.0 h (p<0.045), 1.0–2.0 h (p<0.031) and 0–5.0 h (p<0.001) time-bins and modafinil (600 mg/kg, po) significantly (p<0.02) enhanced locomotor activity in every 1.0 h time-bin during the five-hour observation period and also in the overall 0–5.0 h time-bin.

The increases in locomotor activity were accompanied by dose-dependent behavioural changes. Modafinil (100 mg/kg, po) produced very little overt activation and the rats spent much of the period after drug administration either resting or asleep. At the intermediate dose of 300 mg/kg, po, a range of behaviours was observed, i.e. exploratory activity, sniffing, rearing and especially chewing movements. These effects were evident within 15–40 min after oral dosing. The duration of the effects was variable ranging from 60–90 min to 240–300 min in some cases. Modafinil (600 mg/kg, po) produced the same behavioural changes, but they were more intense and longer lasting. Stereotypy was observed in some animals. Some rats would remain in one place in the cage and perform very intense chewing and sniffing. These periods were interrupted by brief bursts of locomotor activity. The onset of these behaviours was 15–40 min after dosing and in most animals the behaviours lasted for most of the five-hour observation period.

to 24% of baseline (p<0.05) 165 min after dosing (Table 3). There was also a non-significant trend for a decrease in extracellular DOPAC after modafinil (100 mg/kg) dose. However, modafinil (600 mg/kg, po) did not alter the extracellular concentration of DOPAC (Table 3). Modafinil (100, 300 and 600 mg/kg, po) did not induce any change in extracellular HVA (Table 3).

Modafinil (100, 300 and 600 mg/kg, po) did not consistently alter 5-HT efflux in the PFC (Figure 3(c), Table 2) and this lack of effect was confirmed by AUC analyses. The only significant change was an increase of 269% of basal (p<0.05) 75 min after administration of modafinil (600 mg/kg, po) (Figure 3(c)). None of the doses of modafinil altered the extracellular concentration of 5-HIAA (Table 3).

Effects of modafinil on extracellular concentrations of monoamines and metabolites in the striatum. When modafinil (100, 300 and 600 mg/kg, po) was tested, the lowest dose did not significantly alter extracellular dopamine levels in the striatum at any time-point (Figure 4(c), Table 2). The doses of 300 and 600 mg/kg, po of modafinil produced very similar increases in striatal dopamine efflux. Peak increases occurred 60 min post-dosing with values of 134% and 136% of baseline, respectively, for doses of 300 and 600 mg/kg, po (p<0.01 for both doses) (Figure 4(c)). An AUC analysis confirmed that modafinil (300 and 600 mg/kg, po) significantly (p<0.01) increased the overall efflux of dopamine compared to vehicle by ~30% in the 0–1.0 h post-dosing time-bin. Modafinil (100, 300 and 600 mg/kg, po) did not significantly alter extracellular DOPAC or HVA levels in the striatum at any time-point (Table 3).
Correlation between increases in the extracellular concentration of striatal dopamine and locomotor activity

Correlations between the extracellular concentration of dopamine in the striatum and the magnitude of locomotor activity response were determined in the groups of rats given lisdexamfetamine (4.5 mg/kg, po) methylphenidate (30 mg/kg, po) and modafinil (600 mg/kg, po). Significant linear correlations were found between these two independent parameters for the rats given lisdexamfetamine ($r^2=0.450; p=0.002$) and methylphenidate ($r^2=0.875; p<0.001$) (Figure 7), but not modafinil ($r^2=0.002; p=0.857$).

The slope of the linear correlation was significantly steeper ($p=0.008$) for methylphenidate (mean slope=0.0267) than lisdexamfetamine (mean slope=0.0057) demonstrating that the latter could produce larger increments in extracellular dopamine in the striatum without inducing locomotor activation.

Discussion

As set out in the introduction, we have characterised the behavioural and neurochemical effects of lisdexamfetamine, methylphenidate and modafinil across a wide range of pharmacologically equivalent doses (see Methods) to gain an insight into therapeutic and side-effect profiles and sizes of ‘therapeutic windows’ of these stimulants. In the discussion, we first compare the effects of these three compounds on monoaminergic neurochemistry in the PFC and striatum, and second, we explore the possible links between their effects on dopamine efflux in the striatum and locomotor activity.

