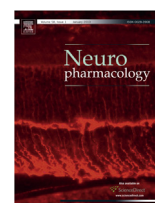




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# A preclinical evaluation of the discriminative and reinforcing properties of lisdexamfetamine in comparison to *D*-amphetamine, methylphenidate and modafinil

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## ABSTRACT

Lisdexamfetamine dimesylate, which consists of *L*-lysine covalently bound to *D*-amphetamine, is the first prodrug for treating ADHD. Its metabolic conversion to yield *D*-amphetamine by rate-limited, enzymatic hydrolysis is unusual because it is performed by peptidases associated with red blood cells. Other stimulants shown to be effective in managing ADHD include *D*-amphetamine, methylphenidate and modafinil. All have the potential for misuse or recreational abuse. The discriminative and reinforcing effects of these compounds were determined in rats using a 2-choice, *D*-amphetamine (0.5 mg/kg, *i.p.*)-cued drug-discrimination test, and by substitution for intravenous cocaine in self-administration. Lisdexamfetamine (0.5–1.5 mg/kg [*D*-amphetamine base], *p.o.*) generalised to saline when tested 15 min post-dosing, but dose-dependently generalised to *D*-amphetamine at 60 min. At 120 min, its *D*-amphetamine-like effects were substantially diminished. At 15 min, methylphenidate (3.0–10 mg/kg, *p.o.*) and *D*-amphetamine (0.1–1.5 mg/kg, *p.o.*) dose-dependently generalised to the intraperitoneal *D*-amphetamine cue. Switching to the intraperitoneal route reduced the interval required for lisdexamfetamine to be recognised as *D*-amphetamine-like, but did not alter its potency. Switching to intraperitoneal injection increased the potency of methylphenidate and *D*-amphetamine by 3.4× and 2.2×, respectively. Modafinil (50–200 mg/kg, *i.p.*) generalised partially, but not fully, to *D*-amphetamine. Methylphenidate (0.1, 0.3, 1.0 mg/kg/injection, *i.v.*) maintained robust self-administration at the 2 highest doses. Neither lisdexamfetamine (0.05, 0.15 or 0.5 mg/kg/injection [*D*-amphetamine base], *i.v.*) nor modafinil (0.166, 0.498 or 1.66 mg/kg/injection, *i.v.*) served as reinforcers. The results reveal important differences between the profiles of these stimulants. Lisdexamfetamine did not serve as a positive reinforcer in cocaine-trained rats, and although it generalised fully to *D*-amphetamine, its discriminative effects were markedly influenced by its unusual pharmacokinetics.

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## 1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a childhood-onset, psychiatric, cognitive and behavioural disorder that is widely treated with the catecholaminergic stimulants, *D*-amphetamine and methylphenidate. These drugs are effective in managing the symptoms of approximately three quarters of children and adults (Spencer et al., 1996; Elia et al., 1999; Heal and Pierce, 2006; Heal et al., 2009, 2012a; Buitelaar and Medori, 2010). Although these stimulants are undoubtedly effective, they have two major shortcomings. First, *D*-amphetamine and methylphenidate have relatively short half-lives that require the drugs to be administered several times a day, which makes them particularly unsuitable for

**Abbreviations:** ADHD, attention deficit hyperactivity disorder; AMF, amphetamine; C-II, Schedule 2 Controlled Drug; C-IV, Schedule 4 Controlled Drug; CD, controlled drug;  $C_{max}$ , maximum plasma drug concentration; DAT, dopamine reuptake transporter; FR, fixed ratio; IACUC, Institutional Animal Care and Use Committee; *i.p.*, intraperitoneal; IR, immediate release; *i.v.*, intravenous; PET, positron emission tomography; PFC, prefrontal cortex; *p.o.*, *per os* (oral); SAL, saline; SR, sustained release;  $t_{max}$ , time to reach maximum plasma drug concentration.

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use by individuals whose disorder is characterised by inattention, distractibility and impulsivity. Second, when these catecholaminergic drugs are taken at doses above those recommended in the prescribing instructions and often by non-clinical routes, e.g. nasal insufflation (“snorting”) or intravenous injection, they have powerful psychostimulant and euphoriant properties which makes them liable to diversion and recreational abuse. Both shortcomings have to some extent been addressed by the development of long-acting formulations and by the use of novel delivery systems, e.g. osmotically controlled release or transdermal patches, that are also tamper deterrent (see reviews by Heal and Pierce, 2006; Heal et al., 2009, 2012a); nonetheless, all formulations of methylphenidate and *D*-amphetamine are classified as Schedule 2 Controlled Drugs (C-II) in the UK, USA and many other countries.

Lisdexamfetamine dimesylate (Vyvanse<sup>®</sup>) is a relatively recent entry to the portfolio of ADHD medications. It is a *D*-amphetamine prodrug, which comprises the naturally occurring amino acid, *L*-lysine, covalently bound to *D*-amphetamine via an amide linking group. Lisdexamfetamine is the first prodrug to have been approved in the USA and Canada for the management of ADHD in children (age 6–12), adolescents (age 13–17) and adults. It is currently undergoing evaluation for the treatment of ADHD in a number of European countries. The metabolic route of conversion of lisdexamfetamine is unusual because after absorption into the bloodstream it is metabolised by red blood cells to yield *D*-amphetamine and the natural amino acid, *L*-lysine, by rate-limited, enzymatic hydrolysis (Pennick, 2010). The prodrug is pharmacologically inert *in vitro* and lacks affinity for a wide range of molecular targets that mediate the effects of drugs of abuse (data on file, Shire Pharmaceuticals). As a prodrug of *D*-amphetamine, lisdexamfetamine has been classified as a C-II in both the USA and UK.

Modafinil is an unusual stimulant with enigmatic pharmacology (see reviews by Minzenberg and Carter, 2008; Heal et al., 2012a). Although its clinical development as a treatment for ADHD was terminated due to safety concerns, modafinil has been shown unequivocally to improve symptoms in children and adolescents with ADHD in several, randomised, double-blind, placebo-controlled, clinical trials (Biederman et al., 2006; Swanson et al., 2006; Greenhill et al., 2006). Modafinil has a C-IV classification in the USA, but it is not a CD in the UK.

Thus, all of these stimulants have to a greater or lesser extent the potential for misuse and/or recreational abuse. Drug-discrimination and self-administration studies are mandated by FDA and EMA for all novel CNS-active drugs for use in man (Center for Drug Evaluation and Research [CDER]/Food and Drug Administration [FDA], 2010; Committee for Medicinal Products for Human Use [CHMP]/European Medicines Agency [EMA], 2006), and for this reason, lisdexamfetamine and the other reference stimulants were tested in two established rodent models in laboratories where these protocols have been in use for more than 20 years and for which a wealth of data and experience with other reference abused and non-abused drugs exists. In this study, we have explored the discriminative effects of lisdexamfetamine in rats trained to discriminate between *D*-amphetamine and saline in a 2-choice lever-pressing model, and its ability to serve as a positive reinforcer in rats trained to intravenously self-administer low-dose cocaine. In these experiments, the profile of lisdexamfetamine has been compared with those of other stimulants that are effective ADHD medications, i.e. *D*-amphetamine, methylphenidate and modafinil.

## 2. Methods

### 2.1. Animals and environment

For the drug-discrimination study, 48 4-week old, female, PVG rats were obtained from Harlan UK. The animals were housed in groups of 4 in polypropylene

cages with sawdust covered floors in a temperature and humidity controlled room. Animals were maintained on 12 h:12 h light–dark cycle with free access to food and tap water at all times when in their home cages. Rats were accustomed to these conditions for 1 week before the start of training.

