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The effects of clinically relevant doses of amphetamine and methylphenidate on signal detection and DRL in rats

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Abstract

Low dose amphetamine (AMPH) and methylphenidate (MPH, Ritalin[®]) are the most widely prescribed and most effective pharmacotherapy for attention-deficit/hyperactivity disorder (ADHD). Certain low, clinically relevant doses of MPH improve sustained attention and working memory in normal rats, in contrast to higher doses that impair cognitive ability and induce locomotor activity. However, the effects of AMPH or MPH on sustained attention and behavioral inhibition remain poorly characterized. The present experiments examined the actions of AMPH (0.1 and 0.25 mg/kg) and MPH (0.5 and 1.0 mg/kg) in a rat model of 1) sustained attention, where signal and blank trials were interspersed randomly and occurred at unpredictable times, and 2) behavioral inhibition, using a differential reinforcement of low rate (DRL) schedule. In a signal detection paradigm, both 0.5 mg/kg and 1.0 mg/kg MPH and 0.25 mg/kg AMPH improve sustained attention, however neither AMPH nor MPH improve behavioral inhibition on DRL. Taken together with other recent studies, it appears that clinically-relevant doses of AMPH and MPH may preferentially improve attention-related behavior while having little effect on behavioral inhibition. These observations provide additional insight into the basic behavioral actions of low-dose psychostimulants and further suggest that the use of sustained attention tasks may be important in the development of novel pharmacological treatments for ADHD.

Keywords

Attention; Behavioral inhibition; Signal detection; DRL; Methylphenidate; Amphetamine; Rats

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is associated with a dysregulation of working memory, sustained attention, behavioral inhibition/impulsivity and hyperactivity. Conservative estimates suggest that 3–5% of both children and adults are affected (Solanto, 1998; Wilens et al., 2004). Low doses of psychostimulants, amphetamine (AMPH) and

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methylphenidate (MPH), comprise the most common and most effective pharmacotherapy for ADHD (Greenhill, 2001). In ADHD-affected individuals, low-dose stimulants reduce motor activity while improving performance in tests of working memory, sustained attention and impulsivity (Solanto, 1998). Interestingly, the cognitive enhancing and behavioral calming actions of low-dose psychostimulants are not limited to individuals with ADHD, but also extends to “healthy” human and animal subjects (A. F. Arnsten and Dudley, 2005; Kuczenski and Segal, 2002; Mehta et al., 2000; J. L. Rapoport et al., 1980; Vaidya et al., 1998). These actions contrast with those of higher doses, which produce pronounced cognitive impairments and locomotor activation (McGaughy and Sarter, 1995; Segal, 1975).

Recently, we demonstrated biphasic dose-dependent actions of MPH on working memory, with beneficial effects observed at low doses but not at modestly higher doses (i.e. 2.0 mg/kg oral vs. 8.0 mg/kg oral). In addition, we showed that a clinically relevant dose of intraperitoneally (IP)-administered MPH (0.5 mg/kg) improved sustained attention in a signal detection task in rats (Berridge et al., 2006). However, little is known regarding the dose-dependency of the effect on sustained attention and the degree to which this is observed with other psychostimulants.

In addition, less information about the actions of low-dose psychostimulants on measures of behavioral inhibition in healthy animals has been gathered. Differential Reinforcement of Low Rates (DRL) schedules of reinforcement have been proposed as an assay of impulsivity (Monterosso and Ainslie, 1999; D. B. Neill, 1976; D.B. Neill and Herndon, 1978; Peterson et al, 2003). This schedule reinforces responding if a certain amount of time has expired between responses (i.e., inter-response times (IRTs) exceed a certain value). DRL schedules test impulsivity because the participant must respond at certain times, and inhibit responding at other times. Seiden and colleagues have shown that both MPH and AMPH disrupt, rather than improve, DRL performance (Balcells-Olivero et al., 1998; Sabol et al, 1995; Seiden et al, 1979), although they tested doses that induce locomotor activity and produce sensitization, thereby limiting the relevance of their findings to the therapeutic actions of these drugs. In humans, deficient DRL performance of ADHD subjects has been observed in one study (McClure and Gordon, 1984) but not others (Avila et al, 2004; Daugherty and Quay, 1991). Nevertheless, DRL schedules may be a useful screening tool for the therapeutic actions of psychostimulants on impulsivity/behavioral inhibition in animals given its face validity, ease of implementation, and amenability for use with non-human animals. Moreover, the effects of low dose psychostimulants (cf. higher doses) on this widely used paradigm are not known.