At what we have classified as pharmacologically relevant doses, lisdexamfetamine increased the extracellular concentration of both catecholamines in the PFC. The increases were gradual in onset reaching a peak after ~120 min and were maintained throughout the course of the experiment. Although 5-HT efflux in the PFC was increased by the highest dose of lisdexamfetamine, this effect was transient and probably not pharmacologically relevant. Lisdexamfetamine did not change the extracellular concentration of DOPAC, HVA, or 5-HIAA. Lisdexamfetamine’s monoaminergic profile did not alter substantially when the dose shifted from the pharmacological to the non-therapeutic range. In contrast, methylphenidate’s effects on noradrenergic and dopaminergic neurotransmission varied markedly according to the dose. In the pharmacological range, methylphenidate selectively increased dopamine efflux in the PFC at the lowest dose, but increased both catecholamines at the intermediate dose. The supra-therapeutic dose of methylphenidate also produced approximately equal increases in the extracellular concentration of both catecholamines. Serotonergic neurotransmission in the PFC was not significantly influenced by methylphenidate, irrespective of whether the doses were within or above the therapeutically relevant dose-range. The actions of methylphenidate were relatively fast in onset even after oral administration, with peak increases observed ~60 min after dosing. The substantial and highly significant decreases in extracellular DOPAC indicate that increased dopamine efflux was accompanied by a concomitant reduction in dopamine turnover and/or dopaminergic neuronal firing-rate.

In the striatum, lisdexamfetamine dose-dependently increased extracellular dopamine and 5-HT. The pattern of gradual and sustained increases in monoamine efflux was the same as seen in the PFC. Reductions of HVA and DOPAC suggested concurrent decreases in dopamine turnover and/or dopaminergic neuronal firing. The effects of orally administered lisdexamfetamine on striatal dopamine efflux were similar to those observed after ip injection (Rowley et al., 2012), which is consistent with its pharmacokinetics and pharmacodynamics remaining unchanged when administration routes are switched (Heal et al., 2013a, b). In contrast, methylphenidate had no effect on extracellular dopamine at the lower pharmacologically relevant dose and it produced only a minimal increase at the higher one. These data complement earlier findings reported by Berridge et al. (2006). It was only when the oral dose of methylphenidate was taken above the therapeutic window that a moderately large and sustained increase in striatal dopamine efflux occurred. Methylphenidate’s actions on the dopamine metabolites indicate that, although it increased dopamine efflux, there was no compensatory decrease in turnover. On the contrary, the marked and significant increase in extracellular HVA produced by all doses of methylphenidate indicate dopamine turnover and/or the rate dopaminergic neuronal firing was increased. These results confirm and extend our earlier findings obtained in the mouse (Cheetham et al., 2010) and are consistent with the hypothesis that methylphenidate induces firing-mediated release of dopamine from dopaminergic nerve terminals in richly-innervated dopaminergic areas like the striatum and nucleus accumbens (Heal, 2008). This mechanism of action is similar to that of cocaine and differs markedly from that of conventional dopamine reuptake inhibitors (Heal, 2008; Heal et al., 2012). In agreement with earlier in vitro and in vivo work (Gatley et al., 1996; Kuczenski and Segal, 1997; Richelson and Pfenning, 1984), we found that methylphenidate did not produce any consistent changes in the extracellular concentration of 5-HT in either the striatum or PFC, thereby confirming the exclusively catecholaminergic profile of this drug. The only note of caution to apply to this conclusion is the consistent increases in extracellular 5-HIAA.