For the self-administration study, 54 male, Sprague–Dawley rats (277–352 g at start of study) were purchased from Charles River UK, and 58 male, Sprague–Dawley rats (277 g–342 g at start of study) from Harlan, USA. Rats were housed individually in plastic cages containing rodent bedding and environmental enrichment on a 12 h:12 h light–dark cycle in a temperature and humidity controlled room. Animals were allowed to acclimatise to these conditions for at least 4 days before the study commenced, during which time they underwent daily weighing and handling. Rats were allowed free access to tap water and standard rodent diet during the acclimatisation period. After the acclimatisation period, food was restricted to 10 g/day over 5 days, after which daily food intake was restricted to ~90% of normal levels (calculation based on the mean daily food intake during the acclimatisation period). Rats were given sufficient food to maintain age-appropriate growth. Body weights were monitored and the amount of food given in home cages was altered when necessary. This regime was maintained throughout the remainder of the study, except during the recovery period after surgery.

In both studies, animals were tested in the light part of the light–dark cycle.

### 2.2. Drug-discrimination training and testing

*D*-Amphetamine-cued drug-discrimination testing in rats was based on the method previously described by Heal et al. (1992). Briefly, female PVG rats were trained to distinguish between *D*-amphetamine (0.5 mg/kg, i.p.) and saline (1 ml/kg, i.p.) in a 2-choice lever-pressing task in response to a sweetened milk reward made available on a FR-5 reward schedule (i.e. 5 lever-presses for 1 reward). Rats were randomly allocated one lever for *D*-amphetamine (0.5 mg/kg, i.p.) and the other for saline. Once a rat had achieved approximately 60% correct lever-presses on most trials, it began the test regime.

On the test regime, rats were injected with drug cue or saline and then placed in the test chamber. The treatments during testing were alternated to prevent rats learning a particular sequence. On a test day, rats were not rewarded during the first 2.5 min of the session for presses on either lever and then rewarded on either lever for the remaining 7.5 min of the session.

The criterion for acceptable performance during testing was  $\geq 75\%$  correct lever-presses in response to the drug cue or saline in the initial 2.5 min of the 10 min test preceding a drug test and a mean of  $\geq 75\%$  correct lever-presses in 4 consecutive drug cue and saline cue tests. When rats had achieved 4 correct saline and amphetamine test sessions they progressed to the test drugs, routes and time periods evaluated in this study. Test compounds were assessed in the same manner i.e. the result for each rat was the percentage of responses on the amphetamine lever in the unrewarded 2.5 min of the test session.

Rats had to correctly complete one saline and *D*-amphetamine test and reinforcement session in a random order between each compound test. These sessions were repeated if a rat showed unacceptable performance in response to saline or *D*-amphetamine.

Training of the rats with saline (i.p.) and *D*-amphetamine (0.5 mg/kg, i.p.) was performed 3–4 days each week, but test compounds were tested only once per week. Prior to each rat being placed in a chamber, the levers and walls were swabbed with 10% ethanol solution to prevent olfactory stimuli from the previous rat influencing the subsequent rat's lever choice (Extance and Goudie, 1981).

In test sessions where the operant responding after administration of a test compound was markedly suppressed, i.e.  $\geq 50\%$  decrease in operant responding compared to the mean number of responses in the previous 4 sessions made by the same rat when tested with the training cue, i.e. *D*-amphetamine 0.5 mg/kg, i.p., the test was repeated 1 day later. If the result of  $\geq 50\%$  decrease in operant responding was confirmed the repeat test, suppressed operant responding was taken as the experimental outcome. On the other hand, if on repeat testing the rat showed an acceptable level of operant responding, the percentage generalisation to *D*-amphetamine was recorded and included in the analysis. When the dose of a test compound selected for testing produced  $\geq 50\%$  decrease in the operant responding for  $\geq 50\%$  of a group of rats, it was classified as “behavioural disruption” and testing at higher doses was not performed. In these experiments, behaviourally disruptive doses of lisdexamfetamine and the reference comparators, methylphenidate and *D*-amphetamine, were not encountered. In the case of modafinil, only the highest 200 mg/kg, i.p. dose of caused behavioural disruption.

### 2.3. Self-administration training and testing

Training sessions were conducted on a FR-1 schedule of food reinforcement (45 mg dustless pellets; F0021-B, Bilaney Consultants Ltd or PJA1-0045, Noyes Precision Pellets, Research Diets Inc., New Brunswick, New Jersey, USA.). Operant training sessions lasted for a maximum of 1.0 h, or finished once a rat had received 50 food pellet rewards. Once rats had learnt to lever-press to receive 50 pellets in a 1.0 h session, the response requirement was increased to FR-2 and the left lever was designated as the active lever. Thereafter only responses on the left lever resulted in the delivery of a reward.

Once operant responding for food was stable under the FR-2 schedule, a chronic in-dwelling intravenous catheter was implanted into the jugular vein of each rat. Surgery was conducted under aseptic conditions using isoflurane anaesthesia. The catheter (11 cm IVSAP40 [3 French] silicone; Camcaths Ltd., Cambridge, UK or 10 cm CBAS-C30 [3 French] heparin-coated polyurethane; Instech Solomon, Plymouth Meeting, PA, USA) was implanted into the right jugular vein, secured to the vessel then tunnelled subcutaneously from the site of insertion to the midscapular region where the access port exited. The wound was closed with sutures and dressed with an appropriate antiseptic spray and plastic dressing. In the case of PMINA-CBAS-C30 access ports (7 mm high; Instech Solomon), rats were fitted with a mesh jacket (RJ02; Lomir Biomedical, Inc., Malone, New York, USA) to which the exteriorised port and catheter were attached.

Catheters were filled with heparinised saline immediately post-surgery and after every experimental session to maintain catheter patency. Catheter patency was confirmed daily by drawing back and observing freely flowing blood in the catheter line. If blood could not be drawn, catheter patency was confirmed by observation of immediate sedation upon intravenous injection of propofol or methohexital. If catheter patency failed during the study, the rat was terminated by a Schedule 1 procedure.

Animals were allowed to recover from surgery for at least 24 h, after which animals began the dose-finding study or the self-administration study. The purpose of the dose-finding study was to identify the doses of test compound to be used in the self-administration study. Rats were given a single i.v. injection of either vehicle or test compound immediately prior to a 1.0 h operant training session, in which they were allowed to respond under a FR-2 schedule for food pellet rewards. During this session, rats were monitored for effects on lever-pressing or other clear evidence of pharmacological activity.

Cocaine was selected as the training drug for this study as it is the "gold standard" stimulant reinforcer in preclinical self-administration studies. It is a powerful reinforcer in humans, primates, rats and many other species. Cocaine is a well known drug of abuse that is a Schedule II Controlled Drug in the USA and UK. *D*-Amphetamine will substitute as a reinforcer for cocaine in rats (Barrett et al., 2004; Liu et al., 2007) and its pharmacodynamic effect on dopamine efflux in the nucleus accumbens when it is self-administered by rats is similar to that of cocaine (Di Ciano et al., 1995).

In the self-administration study, each rat was trained to self-administer multiple injections of a low reinforcing dose of cocaine (0.32 mg/kg/injection, 1 ml/kg/injection, i.v.) on a FR-2 schedule of reinforcement. After an initial non-contingent injection of cocaine (0.32 mg/kg, i.v.), the rats were allowed to lever-press for a maximum of 20 injections (in addition to the non-contingent injection)/1.0 h session/day. After consistent and robust self-administration of cocaine had been established, low dose cocaine was substituted by saline (1 ml/kg i.v.) to demonstrate extinction of self-administration behaviour. A test compound, i.e. lisdexamfetamine, methylphenidate or modafinil, was then substituted into the paradigm. After completing the evaluation of a test compound, the rats were again given access to saline (1 ml/kg, i.v.) under a FR-2 schedule in daily 1.0 h sessions until lever-pressing was non-reinforced. Finally, lever-pressing for cocaine HCl (0.32 mg/kg/injection) was retested under a FR-2 schedule to ensure that rats would still self-administer a known drug of abuse with robust positive reinforcing effects.