The current studies were designed to provide a more complete description of the effects of low-dose psychostimulants on sustained attention, as demonstrated in Berridge et al. (2006), and to assess the degree to which this extends to other commonly used psychostimulants in the treatment of ADHD. A second goal of the current studies was to examine the same psychostimulants, demonstrated to improve sustained attention, in other paradigms posited to assess response inhibition.

While several rodent models of ADHD have been developed, the present experiments used a “normal” rat strain for several reasons. First, MPH and AMPH have been shown to improve

performance in a number of paradigms for both ADHD-affected and non-affected individuals (see citations above). These improvements are perhaps most parsimoniously explained as cognitive-enhancing effects of these drugs on both affected and non-affected individuals, in contrast to “impairment-reducing” effects on individuals with ADHD and cognitive-enhancing effects on non-ADHD individuals. Thus, employing a “normal” rodent model would reveal potentially important effects applicable to both ADHD-affected and non-affected individuals. Secondly, the most common rat models of ADHD suffer from some limitations and interpretive concerns. For example, while substantial data on attention and impulse-control problems have been generated in studies using the Spontaneous Hypertensive Rat (SHR), debates remain surrounding issues of the proper control strain (e.g., the progenitor strain vs. a “normal” strain like the Sprague-Dawley, (Sagvolden et al, 2009)). A recent review concluded that predictive validity of the SHR strain in drug efficacy studies is highly dependent on the behavioral test used (Heal et al, 2008). It seems prudent, then, to construct a more complete understanding of MPH's and AMPH's actions on behavior in an outbred strain before proceeding to the SHR model (or other, even less validated model). Lastly, the research presented here seeks to uncover the influence of psychostimulants on attention and behavioral inhibition, rather than the ameliorative effects of psychostimulants on ADHD. The rationale for studying psychostimulants' effects on attention and inhibition is provided by the fact that they are widely prescribed for the treatment of ADHD.

2. Method

2.1. Subjects

Male Sprague-Dawley rats (Harlan, Madison, WI) were housed in pairs in polyethylene cages in colony room with a 12:12 h light/dark cycle. Upon arrival, rats were approximately 70 days old and weighed approximately 300 g each. They were weighed and handled daily and provided with food ad libitum for 7 days. Each rat was reduced to 85% of its ad libitum weight over the course of approximately 3 weeks. Each rat was then feed between 9 and 15 g of chow per day at least one hour after a day's session. According to growth curves provided by the breeder, Sprague-Dawley rats normal gain about 15 g per week, thus body weights were allowed to increase about 12–13 g per week (roughly 85%). All rats were weighed immediately prior to a day's injection or session, thus ensuring the correct drug dose. Water was freely available in the home cage. Care of rats was in accordance with University of Wisconsin-Madison animal care committee guidelines. One squad of experienced rats ($n = 8$), used in a previous experiment (Berridge et al., 2006), served in the Signal Detection experiment. Prior to the present experiments, this squad of rats had experienced a total of 118 sessions of signal detection: 44 baseline training sessions, 27 drug injection sessions (which provided the data for Berridge et al., 2006), and 47 drug-free, re-establishment of baseline sessions. In that previous study, rats received low doses of MPH (0.5 mg/kg) and AMPH (0.1 mg/kg), in a similar fashion described below. Briefly, injections were never given on consecutive days and the order of injections was random. Because each subject served as its own control and the lengthy drug-free period in between drug testing phases, the prior drug exposure did not likely influence the present drug testing results (e.g., sensitization). Drug testing began with the first group when the rats were approximately 9

months old. The second squad of rats ($n = 8$) used in the DRL experiment were 70 days old, 300 g, and experimentally naïve at the start of that experiment. Care was identical to that of the first group.

2.2. Drugs

D-amphetamine hemisulfate (AMPH, 0.1 mg/ml, 0.25 mg/ml) and Methylphenidate hydrochloride (MPH, 0.5 mg/ml, 1.0 mg/ml) were obtained from Sigma–Aldrich (St. Louis, MO, USA). They were measured as salt, dissolved in sterile saline and administered IP in a volume of 1.0 ml/kg. Doses of MPH were selected because they produce clinically relevant plasma concentrations in rats, lack locomotor-activating effects, and improve spatial working memory and sustained attention (Berridge et al., 2006; Kuczenski and Segal, 2001, 2002). Doses of AMPH were selected because their administration produces increases in prefrontal catecholamine efflux comparable to those observed with clinically relevant doses of MPH while lacking locomotor-activating effects (Berridge and Stalnaker, 2002). AMPH, in the range used here, also produces dose-dependent changes in consummatory, spontaneous and unconditioned behavior, learning, and drug discrimination (see Grilly and Loveland, 2001 for review).