The neurochemical profile of modafinil has not been extensively characterised by intracerebral microdialysis in rodents. Furthermore, in the published studies different administration routes have been employed, i.e. intracerebroventricular (Murillo-Rodriguez et al., 2007) and intravenous injection (Wisor et al., 2001; Zolkowska et al., 2009), or experiments have been conducted in anaesthetised animals (Ferraro et al., 1996). Our findings revealed that the lowest dose modafinil was at the threshold of pharmacological effect making 300 mg/kg, po the only dose representative of its therapeutic actions. This dose of modafinil had a balanced effect of enhancing noradrenaline and dopamine overflow in the PFC and this neurochemical profile is consistent with its effectiveness in treating ADHD (Biederman et al., 2005; Greenhill et al., 2006; Swanson et al., 2006). However, there are aspects of its neuropharmacology that may also have implications for its level of therapeutic efficacy. Unlike lisdexamfetamine or methylphenidate, which produced simultaneous increases in the efflux of both catecholamines in the PFC, modafinil’s effect on dopamine was rapid, but its effect on noradrenaline was relatively slow in onset. This difference may help to explain why the response rate for modafinil in ADHD was ~40–50% (Wigal et al., 2006) which is somewhat lower than the ~70% value for lisdexamfetamine or methylphenidate (see Heal et al., 2012). The supra-therapeutic dose of modafinil increased the extracellular...
concentration of both catecholamines in the PFC but, interestingly, it was noradrenaline and not dopamine efflux that was dose-dependently increased by the drug. The therapeutic dose of modafinil (which increased dopamine efflux in the PFC) substantially decreased extracellular DOPAC indicating a probable concomitant reduction in dopamine turnover and/or dopaminergic neuronal firing rate. This fall in DOPAC was not seen with the higher dose indicating that dopamine turnover had returned to control levels. Modafinil’s lack of effect on 5-HT efflux in the PFC is at variance with increases reported by De Saint Hilaire et al. (2001), but there are important technical differences that could account for this disagreement. First, De Saint Hilaire et al. placed the probes much closer to the midline of the brain than we did. Second, they collected microdialysate samples after only 4h of post-implantation recovery, whereas we continuously infused our microdialysis probes for 16–24 h to allow the efflux of monoamines to stabilise before collecting basal samples. Third, De Saint Hilaire et al. (2001) did not employ a vehicle control in their microdialysis experiments and compared the effects of modafinil to baseline (pre-intervention) 5-HT levels, while in our study all of the effects of modafinil were compared against the vehicle.

Consistent with its lack of effect in the PFC, the lowest dose of modafinil similarly failed to alter dopamine or 5-HT efflux in the striatum. The pharmaco logically relevant dose of modafinil evoked a small, but sustained, increase in striatal dopamine efflux that was not augmented when the dose was taken above the therapeutic window. There were no changes in extracellular DOPAC or HVA. The observed difference between modafinil’s effect on dopamine metabolism the striatum and PFC probably derives from the fact that in the striatum most catabolism of dopamine takes place in dopaminergic nerve terminals, whereas in the PFC, which has few DAT sites, most catabolism of dopamine occurs in noradrenergic neurones (see Heal et al., 2009, 2012). The two higher doses of modafinil did not alter extracellular 5-HT, but the highest dose did substantially increase extraneuronal 5-HIAA.

To summarise the findings, the higher of the two therapeutically relevant doses of modafinil increased dopamine efflux in the PFC and striatum, but it did not consistently or robustly increase the extracellular concentration of noradrenaline and 5-HT in the PFC or 5-HT in the striatum. However, increased noradrenaline efflux was observed when the dose of modafinil was taken above the therapeutically relevant range. In general, the increases in dopamine efflux produced by modafinil were rapid in onset, but there was a clear ceiling to its magnitude of effect in both brain regions.

When the relative abilities of the three compounds to enhance catecholamine efflux in the PFC and striatum are compared, the rank order of efficacy is lisdexamfetamine>methylphenidate>modafinil.

The second objective was to explore the relationship between neurochemistry and behaviour at doses producing non-therapeutic, stimulant effects. The therapeutically relevant doses of lisdexamfetamine, methylphenidate and modafinil increased vigilance and wakefulness, but induced very little overt activity. Typically, the rats were engaged in locomotor activity for ~1.0 min in each 15.0 min observation period. At supra-therapeutic doses, all three stimulants produced approximately the same degree of locomotor activation, though it should be noted that the top dose of modafinil was restricted to 600 mg/kg; 900 mg/kg could not be tested because it evoked profound stereotypy. Locomotor activity induced by lisdexamfetamine was gradual in onset, reaching a plateau 60–75 min after dosing and it remained elevated throughout the 5h observation period. Methylphenidate (30 mg/kg) also increased locomotor activity, but the effect was relatively fast in onset, peaking ~45 min after dosing and gradually declining thereafter. A minority of the animals were profoundly activated for a large proportion of the monitoring period. Modafinil’s locomotor effects were gradual in onset, of lisdexamfetamine, but declined after reaching a peak, comparable to methylphenidate. Significant linear correlations were found between the increases in striatal dopamine efflux and locomotor activity for lisdexamfetamine and methylphenidate, but not modafinil. The slopes of the regression lines revealed that much greater increases in extracellular dopamine could be elicited by lisdexamfetamine without evoking locomotor activation than methylphenidate. This is almost certainly because the rate of increase of extracellular dopamine induced by methylphenidate is much greater than lisdexamfetamine. The lack of a correlation between modafinil’s effects on striatal dopamine efflux and locomotor activity is at variance with results from Zolkowska et al. (2009), who reported highly significant linear correlations between the extracellular dopamine and locomotor activity (distance travelled) and stereotypy. Once again, there are important technical differences that could account for this divergence. First, Zolkowska et al. (2009) measured dopamine efflux in the nucleus accumbens not the striatum and, second, they administered modafinil intravenously as opposed to using the clinically relevant oral route. Since the administration route markedly influences the pharmacological effects of stimulant drugs, this difference may well account for the discordance between the two results.