Stable responding for each rat was accepted when the number of injections/1 h session for each substance (cocaine, vehicle or test compound) did not vary by more than  $\pm 20\%$  of the mean of the 3 previous sessions, or where there was no obvious increasing or decreasing trend in self-administration. The maximum number of 1 h test sessions employed for test compound or comparator compound if stable responding was not observed was 10 sessions. If stable responding was not achieved for an animal by this time, it was eliminated from the study. Positive reinforcement with cocaine was defined as 3 consecutive test sessions where the mean number of cocaine injections per session was  $\geq 15$ . Non-reinforcement with vehicle was defined as 3 consecutive test sessions where the number of saline injections per session was  $\leq 8$ .

#### 2.4. Experimental locations

The drug-discrimination experiments were performed in RenaSci's laboratories at the University of Nottingham. Self-administration experiments to determine the reinforcing potential of lisdexamfetamine and methylphenidate were conducted at the University of Texas Health Science Center in San Antonio. The bridging experiment with methylphenidate and the self-administration experiments with modafinil were performed in RenaSci's laboratories.

All *in vivo* experiments were performed in strict accordance with Home Office Guidelines and licenced under the Animals (Scientific Procedures) Act 1986 or in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC), the University of Texas Health Science Center at San Antonio, and the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare Publication No. (NIH)83-23, revised 1996.

#### 2.5. Drugs and formulation

Lisdexamfetamine was provided by Shire Pharmaceuticals. *D*-Amphetamine sulphate was purchased from Sigma–Aldrich, *dl*-*threo*-methylphenidate hydrochloride

from Sigma–Aldrich and Johnson Matthey-Macfarlan Smith Ltd., cocaine hydrochloride from Sigma–Aldrich and modafinil base from Tocris Bioscience.

The vehicles used for lisdexamfetamine, *D*-amphetamine, methylphenidate and cocaine hydrochloride were 0.9% saline for intraperitoneal (i.p.) or intravenous (i.v.) injection and deionised water for oral (p.o.) administration. Modafinil was injected i.p. as a fine suspension in 1% methylcellulose in 0.9% saline and dissolved in 40% (2-hydroxypropyl)- $\beta$ -cyclodextrin [w/v] in deionised water for i.v. injection.

Doses of *D*-amphetamine and methylphenidate are expressed as the mg/kg of base (correction factors: 1.36 and 1.16, respectively). To facilitate comparisons between lisdexamfetamine and *D*-amphetamine, doses of both compounds are expressed as mg/kg of *D*-amphetamine base (correction factor: 3.37 for lisdexamfetamine).

#### 2.6. Data presentation and statistical analysis

In the drug-discrimination experiments, operant responding after administration of test compounds was calculated as the percentage generalisation to the *D*-amphetamine cue for individual rats.

% generalisation to *d* – amphetamine for compound C

= number of *d* – amphetamine lever

– presses in test of compound C/total lever

– presses in test of compound C  $\times$  100

where total lever-presses = *D*-amphetamine lever-presses + saline lever-presses.

ED<sub>50</sub> values for generalisation to the *D*-amphetamine cue were calculated by non-linear regression using a logistic model with the minimum fixed at 0% and the maximum fixed at 100%. Relative potencies of compounds administered orally to the same compound administered intraperitoneally were calculated using a similar non-linear regression model, but forcing the slope to be equal for the two routes of administration. The relative potency was the ratio of the 2 ED<sub>50</sub> values. If the 95% confidence interval for the relative potency did not include 1.0, it indicated a significant difference ( $P < 0.05$ ) between the 2 ED<sub>50</sub> values.

Results from the self-administration experiments were normally distributed with equal variance in all groups, and therefore, were considered to be appropriate for a parametric analysis. A separate statistical analysis was carried out for each dose of each test compound. Statistical analysis was performed using the mean of last 3 sessions for each animal of the test compounds, cocaine and saline. Analysis was by 2-way analysis of variance with treatment and animal as factors, followed by the multiple *t*-test to compare the 3 treatments (cocaine, saline and test compound, i.e. lisdexamfetamine, methylphenidate or modafinil) to each other. Positive reinforcement or Non-reinforcement for the test compound (lisdexamfetamine) and reference comparators (methylphenidate or modafinil) were compared statistically with the number of vehicle and cocaine injections/session. Positive reinforcement of test compound and reference comparators was defined where the mean number of injections/session was significantly greater than the mean number of vehicle injections and also  $\geq 8$  injections/session. Non-reinforcement of test compound and reference comparators was defined where the mean number of injections/session was  $\leq 8$  injections/session and also not different from the injections/session of vehicle.

### 3. Results

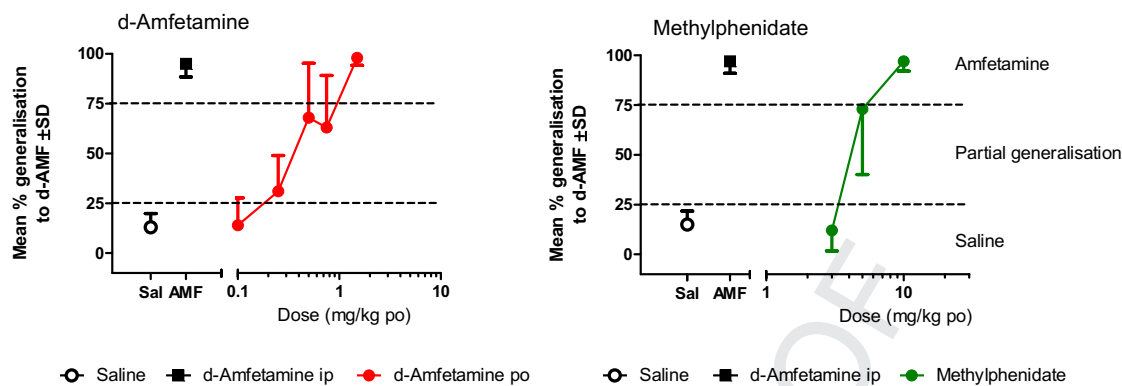
#### 3.1. Discriminative profiles of orally administered *D*-amphetamine and methylphenidate

As shown in Fig. 1, the group of rats employed in this study were highly proficient in distinguishing the discriminative effects of intraperitoneal injection of 0.5 mg/kg *D*-amphetamine from saline.

When rats were given *D*-amphetamine (0.1–1.5 mg/kg) by the oral route, this stimulant was not recognised as *D*-amphetamine at the very low dose of 0.1 mg/kg (Fig. 1). Larger doses of the stimulant were incrementally recognised as *D*-amphetamine with partial generalisation to the intraperitoneal *D*-amphetamine cue at doses of 0.25, 0.5 and 0.75 mg/kg, and full generalisation at a dose of 1.5 mg/kg.

When tested 15 min after oral administration, methylphenidate (3.0–10 mg/kg) dose-dependently generalised to the *D*-amphetamine training cue (Fig. 1). Complete generalisation to the *D*-amphetamine cue was observed at the 10 mg/kg dose of methylphenidate.

None of the oral doses of *D*-amphetamine or methylphenidate influenced the operant response rates of the rats (Table 1).



**Fig. 1.** Discriminative effects of orally administered *D*-amphetamine and methylphenidate in rats trained to discriminate *D*-amphetamine. (0.5 mg/kg, i.p.) from saline. Results are mean values for the percentage generalisation to the *D*-amphetamine (0.5 mg/kg, i.p.) cue  $\pm$  SD. Saline (Sal) and *D*-amphetamine (0.5 mg/kg, i.p.) (AMF) data are the mean  $\pm$  SD from 19 to 24 rats/group for the 4 test sessions preceding the first oral dose of *D*-amphetamine or methylphenidate. Groups of rats ( $n = 6–11$ ) were tested with oral dose of *D*-amphetamine or methylphenidate 15 min after oral dosing. Generalisation was classified as: Amphetamine ( $\geq 75\%$  responses on the *D*-amphetamine lever), Partial generalisation to *D*-amphetamine (26–74% responses on the *D*-amphetamine lever) or Saline ( $\leq 25\%$  responses on the *D*-amphetamine lever).