2.3. Experimental chambers

Sessions were conducted in standard tall operant conditioning chambers (Med Associates, St Alban, VT, model ENV-007, interior dimensions: 305 mm wide, 241 mm deep, and 292 mm high) made of sheet metal and plexi-glass and enclosed in ventilated chests. Fans provided some masking noise continuously throughout sessions. Two retractable levers (Med Associates model ENV-112CM, 48 mm wide $\times$ 19 mm deep) could be projected into the chamber on the right-side wall. A force of approximately 0.20 N was required to depress the levers and register a response. Spaced equally between the two levers was a feeder trough into which 45 mg sucrose pellets (reinforcers, Bio-Serv, Dustless Precision Pellets, #F0042) could be delivered. Above each trough was a row of three stimulus lights (red, yellow, and green LEDs, $\sim$ 1 lux when illuminated, Med Associates model ENV-222M) and above them was a 28V houselight (Med Associates model ENV-215M). Experimental events were arranged and recorded via a personal computer in the same room as the chambers, running Med-PC for Windows Version IV (Med Associates, St. Alban, VT).

2.4. Lever-press training

All rats were initially trained to lever press during 9 daily, 30-min sessions, as described elsewhere (Andrzejewski et al., 2007). All rats were lever pressing by the end of training.

2.5. Experimental design

Rats received extensive training and when performance was judged stable drug testing was started. Drug testing did not start until all of the rats in an experiment met stability criteria. In addition, rats were habituated to the injection procedure by: 1) lightly restraining them, exposing their peritoneal area, and gently poking them with a capped syringe (for 5–10 sessions), and then 2) giving 3 separate vehicle injections (spaced at least 2 days apart) after stability criteria were met (data from these sessions were not used). All rats then went

through drug testing. During this phase, rats received injections 30 min prior to the start of the session. Each rat experienced a different, randomized order of drug injections with the constraint that they receive all 5 doses (vehicle, 0.1 AMPH, 0.25 AMPH, 0.5 MPH, and 1.0 MPH) at least once before a dose was repeated. All rats received 2 rounds of drug injections, however each round was randomly determined. Drug testing occurred every other session; there was at least one “drug free” session between injection sessions. Means were computed over the course of all drug testing, differentiated by rat and condition, facilitating a repeated-measures statistical analysis. Drug free data were compared to that from vehicle sessions; the results were not statistically different and are not shown. Additionally, there were no trends in the results dependent on the replication number (e.g., increasing or decreasing efficacy of the drug). The replications were deemed necessary because, in our experience, the facilitative effects of these drugs are fairly small and variable. Therefore, the replications were needed to ensure a good estimate of the drug effects.

2.6. Signal detection (Sustained attention) task

The signal detection procedure was based on those described by Bushnell, Sarter and colleagues (Bushnell, 1998; McGaughy and Sarter, 1995). It was identical to that used in Berridge et al. (2006). Briefly, sessions lasted 100 trials (about 35 min). “Signal trials” (green LED, ~1 lux) were randomly interspersed with blank (no signal) trials; there were equal numbers of signal and no signal trials. Following signal presentation (or the end of the no signal period), the levers were projected into the chamber. A right lever press was a “signal present” report and a left lever press was a “signal absent” report. Correct responses were reinforced with two sucrose pellets, incorrect responses were followed by a 5s blackout. If the rat failed to respond with 5s, the levers were retracted, followed by a 5s blackout. “Omitted” trials occurred infrequently (<1 trials per session, on average) and were not included in the analysis. The start of the next trial began unpredictably (sampled every second after 5s with a 0.125 probability). This arrangement permitted about 3 trials per minute. There were no correction trials.

Rats began training with a 4s signal until performance was stable through visual inspection of the data. The signal length was then shortened to 0.25s–2.0s in length. Performance was judged stable during this phase if the proportion of correct responding was within $\pm 5\%$ of the previous 3 days. In the drug phase of this experiment, the signal length was randomly selected from the list: 0.25s, 0.5s, 0.75s, 1.0s, 1.5s, and 2.0s with replacement (signal lengths were programmed to occur with equal probability). Because the signals occur unpredictably and vary in length, individual rats must remain attentive to the stimulus array; this preparation is considered a test of sustained attention (Bushnell, 1998). In addition, it seems reasonable to assume that attentional demands varied because the length of the signal varied, with long signals proving easy to detect relative to short ones.