Our findings may help to explain why modafinil has such an unusual pharmacological profile. First, it has a much greater ability to enhance catecholaminergic function in the PFC than dopaminergic neurotransmission in the striatum; the implication being that although modafinil is a stimulant, it has a lower potential to activate dopaminergic pathways linked to reinforcement and reward. Second, the lack of correlation between increased dopamine efflux and locomotor activity indicates that although this neurotransmitter may be involved in mediating the response, another as yet unknown neurochemical mediator is the primary driver of modafinil’s stimulant actions. Thus, not only does modafinil have a pharmacological mechanism that is only partly explained by its actions on monaminergic neurotransmission, but also it appears to be unique because of the capacity for preferentially activating the PFC.

Although the pharmacological profiles of lisdexamfetamine, methylphenidate and modafinil were identical by virtue of their ability to enhance the extracellular concentrations of dopamine and noradrenaline in the PFC, substantial differences were also observed that may have implications for their therapeutic and side-effect potential when used as treatments for ADHD. Interestingly, when given by the oral route, therapeutically relevant doses of modafinil failed to increase extracellular dopamine in the striatum. In contrast, large, dose-dependent increases were observed after administration of lisdexamfetamine. Also, lisdexamfetamine was the only one of the stimulants tested that consistently increased the extracellular concentration of 5-HT in the PFC and striatum. Since 5-HT is believed to exert a tonic
inhibitory action on dopaminergic behaviours (Baumann et al., 2011a, b; Cervo et al., 1981), this effect may also have contributed to the comparatively low level of locomotor activation that resulted from increased striatal dopamine efflux. The monoaminergic profile of lisdexamfetamine did not vary with dose, whereas those of methylphenidate and modafinil did. Lisdexamfetamine produced much larger increases in striatal dopaminergic neurotransmission than methylphenidate or modafinil but, paradoxically, it was less stimulant than either methylphenidate or modafinil. Thus, the non-stimulant, lowest dose of lisdexamfetamine produced an increase in striatal dopamine overflow that was equivalent to that produced by the profoundly stimulant, highest dose of methylphenidate (1.5 mg/kg vs 30 mg/kg, po, respectively). Modafinil only moderately enhanced extracellular striatal dopamine even when tested at a supra-therapeutic dose.

In summary, the data demonstrate that lisdexamfetamine produces more substantial and sustained increases in catecholaminergic neurotransmission in the PFC and striatum than either methylphenidate or modafinil. Moreover, the observation that substantial increases in extracellular dopamine in the striatum can be achieved without inducing locomotor activation are consistent with clinical observations that lisdexamfetamine has a long duration of action and a good separation between its beneficial effects in treating ADHD and the induction of psychostimulant adverse events. It would be interesting to apply this technique of combining dual-probe microdialysis to study multiple monoamine neurotransmitters with simultaneous behavioural measurements in order to provide a greater insight into the marked differences between the therapeutic and stimulant profiles of lisdexamfetamine and its active metabolite, d-amphetamine.

**Highlights**

- Monoamine neurochemistry of lisdexamfetamine determined by microdialysis
- Lisdexamfetamine increased dopamine and noradrenaline efflux in prefrontal cortex
- Lisdexamfetamine produced dose-dependent increases of dopamine and 5-hydroxytryptamine (5-HT) efflux in the striatum
- Lisdexamfetamine was profiled against methylphenidate and modafinil
- Lisdexamfetamine’s pharmacokinetics expand the window between efficacy and unwanted stimulation

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**Conflict of interest**

The authors declare that there are no conflict of interest.

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