### 3.2. Discriminative profile of orally administered lisdexamfetamine

The discriminative effects of orally administered lisdexamfetamine were investigated in rats trained to discriminate *D*-amphetamine (0.5 mg/kg, i.p.) from saline using various time intervals between compound dosing and testing. When the interval was 15 min, of the interval used for testing orally administered *D*-amphetamine and methylphenidate, lisdexamfetamine generalised to the saline cue at various pharmacologically active doses ranging from 0.5 mg/kg to 1.5 mg/kg (Fig. 2). When the interval was extended to 60 min, lisdexamfetamine partially generalised to *D*-amphetamine at doses of 0.5, 0.75 and 1.0 mg/kg, p.o., and fully generalised at 1.5 mg/kg, p.o. (Fig. 2). When the interval between dosing and testing was further increased to 120 min, lisdexamfetamine generalised to saline at doses of 0.5 and 0.75 mg/kg, p.o. and

partially generalised to *D*-amphetamine at the higher doses of 1.0 and 1.5 mg/kg, p.o. (Fig. 2).

None of the oral doses of lisdexamfetamine influenced operant response rates of the rats (Table 1).

### 3.3. A comparison of the discriminative profiles of *D*-amphetamine, methylphenidate and lisdexamfetamine when given by the oral and intraperitoneal routes

Employing the usual 15 min interval between dosing and testing, intraperitoneal injection of *D*-amphetamine (0.1–0.5 mg/kg) produced dose-dependent generalisation to *D*-amphetamine (Fig. 3). Switching from the oral to the intraperitoneal route produced a leftward shift in the dose–response curve (Fig. 3). The  $ED_{50}$  for generalisation to the *D*-amphetamine training cue was 2.2-fold lower

**Table 1**

Operant response rates of rats after administration of lisdexamfetamine, *D*-amphetamine, methylphenidate, modafinil or vehicle.

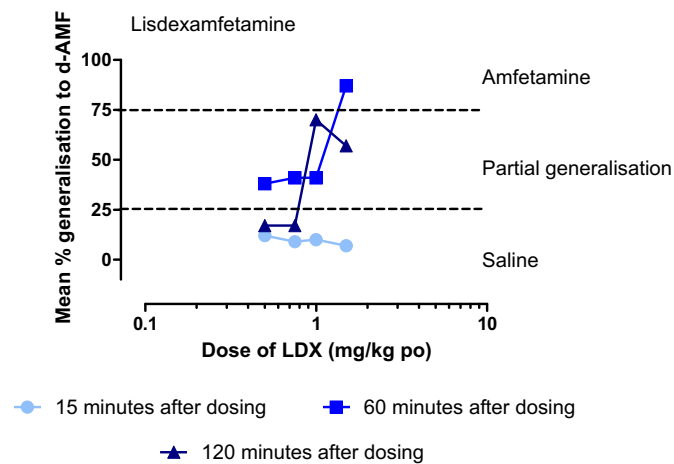
Compound	Dose (mg/kg)	Response rate (mean number of lever-presses/2.5 min $\pm$ S.D. [ $n$ value in parentheses])					
		Oral administration			Intraperitoneal injection		
		15 min	60 min	120 min	15 min	30 min	60 min
<i>D</i> -Amphetamine	0.5	31 $\pm$ 16 [38]					
Saline	–	69 $\pm$ 19 [38]					
Lisdexamfetamine	0.5	79 $\pm$ 15 [8]	57 $\pm$ 14 [7]	75 $\pm$ 25 [6]	77 $\pm$ 20 [6]		
	0.75	88 $\pm$ 29 [8]	79 $\pm$ 41 [8]	88 $\pm$ 30 [6]	82 $\pm$ 37 [7]		
	1.0	74 $\pm$ 34 [9]	95 $\pm$ 37 [9]	76 $\pm$ 20 [6]	55 $\pm$ 20 [7]		
	1.5	100 $\pm$ 26 [6]	57 $\pm$ 30 [9]	76 $\pm$ 46 [7]	57 $\pm$ 34 [6]		
<i>D</i> -Amphetamine	0.7	82 $\pm$ 44 [8]			55 $\pm$ 22 [6]		
	0.25	61 $\pm$ 21 [8]			63 $\pm$ 34 [6]		
	0.5	43 $\pm$ 18 [9]			41 $\pm$ 17 [10]		
	0.75	70 $\pm$ 57 [11]			–		
	1.5	40 $\pm$ 20 [10]			–		
Methylphenidate	0.75	–			57 $\pm$ 26 [6]		
	1.0	–			63 $\pm$ 24 [6]		
	1.5	–			46 $\pm$ 31 [8]		
	3.0	71 $\pm$ 23 [6]			52 $\pm$ 28 [6]		
	5.0	54 $\pm$ 20 [7]			–		
	10.0	54 $\pm$ 31 [7]			–		
Modafinil	50					73 $\pm$ 25 [6]	–
	100					59 $\pm$ 26 [6]	55 $\pm$ 14 [6]
	150					95 $\pm$ 55 [5/6]	94 $\pm$ 38 [6]
	200					–	Dis

Results show the mean operant response rates (number of lever-presses/2.5 min  $\pm$  S.D. [ $n$  value in parentheses]) when tested at various time-points after administration of lisdexamfetamine, *D*-amphetamine, methylphenidate, modafinil or vehicle.

– = not tested.

Dis = Behavioural disruption ( $\geq 50\%$  of rats with  $\geq 50\%$  decrease of operant responding).

Saline and *D*-amphetamine (0.5 mg/kg ip) data are the mean of 4 sessions preceding the first dose of test compound for each rat.



**Fig. 2.** Discriminative effects of orally administered lisdexamfetamine in rats trained to discriminate *D*-amphetamine (0.5 mg/kg, i.p.) from saline. Results are mean values for the percentage generalisation to the *D*-amphetamine (0.5 mg/kg, i.p.) cue;  $n = 6-9$  rats/group. Groups of rats were tested with lisdexamfetamine (0.3–1.5 mg/kg, p.o.) 15, 60 or 120 min after dosing in separate experiments. Generalisation was classified as: Amphetamine, Partial generalisation to *D*-amphetamine or Saline (For definitions see Fig. 1 legend). SD values ranged between 26% and 192% of the mean.

when *D*-amphetamine was administered intraperitoneally ( $ED_{50}$  [mg/kg] with 95% confidence intervals [95% CIs]); Oral = 0.39 [0.31; 0.49]; Intraperitoneal = 0.17 [0.13; 0.23]). Relative potency with 95% CIs = 0.419 [0.295, 0.595]. Because the 95% CIs do not include 1.0, the 2  $ED_{50}$  values were significantly ( $p < 0.05$ ) different.

When tested 15 min after dosing, intraperitoneal injection of methylphenidate (0.75–3.0 mg/kg) dose-dependently generalised to *D*-amphetamine (Fig. 3). Methylphenidate was 3.4-fold more potent when administered by the intraperitoneal versus the oral route ( $ED_{50}$  [mg/kg] with 95% CIs; Oral = 4.26 mg/kg [3.75; 4.84]). Intraperitoneal = 1.27 [0.98; 1.63]. Relative potency with 95% CIs = 0.299 [0.228, 0.394] indicating a significant difference ( $p < 0.05$ ) between the 2  $ED_{50}$  values.

When the discriminative effects of lisdexamfetamine were determined 15 min after intraperitoneal injection, the prodrug dose-dependently generalised to *D*-amphetamine (Fig. 3). Partial generalisation was observed at 0.75 and 1.0 mg/kg, i.p. with full generalisation at 1.5 mg/kg, i.p. To determine the influence of dose route on the potency of lisdexamfetamine, the intraperitoneal  $ED_{50}$  was compared against the oral  $ED_{50}$  obtained at the prodrug's time of peak effect, i.e. with a 60 min interval between dosing and testing. Switching from the oral to the intraperitoneal route of administration did not increase the potency of lisdexamfetamine

( $ED_{50}$  [mg/kg] with 95% CIs; Oral  $ED_{50} = 0.86$  [0.66; 1.14]; Intraperitoneal  $ED_{50} = 0.83$  [0.65; 1.07]). Relative potency with 95% CIs = 0.943 [0.656; 1.356] indicating no significant difference between the two  $ED_{50}$  values.