2.6.1. Dependent measures—Dependent measures include the conditional probability of a hit (the proportion of signal trials in which the rat reported a signal present), conditional probability of a false alarm (the proportion of no signal trials in which the rat reported a signal present), d' (sensitivity), c (response bias) and response latencies. d' was computed by taking the z transformation of the $\text{Pr}(\text{Hit})$ and subtracting the z transformation of $\text{Pr}(\text{FA})$: d'

$= z(\text{Pr}(\text{Hit})) - z(\text{Pr}(\text{FA}))$). The theoretical range of d' is $-\infty$ to ∞ , however it was mathematically constrained to -4.107 to 4.107 (at least 1 error detecting a signal and 1 error on no signal). The bias parameter c , which is completely orthogonal to d' , was computed using the formula: $c = -0.5(z(\text{Pr}(\text{Hit})) \pm z(\text{Pr}(\text{FA})))$; it was constrained in the same way as d' .

Measures of d' and c were chosen because of their extensive use in the signal detection literature, both theoretical and experimental. The utility of d' and c have been reviewed extensively elsewhere (Gescheider, 1985; Green and Swets, 1966; Macmillan and Creelman, 2005; Swets et al., 1961). Generally, d' is considered a pure measure of stimulus detectability (sometimes referred to as a discriminability index) because it takes into account both hits and false alarms. d' is a function of stimulus intensity and capacity of sensory system, but most importantly, is uncontaminated by the participant's decision criterion. Thus, changes in d' in the current experiment will be interpreted as changes in sensitivity, not changes in decision criteria. c is a completely orthogonal measure to d' and indicates if a rat is more or less likely to respond in a particular way (e.g., report signal present), regardless of the type of trial (i.e., signal vs. non-signal) and is not expected to change in the present experiments. Response latencies (time between lever insertion and a response) were computed to assess the possible locomotor activating effects of the psychostimulants used here.

2.7. Differential Reinforcement of Low Rates (DRL)

A DRL schedule reinforces a response after a set amount of time has expired in which no response has occurred. In the present experiment, the rat had to make a lever press, wait (the delay interval), and then make another lever press to produce a reinforcer; lever presses during the delay interval reset the timer. The second squad of rats, used in the DRL experiments, received two phases of training prior to drug testing.

2.7.1. Preliminary training—Sessions were 30 min in length. After lever press shaping, the reinforcement schedule was thinned directly from an FR-1 to an RI-30s (a reinforcer was set up, on average, every 30s, no intermediate values between FR-1 and RI-30s were used). This training lasted until a current session's response total was within $\pm 10\%$ of the previous three session's average. When all rats had met criteria, they were moved to a Multiple RI-30s DRL-30s schedule.

2.7.2. Multiple RI-30s DRL-30s—Sessions lasted 48 min sessions and were divided into 8 components (4 DRL and 4 RI). A session began with the insertion of either the left or right lever into the chamber, chosen randomly, and the illumination of the houselight. The first component lasted 5 min and lever presses were reinforced on either an RI-30s or DRL-30s, depending on lever assignment. There was no limited hold on the DRL-30s contingency. At the end of a component, the levers were retracted and the house-light was extinguished for 1 min. The second component was also 5 min, with the houselight on, but the opposite lever to the one used in the first component, was inserted. Responses on this lever were reinforced on the other schedule. Another 1 min time out followed, then a 5 min component identical to the first, and so on. Which lever was inserted during the first component was selected

randomly each day, although for individual rats, lever assignment (e.g., Left – DRL, etc.) was constant. Lever-assignment was counterbalanced across rats. A schematic of a typical session for a rat running on the DRL-Left/RI-Right is diagrammed in Fig. 1.

Multiple schedule training ensued until responding was stable ($\pm 25\%$ of the previous 3-day average). When stability was reached, drug testing began.

The rationale for running a multiple schedule, rather than a simple DRL schedule was that the drug-RI-30s components would provide the most stringent controls for the DRL-30s components. In order to control for the possible response rate-altering effects of psychostimulants, performance on the DRL-30s under the effects of a drug would be compared to RI-30s performance under the same drug, during the same session. Moreover, since the two schedules were presented in the same session, concerns about the integrity of a drug injection would be mitigated.