When comparing the influence of route of administration of lisdexamfetamine, *D*-amphetamine and methylphenidate on rates of operant responding, no differences were observed between the oral administration and intraperitoneal injection (Table 1).

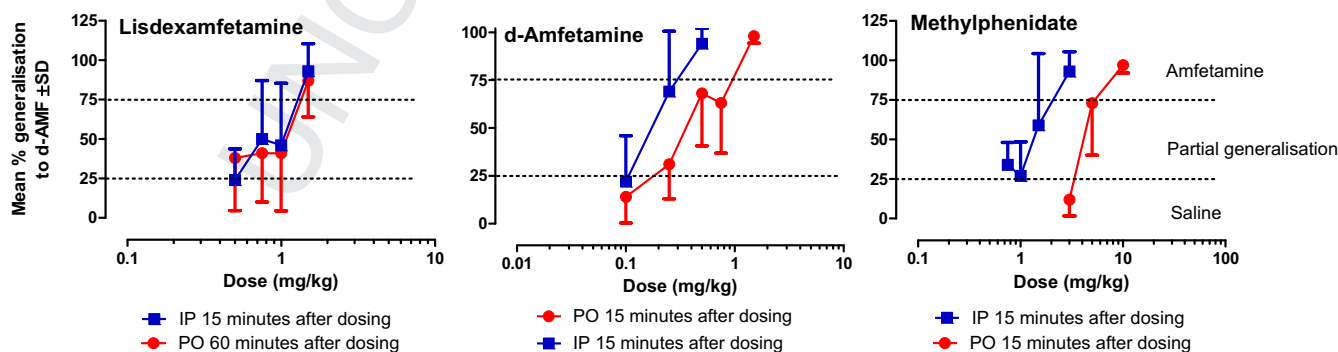
### 3.4. Discriminative profile of modafinil determined after intraperitoneal administration

Based on published descriptions of the time-course of modafinil's pharmacological effects after intraperitoneal injection (de Saint Hilaire et al., 2001; Paterson et al., 2010; Heal et al., 2012b), intervals of 30 min and 60 min between dosing and drug-discrimination testing were selected. When tested 30 min after dosing, all of the doses of modafinil, i.e. 50, 100 and 150 mg/kg, i.p., partially generalised to *D*-amphetamine (Fig. 4). When tested at 60 min, the pharmacological effect of modafinil was reduced (Fig. 4). Modafinil (100 mg/kg, i.p.) produced only 28% generalisation to amphetamine (2/6 Saline, 2/6 Partial Generalisation; 2/6 Amphetamine) compared with 45% generalisation to *D*-amphetamine at 30 min (5/8 Saline; 2/8 Partial Generalisation; 1/8 Amphetamine). Modafinil differed from all of the other stimulants investigated by virtue of the fact that irrespective of the dose tested, there were substantial inter-animal differences in the perception of its amphetamine-like discriminative effects.

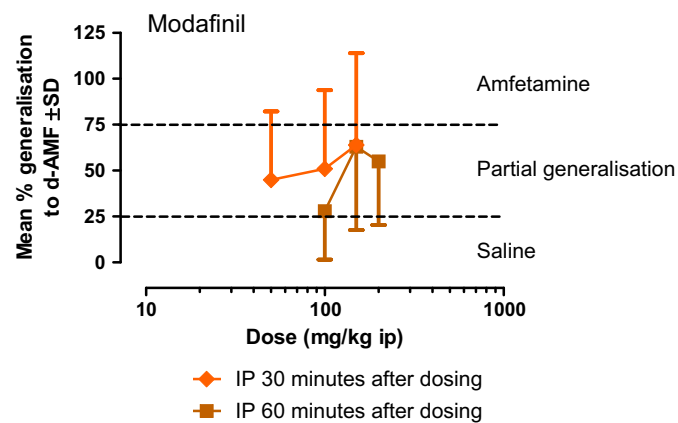
Intraperitoneally injected doses of modafinil  $\leq 150$  mg/kg had no effect the operant response rates of the rats (Table 1).

### 3.5. Reinforcing potential of methylphenidate, lisdexamfetamine and modafinil determined in rats using an intravenous self-administration model

The doses for methylphenidate (0.03, 0.1 and 0.3 mg/kg/injection, i.v.) were selected from previously published scientific data (e.g. Nielsen et al., 1984; Marusich and Bardo, 2009; Burton et al., 2010). An experiment was performed to select pharmacologically active doses of lisdexamfetamine and modafinil to be tested in the intravenous self-administration model. Groups of rats which had been trained to lever-press consistently for food rewards under a FR-2 schedule of reinforcement were given a single bolus injection of the test compounds and the rate of operant responding for food was monitored over the following 60 min or until 50 food pellet rewards had been collected. Lisdexamfetamine decreased the rate of operant responding by 20% and 28% at doses of 0.05 and 0.15 mg/



**Fig. 3.** Comparison of oral versus intraperitoneal potency of lisdexamfetamine, *D*-amphetamine and methylphenidate in rats trained to discriminate *D*-amphetamine (0.5 mg/kg, i.p.) from saline. Results are mean values for the percentage generalisation to the *D*-amphetamine (0.5 mg/kg, i.p.) cue  $\pm$  SD;  $n = 6-11$  rats/group. For lisdexamfetamine, its potency when administered by the intraperitoneal route was compared against its oral potency determined at the prodrug's time of peak effect, i.e. 60 min after dosing. Generalisation was classified as: Amphetamine, Partial generalisation to *D*-amphetamine or Saline (For definitions see Fig. 1 legend).



**Fig. 4.** Discriminative effects of modafinil determined in rats trained to discriminate *D*-amphetamine (0.5 mg/kg, i.p.) from saline. Results are mean values for the percentage generalisation to the *D*-amphetamine (0.5 mg/kg, i.p.) cue  $\pm$  SD;  $n = 6$ –9 rats/group. Groups of rats were tested 30 or 60 min after intraperitoneal dosing in separate experiments. Generalisation was classified as: Amphetamine, Partial generalisation to *D*-amphetamine or Saline (For definitions see Fig. 1 legend).

kg, i.v. (Table 2). Lisdexamfetamine showed a non-linear dose–response curve as it did not change the rate of operant responding when administered at the lowest and highest doses of 0.015 and 0.5 mg/kg, i.v. (Table 2). Based on these results, lisdexamfetamine doses of 0.05, 0.15 and 0.5 mg/kg/injection were selected for the self-administration experiment. Modafinil (0.166, 0.498 and 1.66 mg/kg, i.v.) incrementally increased the rate of operant responding across the 3 doses (Table 2), and therefore, these doses of modafinil were selected for the self-administration experiment.

Cocaine (0.32 mg/kg/injection, i.v.) maintained high rates of self-administration in all rats ( $p < 0.001$  versus saline for all groups of test compounds), whereas saline (i.v.) maintained only low rates of self-administration ( $\leq 8$  injections/session) in all rats before and after testing of lisdexamfetamine, methylphenidate or modafinil (Fig. 5).

Although methylphenidate was not self-administered when tested at 0.03 mg/kg/injection, doses of 0.1 and 0.3 mg/kg/injection maintained levels of self-administration significantly above saline (Fig. 5).

To ensure that the data were consistent across both laboratories participating in this study, methylphenidate (0.1 mg/kg/injection, i.v.) was retested as a validation experiment. Cocaine (0.32 mg/kg/injection, i.v.) and saline respectively served as a positive reinforcer and non-reinforcer in this group of rats, and as shown in Table 3, methylphenidate (0.1 mg/kg/injection, i.v.) was found to act as a

**Table 2**  
Effect of various doses of lisdexamfetamine and modafinil on FR-2 operant responding for food rewards.

Treatment	<i>n</i>	Mean number of lever-presses/min $\pm$ SEM	% Baseline
Vehicle (Saline, i.v.)	4	39.0 $\pm$ 2.4	–
Lisdexamfetamine (0.015 mg/kg, i.v.)	4	39.0 $\pm$ 2.4	100
Lisdexamfetamine (0.05 mg/kg, i.v.)	4	31.2 $\pm$ 3.0	80
Lisdexamfetamine (0.15 mg/kg, i.v.)	4	28.2 $\pm$ 4.8	72
Lisdexamfetamine (0.5 mg/kg, i.v.)	4	39.0 $\pm$ 1.2	100
Vehicle (40% (2-hydroxypropyl)- $\beta$ -cyclodextrin, i.v.)	3	7.5 $\pm$ 3.5	–
Modafinil (0.166 mg/kg, i.v.)	3	11.7 $\pm$ 1.9	155
Modafinil (0.498 mg/kg, i.v.)	3	14.7 $\pm$ 3.7	195
Modafinil (1.66 mg/kg, i.v.)	3	17.2 $\pm$ 2.2	228

positive reinforcer and it supported the same level of self-administration responding in both laboratories.

Modafinil (0.166, 0.498 or 1.66 mg/kg/injection, i.v.) did not maintain self-administration at levels significantly above saline at any dose (Fig. 5) showing that at these doses it did not serve as a positive reinforcer in rats. When the data were assessed in individual animals, 3/9 (33%) rats self-administered the high dose of modafinil (1.66 mg/kg/injection, i.v.) at levels above saline. However, in each case, the rats showed highly variable intakes of modafinil and all rats were tested for the maximum of 10 sessions without achieving consistent responding on the drug.

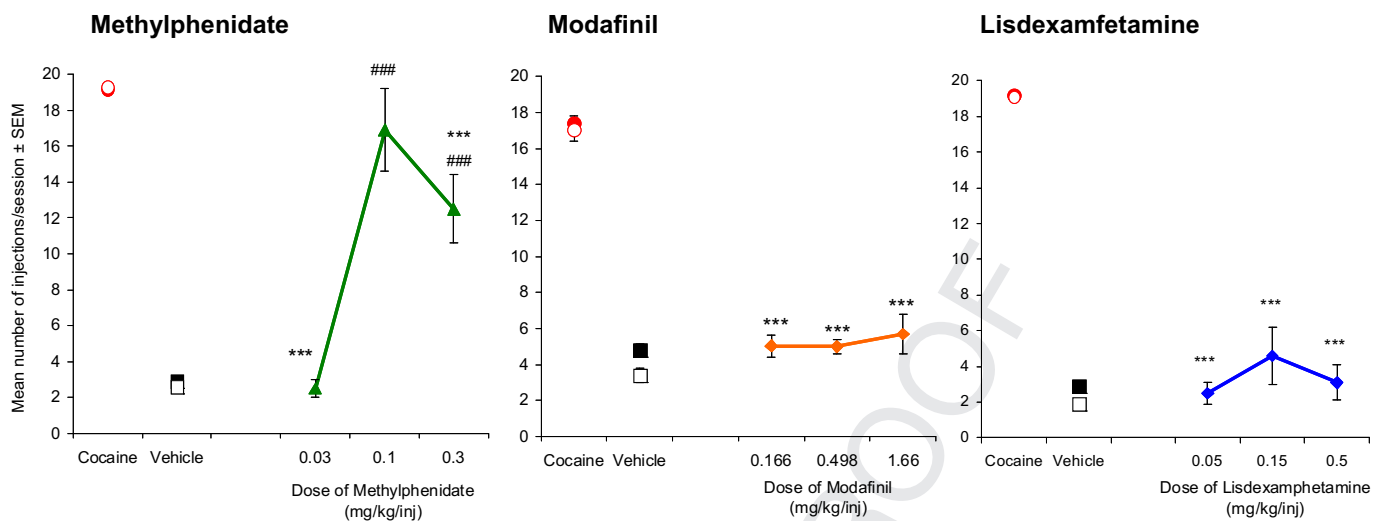
Lisdexamfetamine (0.05, 0.15 or 0.5 mg/kg/injection, i.v.) did not maintain self-administration at levels significantly above saline at any dose, (Fig. 5) showing that it did not serve as a positive reinforcer in rats. When the data for individual animals were analysed 2/10 (20%) and 1/9 (11%) rats self-administered the 0.15 mg/kg and 0.5 mg/kg doses of lisdexamfetamine at numerically greater levels than saline.

#### 4. Discussion

Drug-discrimination and intravenous self-administration are well established models to compare the similarity of the discriminative and positive reinforcing effects of novel centrally-acting drugs to those of known substances of abuse (Johanson, 1990; Balster, 1991; Ator and Griffiths, 2003; Solinas et al., 2006).

The drug-discrimination procedure employing female, PVG rats trained to discriminate *D*-amphetamine from saline has been extensively characterised with a range of monoaminergic drugs with and without liability for recreational abuse (Heal et al., 1992; Gosden et al., 1996). In general, this model has good predictive validity for detecting stimulants with high to low levels of recreational abuse liability; however, some catecholamine reuptake inhibitors, e.g. bupropion and nomifensine, show up as false positives (Heal et al., 1992; Gosden et al., 1996). In this model, *D*-amphetamine and methylphenidate both dose-dependently generalised to the discriminative cue elicited by intraperitoneal injection of *D*-amphetamine. This finding is consistent with the cross-generalisation of the discriminative cues of these two stimulants as determined in both rats (Witkin et al., 1991; Gosden et al., 1996; Craft and Stratmann, 1996; Kollins et al., 2001; Stadler et al., 2001; Desai et al., 2010) and human subjects (Martin et al., 1971; Smith and Davis, 1977; Heishman and Henningfield, 1991; Rush et al., 1998).

As a prodrug, lisdexamfetamine is pharmacologically inactive and its enzymatic conversion to yield the active moiety, *D*-amphetamine, and the naturally occurring amino acid *L*-lysine is unusual because it is mediated by a rate-limited enzymatic hydrolysis that is almost exclusively carried out by red blood cells (Pennick, 2010). The metabolic route profoundly influences the pharmacokinetics of lisdexamfetamine's active metabolite, which in turn influences its pharmacodynamic profile. In rats, plasma exposure to *D*-amphetamine after administration of lisdexamfetamine was not different when compared with immediate release (IR) *D*-amphetamine, but the  $C_{max}$  was 50% lower and the  $T_{max}$  was doubled (Rowley et al., 2012). As a result of its unusual pharmacokinetics, lisdexamfetamine was shown to be markedly less stimulant than IR *D*-amphetamine when tested at equivalent doses in terms of *D*-amphetamine base and the time of maximum activation was substantially delayed (Rowley et al., 2012). However, this prodrug produced substantial motor activation in rats by administering very high doses of lisdexamfetamine (Rowley et al., 2012). Intracerebral microdialysis experiments performed in rats have revealed that lisdexamfetamine dose-dependently increases the extraneuronal concentrations of dopamine and noradrenaline in the prefrontal cortex (PFC) with effects of equal magnitude on both catecholamine



**Fig. 5.** Evaluation of the possible reinforcing effects of various doses of methylphenidate, lisdexamphetamine and modafinil using a FR-2 schedule of drug reinforcement in rats compared with cocaine (positive reinforcer) and saline (nonreinforcer). Results are the mean number of injections/session  $\pm$  SEM for rats responding under a FR-2 schedule of intravenous drug reinforcement obtained either (a) in the last 3 sessions for each condition where responding was stable (see Methods for criteria) or (b) over 10 test sessions where responding was unstable. Results were analysed by multiple *t*-test using the average values from before and after test compound administration. Significantly different from saline: ###*p* < 0.001. Significantly different from cocaine: \*\*\**p* < 0.001. Cocaine and saline: closed symbols = result obtained before evaluation of test compound; open symbols = result obtained after evaluation of test compound.

neurotransmitters (Heal et al., 2012b). The *D*-amphetamine prodrug also dose-dependently increased dopamine efflux in the striatum (Rowley et al., 2012; Heal et al., 2012b). Although this profile is consistent with lisdexamphetamine producing its pharmacological effects via *D*-amphetamine, clear differences between lisdexamphetamine and the IR formulations of *D*-amphetamine and methylphenidate were observed in these experiments. In both microdialysis studies, the locomotor activity of the rats was monitored simultaneously with dialysate collection and the results unequivocally demonstrated that lisdexamphetamine was different from either *D*-amphetamine and methylphenidate by its ability to produce large and sustained increase in striatal dopamine efflux whilst producing only minimal behavioural activation (Rowley et al., 2012; Heal et al., 2012b).