2.7.3. Dependent measures—The primary dependent variable was the ratio of total responses in the DRL-30s components of a session to responses in the RI-30s (suppression ratio), after injection of the same drug (or vehicle). Other dependent variables response rates during DRL-30s and RI-30s components and inter response time (IRT) distributions.

2.8. Data analysis

Data were analyzed using repeated measures, two-way ANOVA (with drug and signal length as factors), or paired *t*-tests. *A priori*, our hypotheses were focused on comparing the individual drug doses to saline. Because an omnibus repeated-measures ANOVA would have to account for 10 possible comparisons, the restriction of our analyses to 4 comparisons (each of the drug doses vs. saline) increased our statistical power, and moreover, satisfies a conservative criterion for conducting multiple comparisons. Significance level was set at $\alpha = 0.05$ for all statistical tests.

3. Results

3.1. Sustained attention

Fig. 2 shows that both AMPH (at the 0.25 mg/kg dose, Panels A and B, $F(1, 7) = 5.99$, $p < 0.05$) and MPH (at both doses, Panel G and H, 0.5 mg/kg: $F(1, 7) = 15.92$, $p < 0.05$, 1.0 mg/kg: $F(1, 7) = 12.23$, $p < 0.05$) improved stimulus detectability, replicating our earlier effects of the 0.5 mg/kg dose of MPH, and extending those effects to the 1.0 mg/kg dose of MPH and 0.25 mg/kg of AMPH. There were no statistically significant effects of either drug on response bias, *c.* (Panels C and F).

In the analyses of *d'*, a significant effect of signal length was found (all $F_s > 30.00$, all $p_s < 0.05$), but no interactions were statistically reliable. The fact that *d'* varied as a function of signal length lends credence to the assumption that attentional demands vary in this arrangement.

In comparison to the results of Berridge et al. (2006), the effects of 0.5 mg/kg MPH, relative to their respective saline conditions, were nearly identical. For example, 0.5 mg/kg afforded,

on average, a 10.75% improvement in d' from saline during the first experiment (results published in Berridge et al. (2006)) and on average, a 14.51% improvement in the present experiment. There was no statistically significant difference between the percent improvement between Berridge et al. (2006) and the present. Similarly, 0.1 mg/kg AMPH improved performance, on average, 9.61% over saline during the first experiment (not published) and 7.78%, on average over saline, during the present experiment. Again, there was no significance difference between the percent improvement between the 2 experiments.

Fig. 3, Panels A and C, show that Pr(Hit) systematically increased as a function of signal length, as expected. In addition, 0.25 mg/kg AMPH increased the Pr(Hit) ($F(1, 7) = 7.37$, $p < 0.05$, Panel A) independently of signal length (no significant interaction) but did not affect Pr(FA) (Panel B). In contrast, neither dose of MPH statistically improved the Pr(Hit), but both doses of MPH reduced the Pr(FA), as shown in Panel C (0.50 mg/kg: $t(7) = 2.42$, $p < 0.05$; 1.0 mg/kg: $t(7) = 5.56$, $p < 0.05$). The Pr(FA) could not be broken out by signal length, because, by definition, a false alarm is a signal report when no signal was present, and hence, no-signal trials have no length.

Lastly, there was no evidence that latencies to Hit, Miss, FA, or Correct Rejection were affected by AMPH or MPH, when compared to saline (not shown). Latencies to hit were influenced by signal length ($F(5, 35) = 11.702$, $p < 0.05$), with longer signals producing shorter latencies, but latencies to miss were not.

3.2. DRL

The first phase of this experiment, thinning the reinforcement schedule from an FR-1 to an RI-30s, lasted for 22 sessions. All rats then received 32 sessions of the multiple DRL-30s/RI-30s schedule. Fig. 4 demonstrates that by 32 sessions, response rates were stable, and that discriminative control on the multiple schedule was established. Support for that conclusion comes from the fact that response rates covaried with the contingency during the first minute of exposure to each of the components (cf. panel B and C).

The effects of pre-session AMPH, MPH, or saline administrations on mult DRL-30s/RI-30s performance are also shown in Fig. 4, Panels D and E. There were no statistically significant effects of AMPH or MPH on DRL responding when compared to saline.

4. Discussion

The present experiments demonstrate that low doses of MPH (0.5 mg/kg and 1.0 mg/kg) and AMPH (0.25 mg/kg) improve signal detection, but not behavioral inhibition, in rats. Importantly, the doses examined here are considered clinically relevant on the basis of pharmacokinetic, dialysis and other behavioral/cognitive studies (Berridge et al., 2006; Kuczenski and Segal, 2001, 2002). Moreover, the doses tested are devoid of locomotor-activating effects, which is consistent with the lack of effects on latencies to respond in the present experiments. Overall, these observations provide further evidence that low-dose psychostimulants enhance dissociable behavioral processes in healthy animal subjects. Therefore, these results are consistent with a large literature demonstrating that the cognition-enhancing actions of low-dose psychostimulants are not unique to ADHD-affected

individuals (i.e. are not paradoxical). In addition, these observations suggest that, in addition to tests of working memory, tests of signal detection may be useful for identification of novel compounds for use in the treatment of disorders with attentional dysfunction.

4.1. Sustained attention

Low doses of MPH and AMPH improved sustained attention in unimpaired rats, comparable to that observed in normal and ADHD human subjects. Consistent with the present results, 0.1 mg/kg AMPH did not affect signal detection in a similar procedure (Oshiro et al., 2004), whereas slightly higher doses of 0.3, 0.4 and 0.8 mg/kg, impair signal detection (McGaughy and Sarter, 1995; Oshiro et al., 2004). Paolone et al. (2013) recently reported a 17.07% improvement in Sustained Attentions scores (0.41–0.48) after administration of 0.25 mg/kg AMPH, however this increase did not reach statistical significance ($p = 0.12$). Thus, it appears that the facilitative range of AMPH on signal detection is fairly small and possibly sensitive to differing task demands. While a full dose–response function for MPH on signal detection is beginning to emerge (Berridge et al., 2012), the present results clearly demonstrate beneficial effects for doses up to 1.0 mg/kg.

Increases in d' , reflecting improved sustained attention, however, are influenced by both increases in $\text{Pr}(\text{Hit})$ and decreases in $\text{Pr}(\text{FA})$. In the current experiments, MPH reduced the false alarm rate (i.e., $\text{Pr}(\text{FA})$) while AMPH increased the hit rate (i.e., $\text{Pr}(\text{Hit})$). These results are consistent with our previous study (some of these results have not been published): 0.5 mg/kg MPH reduced $\text{Pr}(\text{FA})$ ($t(7) = -3.605$, $p < 0.05$), but did not increase $\text{Pr}(\text{Hit})$, while the effects of 0.1 mg/kg AMPH on $\text{Pr}(\text{Hit})$ were nearly significant ($t(7) = 2.016$, $p = 0.08$), but not so on $\text{Pr}(\text{FA})$. In previous research with prepubertal boys, a significant decrease in the number of “omission” errors was reported in a continuous performance test following low-dose d-amphetamine administration, but no effect on “commission” errors (J.L. Rapoport et al., 1978). The current results show that AMPH preferentially decreased omission errors or “misses” (identical to increasing hits), but left false alarms (commission errors) unaffected. Indeed, the actions of AMPH and MPH on a variety of neurobehavioral measures differ in subtle, yet important ways. For example, while it is well-established that both AMPH and MPH inhibit monoaminergic reuptake in frontal-striatal brain regions, their respective efficacies and mechanisms differ (Heal et al., 2008). For example, AMPH affects DA and NE levels via agonism of trace amine-associated receptor 1 (TAAR1), while MPH blocks DA and NE transport via different mechanisms. These data, in concert with others, point to possible differences in the efficacies of these psychostimulants and suggest further research.

Extensive research on the variables that affect signal detection have been reviewed elsewhere (Gescheider, 1985; Green and Swets, 1966; Macmillan and Creelman, 2005; Swets et al., 1961). Briefly, sensitivity (d') is most affected by changes in the rate of signal presentation (trials) and the signal intensity. On the other hand, the orthogonal measure c (bias) is most affected by the payoff matrix and/or decision criterion used by the subject. The present experiments held the payoff matrix constant and found no change in c , thereby suggesting that the decision criterion was constant across drug treatments. The rate of trial presentation and the signal intensity (duration) were also held constant, yet sensitivity (d') increased.

Therefore, it appears reasonable to conclude that low dose psychostimulants and not other features of the experiment improve sustained attention.