Consistent with these findings, oral doses of lisdexamphetamine  $\leq 5.06$  mg/kg (equivalent to 1.5 mg/kg of *D*-amphetamine base), were not recognised as amphetamine-like on the *D*-amphetamine-cued drug-discrimination model when tested 15 min after dosing. When the interval between dosing and testing was increased to 60 min, the same doses of lisdexamphetamine dose-dependently generalised to the *D*-amphetamine cue. Extending the interval to 120 min diminished the stimulant discriminative effect of lisdexamphetamine as shown by the result that the prodrug no longer generalised fully to the *D*-amphetamine cue. Together, these findings indicate that the amphetamine-like discriminative effects of orally administered lisdexamphetamine are delayed in onset and of relatively short duration.

Modafinil has an enigmatic pharmacological mechanism of action (Minzenberg and Carter, 2008), but neurochemical

experiments performed *in vitro* and *in vivo* indicate that it has  $\sim 5$   $\mu$ M affinity for the human dopamine reuptake transporter (DAT) (Madras et al., 2006; Zolkowska et al., 2009). Despite this fact, Volkow et al. (2009) has reported that clinical doses of modafinil, i.e. 200 and 400 mg, occupied  $\sim 46\%$  of DAT sites in the caudate,  $\sim 53\%$  in the putamen and  $\sim 60\%$  in the nucleus accumbens in human subjects *in vivo*. Intracerebral microdialysis experiments have revealed that modafinil increased the extracellular concentrations of noradrenaline and dopamine in the PFC (de Saint Hilaire et al., 2001; Rowley et al., 2012), dopamine and 5-HT in the nucleus accumbens (Zolkowska et al., 2009), dopamine in the striatum (Rowley et al., 2012), and noradrenaline and dopamine in the rostromedial hypothalamus (de Saint Hilaire et al., 2001). The finding that modafinil can enhance the extracellular concentration of dopamine in the rat brain are supported by microdialysis determinations of striatal dopamine in rhesus monkeys (Andersen et al., 2010) and positron emission tomography (PET) experiments showing the displacement of [ $^{11}$ C]raclopride from striatal  $D_2$  receptors by dopamine in human subjects (Volkow et al., 2009).

Because of its weak potency and very poor solubility, the discriminative effects of modafinil in the *D*-amphetamine-cued drug-discrimination test were determined only after intraperitoneal injection of the drug in suspension. In this model, modafinil partially generalised to *D*-amphetamine at doses ranging from 50 to 200 mg/kg indicating that it produces some *D*-amphetamine-like discriminative effects. However, this atypical stimulant never generalised fully to the *D*-amphetamine cue. In contrast to all of the other drugs tested, substantial inter-animal variability in responding was present for all of the doses tested revealing that modafinil was clearly recognised as *D*-amphetamine-like by some rats, but different from *D*-amphetamine by others.

The only other comparison of the discriminative effects of modafinil and *D*-amphetamine was performed by Dopheide et al. (2007), who also observed that modafinil partially, but not fully, generalised to *D*-amphetamine with considerable inter-individual variability in the responses of the rats. (Dopheide et al., 2007) also observed that modafinil partially generalised to the cocaine discriminative cue in a separate group of rats trained to distinguish between cocaine and saline. Partial or full substitution for cocaine

**Table 3**

A comparison of the intravenous self-administration profile of cocaine, methylphenidate and saline in the two participating laboratories.

Research facility	Number of injections/session $\pm$ SEM		
	Cocaine (0.32 mg/kg/inj)	Saline	Methylphenidate (0.1 mg/kg/inj)
University of Texas	19.4 $\pm$ 0.2 ( <i>n</i> = 8)	2.8 $\pm$ 0.6 ( <i>n</i> = 8)	16.9 $\pm$ 2.3 ( <i>n</i> = 8)
RenaSci University of Nottingham	18.6 $\pm$ 1.1 ( <i>n</i> = 4)	5.3 $\pm$ 0.7 ( <i>n</i> = 4)	18.0 $\pm$ 2.4 ( <i>n</i> = 4)

by modafinil has been observed in several other investigations performed in rats (Gold and Balster, 1996; Newman et al., 2010; Paterson et al., 2010) and in rhesus monkeys (Gold and Balster, 1996). It has previously been reported that *D*-amphetamine and cocaine share a common discriminative cue in rats (Witkin et al., 1991; Gosden et al., 1996; Craft and Stratmann, 1996; Gold and Balster, 1996; Stadler et al., 2001).

In drug-experienced human volunteers, who had been trained to discriminate between cocaine and placebo, modafinil generalised partially (~60%) to the cocaine cue whilst methylphenidate produced high levels of generalisation to cocaine in most subjects (Rush et al., 2002a). Modafinil also evoked subjective ratings of “high”, “take again”, “good effects” and moderate “drug liking” (Rush et al., 2002a). Two other studies performed in drug-experienced humans also found that modafinil produced stimulant-like subjective effects and had positive reinforcing properties (Jasinski, 2000; Stoops et al., 2005). When all of the animal and human data are taken into consideration, they lead to the conclusion that modafinil differs from *D*-amphetamine, methylphenidate and lisdexamfetamine by virtue of eliciting a discriminative stimulus that is much weaker than that evoked by these other stimulants.

The rate at which stimulants and other substances of abuse enter the brain is an important factor in determining their liability for recreational abuse (Volkow et al., 1996, 2003; Kollins et al., 1998; Gorelick, 1998; Cone, 1998). To maximise both the quantity and rate of entry of drugs into the brain, some abusers resort to non-clinical routes for self-administration including intravenous injection, “snorting” and smoking. These approaches increase the chance of overdose and expose the individual to other serious hazards, e.g. hepatitis or HIV infection from unclean needles. In this investigation, the influence of dosing route on potency was explored in the rat drug-discrimination model by comparing dose–response curves obtained when the stimulants were given orally and by intraperitoneal injection. When given by the oral route, the ED<sub>50</sub> values for generalisation to the training cue revealed that *D*-amphetamine was ~10-fold more potent than methylphenidate. When the route was switched to intraperitoneal injection, the potency of *D*-amphetamine increased 2.2-fold, but the potency of methylphenidate was increased by 3.4-fold. When the finding that methylphenidate maintained robust self-administration at a dose of 0.1 mg/kg is also taken into consideration, it indicates that even greater increases in methylphenidate’s potency would occur when the intravenous route is selected. In lisdexamfetamine’s case, switching from the oral to the intraperitoneal route decreased the delay before the prodrug’s amphetamine-like discriminative effects could be detected by the rats, but intraperitoneal injection did not enhance the prodrug’s potency. This lack of influence of the dose route on the potency of lisdexamfetamine extends to humans where the pharmacokinetics of lisdexamfetamine’s active metabolite, *D*-amphetamine, are not altered when nasally insufflated compared with the clinically relevant oral route (Ermer et al., 2011). Jasinski and Krishnan performed trials in drug-experienced, human volunteers where the subjective and reinforcing effects of lisdexamfetamine were evaluated when it was given orally (Jasinski and Krishnan, 2009a) or by intravenous injection (Jasinski and Krishnan, 2009b). A recent *post hoc* analysis of results taken from these two trials showed that the potency of lisdexamfetamine was unchanged in human subjects irrespective of whether the oral or the intravenous route was employed (Heal et al., 2013). The explanation is the rate-limited enzymatic hydrolysis of lisdexamfetamine that is carried out by peptidases associated with red blood cells dictates that the gradual and sustained liberation of *D*-amphetamine cannot be accelerated by loading the blood-stream with the parent compound. The outcome is lisdexamfetamine’s potency remains relatively constant irrespective of its route of administration.