Sustained attention has been assessed in procedures other than signal detection. In the five choice serial reaction time task (5CSRTT), systemic AMPH (0.1–0.4 mg/kg) and MPH (2.5–10.0 mg/kg) improved some measures of performance (Bizarro et al., 2004), as did dopamine agonism in the nucleus accumbens core (Pezze et al., 2007). In a simultaneous discrimination preparation, AMPH improved performance (Grilly, 2000) at a dose identical to that used here (0.25 mg/kg). In contrast, Milar (Milar, 1981), using a single lever discrimination procedure and a signal detection analysis, found no effect of AMPH (0.1 and 0.25 mg/kg) on the performance measure A' (Grier, 1971). However, A' was greater than 0.90 in all rats (1.0 indicates a perfect discrimination), thus it is possible that any facilitative effects of AMPH were mitigated by a ceiling effect. Thus, the present data conform closely to other reports suggesting a facilitative effect of low-dose AMPH on attention-related behavior, tested in a number of different procedures, but also extend those effects to MPH on sustained attention.

The 5CSRTT test, simultaneous discrimination procedures, and signal detection differ in critical ways and thus may help fully triangulate the therapeutic actions of AMPH and MPH or attention-related abilities. The 5CSRTT procedure assesses sustained attention by requiring a rat to poke in one of 5 holes, each equipped with an infrared beam, if a brief light flashed in that hole. The signals and the response devices are in the same physical location (i.e., the signal and the place where the rat responds are in the same spatial position). A typical simultaneous discrimination procedure employs two concurrently available response devices (e.g., levers), with the correct lever signaled by separate signals in a different spatial location (e.g., lights). The signal detection procedure utilizes a conditional discrimination, with one signal but two response devices, in separate locations. The spatial relationship of the signals and response devices is critical here, for 5CSRTT performance may be influenced by Pavlovian contingencies (e.g., autoshaping), whereas the signal detection and simultaneous discrimination are less so. In addition, reinforcer delivery during a simultaneous discrimination procedure could itself serve a discriminative function (i.e., reinforcer delivery signals the availability of reinforcement), while reinforcer delivery during signal detection or 5CSRTT does not (i.e., reinforcer delivery does not signal the correct response device). In addition, 5CSRTT performance is typically presented as changes in accuracy and not changes in correct (hit) and incorrect (miss) choices. Indeed, the 5CSRTT cannot distinguish errors of omission and commission, thereby limiting the potential sensitivity of this model to detect differences in drug effects. In the current experiments, no differences between MPH and AMPH on proportion correct were found, consistent with Bizarro et al (2004) on 5CSRTT accuracy. However, signal detection analysis affords another level of analysis that yielded a potentially important distinction. Therefore, each of the various preparations that assay attention-related behavior likely have overlapping components as well as crucial differences such as the response requirements, reinforcer delivery effects, and influence of Pavlovian contingencies. Depending on the prevalence of symptoms like inattentiveness, restlessness, and distractibility, ADHD may now be distinguished between 3 subtypes: predominantly inattentive (PI), predominantly hyperactive-impulsive (PHI) and combined. The possibility that MPH and AMPH affects

different behavioral processes underlying different subtypes could lead to more effective use of these pharmacotherapies and seems likely to be informed by studies using signal detection.

4.2. DRL

In contrast to that observed with sustained attention, low-doses of AMPH and MPH did not improve DRL performance. DRL procedures are considered good assays of behavioral inhibition because participants must withhold, or inhibit, prepotent responses for a period of time before one produces a reinforcer. The multiple schedule is a novel arrangement to test the effects of psychostimulant drugs because the RI-30 component provides a stringent within-session, within-subject control condition. Regardless, AMPH and MPH failed to change DRL responding when compared to RI responding. Additionally, neither AMPH nor MPH systematically changed the distribution of IRTs (data not shown).

DRL procedures have been used extensively in preclinical screening for potential antidepressants (O'Donnell et al., 2005). While tricyclic antidepressants and selective serotonin reuptake inhibitors have been shown to improve DRL performance, MPH (2.50–20.0 mg/kg) and AMPH (0.31–3.0 mg/kg), in higher dose ranges than the present experiments, impair DRL (Jackson et al., 1995; Seiden et al., 1979). The present results, therefore, extend our knowledge to even lower doses of these psychostimulants. Moreover, even though the DRL schedule may be a useful tool in evaluating potential antidepressants, its utility in assessing the actions of psychostimulants on behavioral inhibition appear extremely constrained.

4.3. Possible neurobiological mechanisms of the facilitative effects of AMPH and MPH

Although the physiological actions of AMPH and MPH were not directly tested in the present studies, it is important to consider the present results in the context of our current understanding of the actions of these psychostimulants. AMPH and MPH are generally thought to increase dopamine (DA) and norepinephrine (NE) efflux throughout the brain. However, when administered in low doses, AMPH and MPH preferentially increases DA and NE efflux in the prefrontal cortex (PFC) of the rat, but not in the ventral striatum (Berridge et al., 2006; Berridge and Stalnaker, 2002). The prefrontal cortex (PFC) plays a prominent role in attention, and self-control, and is involved in the therapeutic actions of low-dose psychostimulants. Dysregulation of the PFC is widely held to underlie many of the symptoms of ADHD (A. F. T. Arnsten, 2001; Robbins and Arnsten, 2009). PFC-dependent behavioral tasks are improved by MPH, through actions on DAergic and noradrenergic processes (A.F. Arnsten and Dudley, 2005) including attention-related processes assayed in the 5CSRTT (Pezze et al., 2009). If we assume that the present experiments also produced a preferential increase of DA and NE in the PFC, it follows that these levels are responsible for the improvement in sustained attention, but are not in behavioral inhibition. Indeed, DRL performance is most profoundly affected by DAergic manipulations in the ventral striatum, an area that shows relatively less DA and NE efflux in response to psychostimulants. While the current experiments did not directly test the hypothesis that increases in DA and NE efflux in the PFC produced by AMPH and MPH are responsible for the improvement in sustained attention, they do provide corroborative evidence of an “emerging and cohesive story” of attention, PFC function, and psychostimulant action (A. F. Arnsten, 2009).

5. Conclusions

The widespread use of AMPH and MPH in the treatment of ADHD raises a variety of questions that would benefit from animal models. For example, substantial data exist showing the beneficial effects of low dose psychostimulants in humans, but limited information is available on the neural mechanisms involved in these therapeutic actions. While the actions of these psychostimulants have been a source of a large body on research in animals, generally they have been tested in dose ranges that are significantly higher than those used therapeutically. The doses of AMPH and MPH used in the present experiments with rats are within the range used therapeutically with humans (Berridge et al., 2006; Grilly and Loveland, 2001) and they demonstrate that low dose AMPH and MPH improve sustained attention, but have little effect on behavioral inhibition. Sustained attention recruits prefrontal circuitry (Wilkins et al., 1987) via catecholaminergic mechanisms (Puumala and Sirvio, 1998) and clinically relevant doses of MPH increase catecholamine efflux preferentially in the medial prefrontal cortex (Berridge et al., 2006). Therefore, the improvement in sustained attention following AMPH or MPH administration likely is a result of increased catecholamine release in the mPFC. Conversely, with the doses used here, behavioral inhibition did not improve following AMPH or MPH administration. DRL appears to recruit ventral striatal circuitry (Cardinal et al., 2001) and low-dose MPH has minimal effects on catecholamine efflux in the ventral striatum (Berridge et al., 2006). Given that inattentiveness and impulsivity are two of the major symptom clusters in ADHD, the present result help us in aligning the animal and human literature and provide a better understanding of the neurobiology of attention and the facilitative effects of psychostimulants.

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Fig. 1.
Schematic of the Multiple DRL-30s/RI-30s schedule. The graphics represent the state of the intelligence panel (front wall) in a chamber over the course of the session.

Fig. 2. The effects of 0.1 and 0.25 mg/kg Amphetamine (AMPH) and 0.5 and 1.0 mg/kg Methylphenidate (MPH) on sustained attention. Panels A and B show that 0.25 mg/kg AMPH and both doses of MPH but not 0.1 mg/kg AMPH, improve d'. Panels C and D shows that c was not affected by AMPH or MPH.

Fig. 3.

The effects of 0.1 and 0.25 mg/kg AMPH and 0.5 and 1.0 mg/kg MPH on components of sustained attention. Panel A shows that 0.25 mg/kg significantly improved Pr(Hit). Panel B shows that neither dose of AMPH affected the Pr(FA). Panel C shows that MPH did not affect the Pr(Hit). However, Panel D shows that both doses of MPH reduced the Pr(FA).

Fig. 4.

Performance on the Multiple DRL RI schedule during training and testing. Panel A shows the mean response rate during a session over the course 32 training sessions, differentiated by component (DRL or RI). The two right panels show the mean response rate per minute during session 1 of the multiple schedule (Panel B) and session 32 of the multiple schedule (Panel C). Panels D and E show the effects of AMPH and MPH on DRL performance relative to RI responding.