The ability of lisdexamfetamine, methylphenidate and modafinil to serve as positive reinforcers were compared in rats trained to intravenously self-administer low dose cocaine. In self-administration experiments, this route is routinely employed because it is the one that carries the greatest safety risks for recreational drug abusers. Cocaine served as a robust positive reinforcer in all of the rats with animals consistently taking >15 injections/session. In contrast, saline, which was used as the placebo control in these experiments, maintained only low levels of self-administration, i.e. generally <5 injections/session. Methylphenidate served as a robust positive reinforcer at very low doses, i.e. 0.1 and 0.3 mg/kg/injection and this finding agrees with numerous previous reports that this stimulant maintains high levels of self-administration in rodents (Nielsen et al., 1984; Botly et al., 2008; Marusich et al., 2010), and primates (Johanson and Schuster, 1975; Bergman et al., 1989; Schindler et al., 2011). Consistent with the preclinical findings, methylphenidate has been found to be a powerful reinforcer in many double-blind, placebo-controlled trials in human subjects (Smith and Davis, 1977; Kollins et al., 1998; Volkow et al., 1999; Jasinski, 2000; Rush and Baker, 2001; Stoops et al., 2003, 2004; Spencer et al., 2006). In an interesting study, Kollins et al. (1998) compared the subjective and reinforcing properties of a sustained release (SR) and IR formulation of methylphenidate in healthy volunteers and observed that the stimulant and reinforcing effects of the former were attenuated and transient compared with the latter leading the authors to conclude that the SR formulation posed a reduced risk for recreational abuse. To summarise, therefore, methylphenidate produces both the subjective and reinforcing effects in humans that are typical of stimulants like cocaine and *D*-amphetamine supporting the view that the preclinical results obtained in these drug-discrimination and self-administration experiments have good translational validity.

Modafinil did not maintain self-administration at levels above saline when tested across a range of pharmacologically active doses indicating that this atypical stimulant does not serve as a positive reinforcer in cocaine-trained rats. Analogous to results obtained from the drug-discrimination model, several of the rats showed highly variable intakes of modafinil over the 10 sessions of the self-administration experiment. In these individuals, the average number of injections/session taken was numerically greater than the saline control value, which hints that modafinil may act as a weak reinforcer in a minority of rats. Although modafinil’s very poor solubility limited the dose that could be evaluated in the rats, there was no suggestion in the results that higher doses of modafinil elicited greater levels of self-administration. That being said, it would be unwise to exclude the possibility that modafinil would not serve as a positive reinforcer in rats under any circumstance. The results reported here are in general agreement with those of other determinations of modafinil’s reinforcing effect in rats. Modafinil failed to serve as a positive reinforcer in an intravenous self-administration procedure employing drug-naïve rats, and in addition, it did not induce place preference in rats (Deroche-Gamonet et al., 2002). In contrast, *D*-amphetamine, which was employed as the positive control, produced a clear preference for the drug-associated compartment (Deroche-Gamonet et al., 2002). On the other hand, Gold and Balster (1996) reported that modafinil unequivocally maintained intravenous self-administration at levels above saline in a group of 4 cocaine-trained rhesus monkeys. Additional evidence for similarity between the reinforcing properties of modafinil and cocaine come from the findings that modafinil reinstated both cocaine-conditioned place preference in rats (Bernardi et al., 2009) and cocaine self-administration in rhesus monkeys (Andersen et al., 2010). In drug-experienced volunteers, modafinil produced stimulant-like subjective effects and acted as a

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reinforcer (Jasinski, 2000; Rush et al., 2002a). Stoops et al. (2005) reported that modafinil served as a reinforcer in human volunteers performing a cognitive task, although not when they were relaxing. However, the weakness of modafinil's reinforcing effect relative to cocaine was demonstrated by its failure to serve as a positive reinforcer in stimulant abusers (Rush et al., 2002b; Vosburg et al., 2010) and its lack of clinical efficacy in the treatment of cocaine dependence, showing that it has low reinforcing effects of its own (Anderson et al., 2009).

Lisdexamfetamine did not maintain intravenous self-administration at levels greater than saline when tested across a range of doses indicating that this prodrug does not serve as a positive reinforcer in cocaine-trained rats. As indicated by the reduced operant responding for food rewards, the doses employed were pharmacologically active. Moreover, as the potency of this prodrug is not materially influenced by its route of administration, a non-contingent injection of the highest dose, i.e. 0.5 mg/kg/injection, which was employed to initiate the self-administration session, would almost certainly have elicited partial generalisation to the training cue if it had been tested in the *D*-amphetamine-cued drug-discrimination model. Although the reinforcing properties of lisdexamfetamine's metabolite, *D*-amphetamine, were not determined in this study, the latter has been consistently reported to maintain robust intravenous self-administration in rats (Yokel and Wise, 1978; Di Ciano et al., 1995; Carroll and Lac, 1997; Crombag et al., 2008) and primates (Johanson et al., 1976; Aigner and Balster, 1979). Furthermore, *D*-amphetamine substituted for cocaine as a positive reinforcer in primates (Johanson et al., 1976) and also reinstated cocaine self-administration behaviour in rats (de Wit and Stewart, 1981; Suto et al., 2002).

In drug-experienced human volunteers, lisdexamfetamine was less potent than *D*-amphetamine (Jasinski and Krishnan, 2009a) in producing amphetamine-like stimulant effects and there was a substantial delay in their appearance. However, consistent with the data from the rodent experiments (Rowley et al., 2012; Heal et al., 2012b; this study), lisdexamfetamine nonetheless has the ability to elicit stimulant pharmacological effects in man when given at high doses (Jasinski and Krishnan, 2009a).

Predictions on whether a compound will be subjected to diversion and recreational abuse based on results from either preclinical experiments or trials in drug-experienced human volunteers is uncertain because many other factors come into play in the real world. Examples include how well the euphoriant and stimulant profile of the drug fits with the culture and context of recreational abuse which varies widely from country to country, the availability and cost of alternatives, its pharmacokinetic profile, and the ability to substantially increase the drug's psychostimulant experience by employing non-clinical routes of self-administration. In the case of modafinil, the preclinical and clinical data reveal that its pharmacological profile is unlikely to make it attractive as a recreational drug of abuse, but it does possess cognitive enhancing and wake-promoting properties that would support non-medical misuse. In contrast, methylphenidate is a well established, stimulant euphoriant with powerful reinforcing properties that present a substantial risk for recreational abuse. To some extent, this has been counterbalanced by the development of long-acting preparations of methylphenidate that attenuate its stimulant potential (Kollins et al., 1998) and tamper-deterrent formulations and delivery systems that increase the difficulty of recreational abuse by snorting or intravenous injection. The unusual metabolic activation of lisdexamfetamine predicts that its recreational abuse liability will not be influenced by changing its route of administration (Jasinski and Krishnan, 2009a,b; Ermer et al., 2011; Heal et al., 2013; this study). Furthermore, although lisdexamfetamine generalised fully to *D*-amphetamine in the drug-discrimination test, its stimulant

effects were transient and this prodrug did not serve as a positive reinforcer in rats. In all of the above respects, lisdexamfetamine was clearly differentiated from IR methylphenidate. Although these preclinical experiments have explored lisdexamfetamine's potential for recreational abuse, they do not address the issue of non-clinical misuse.

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The authors wish to state that the opinions expressed in this manuscript reflect their own views and do not necessarily reflect those of Shire Pharmaceuticals.

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