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Amphetamine improves mouse and human attention in the 5-Choice Continuous Performance Task

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Abstract

Non-medical use of prescription stimulants amongst college students is common, with claims of cognitive and academic benefits. The mechanism, magnitude, and pervasiveness of the cognitive enhancing effects of stimulants in healthy adults remain poorly understood however. The present study determined the effects of dextroamphetamine (D-amp) on the 5-choice continuous performance test (5C-CPT) of attention in healthy young adult humans and mice. A mixed gender sample received placebo (n=29), 10 (n=17) or 20 mg D-amp (n=25) in a double-blind fashion before 5C-CPT testing. In addition, male C57BL/6J mice were trained on a touchscreen adaptation of the 5C-CPT and tested after receiving saline or D-amp (0.1, 0.3, 1.0 mg/kg; n=8/dose). In humans, D-amp significantly improved 5C-CPT performance. Both doses improved signal detection driven by increased hit rate (reduced omissions). Both doses also improved response accuracy and reduced hit reaction time (HRT) variability. In mice, similar effects (improved signal detection, hit rate, and response accuracy) were observed at the moderate dose (0.3 mg/kg). In contrast to human participants however, no effect on HRT variability was detected in mice, with no effect on HRT in either species. Human 5C-CPT performance was consistent with prior studies and consistent with alternative CPT paradigms. The performance of C57BL/6J mice on the touchscreen 5C-CPT mirrored performance of this strain on 5-hole operant chambers. Importantly, comparable facilitation of attention with D-amp was observed in both species. The 5C-CPT provides a cross-species paradigm by which the cognitive enhancing properties of stimulants and the neural underpinnings of attention can be assessed.

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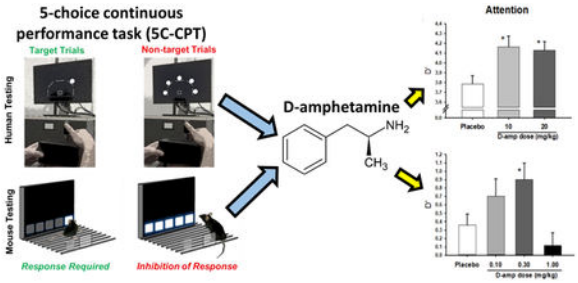
Disclosure of Biomedical Financial Interests and Potential Conflicts of Interest

Dr. Geyer has received consulting compensation from Lundbeck, Omeros, Otsuka, and Sunovion, and holds an equity interest in San Diego Instruments. Dr. Young has received funding from Cerca Insights and Lundbeck Ltd, and has received consulting compensation for Amgen, and honoraria from Arena Pharmaceuticals and Sunovion. Drs. MacQueen, Perry, Minassian and Brigman, and Mr. Kenton, report no biomedical financial interests or potential conflict of interest.

Graphical Abstract



Keywords

Attention; Vigilance; Stimulant; ADHD; Amphetamine; Translation; *Chemical compounds*: dextroamphetamine (PubChem CID: 5826)

1. Introduction

Amphetamine has been used off-label as a “cognitive-enhancer” since its development in the 1920s. The use of amphetamine by students to improve scholastic performance has been a consistent theme over this period (McCabe et al., 2005). Students using amphetamine without a medical recommendation primarily report that use “helps me concentrate” (56.9%), or “helps increase my alertness” (41.3%; Teter et al., 2005). Despite these recurrent reports, questions remain regarding the cognitive domains improved by amphetamine, which populations benefit from amphetamine, and the mechanism of action for its cognitive effects (Farah, 2015; Ilieva and Farah, 2013).

Stimulants such as Dextroamphetamine (D-amp; the more potent enantiomer of racemic amphetamine) and methylphenidate (MPD) remediate symptoms in attention deficit hyperactivity disorder (ADHD) in children/adolescents (Punja et al., 2016; Van der Oord et al., 2008) and adults (Castells et al., 2011; Faraone et al., 2004), with long-term efficacy (Fredriksen et al., 2013; Gillberg et al., 1997; Shaw et al., 2012). These drugs also improve cognition in ADHD patients on a variety of neuropsychological tasks and computerized assessments of cognition. For example, a recent review of MPD effects in children and adolescents with ADHD observed enhanced task performance including domains of executive memory, non-executive memory, reaction time (RT), RT variability, and response inhibition (Coghill et al., 2014). Although there is support for enhancement across domains, effects appear most prominent for tasks which do not have an executive function component (e.g., planning, strategy formulation, or set-shifting; Swanson et al., 2011). Tasks providing RT measures, such as the Stop Signal Task, the Attentional Network Task, and Go/No-go tasks, have been particularly useful for characterizing lapses of attention, which result in grossly delayed responses to task stimuli. Stimulants attenuate ADHD-related deficits on these tasks (Bedard et al., 2003; Konrad et al., 2004; Scheres et al., 2003; Coghill et al., 2007) and also enhance the performance of healthy individuals.

In controlled studies, D-amp (10 and 20 mg, administered to 36 healthy adults) decreased stop signal RT, reduced false alarms on a Go/No-go task, reduced discounting of delayed rewards (de Wit et al., 2002), and reduced the incidence of long RTs (inferred to reflect attention lapses) in a simple RT task (Weafer and de Wit, 2013). More complex attentional tasks such as the continuous performance tests (CPT) have also been used to assess stimulant effects. A benefit of modern CPT paradigms is that they allow for signal detection analysis in a format that also provides sufficient trial data to evaluate RTs by trial outcome (Young et al., 2009). In general, MPD improves CPT performance in healthy adults (Linssen et al., 2014). Although CPT designs have varied across studies, MPD reduced false alarms (Aman et al., 1984) and sped RT (Camp-Bruno and Herting, 1994). Others reported linear dose-dependent decreases in omissions and RT (Cooper et al., 2005; Hermens et al., 2007). Stimulants also improve vigilance, the ability to maintain performance on an attentional task across time. In healthy subjects, MPD prevented increases in omission errors with time on task (Coons et al., 1981; Strauss et al., 1984) and the progressive decline of signal detection (Strauss et al., 1984). Although D-amp has received considerably less attention than MPD we recently reported similar effects for a 20 mg dose of D-amp on the Conners' CPT-II, a modern standardized version of the task (MacQueen et al., 2017). In a placebo-controlled, within-subject design, D-amp improved vigilance by preventing decline in signal detection (d'), as well as increased hit (H)RT variability, with time on task. Understanding the mechanism by which these stimulants improve attention is important both for delineating the neural mechanisms of attention and for the development of therapeutics to address disorders of attention.

The effect of stimulants on attention and vigilance in healthy participants are strikingly similar to those observed in patients with ADHD; although generally less pronounced likely due to ceiling effects (healthy individuals exhibit high levels of performance). These findings indicate that D-amp likely augments networks of attention/vigilance broadly, rather than exclusively correcting ADHD-related neuropathology. Stimulant trials may therefore, be utilized to delineate the neurocircuitry of these cognitive processes. Both D-amp and MPD inhibit dopamine transporters (DAT) and norepinephrine transporters (NET), blocking dopamine and norepinephrine reuptake respectively, thereby increasing transmitter concentrations in the synaptic cleft. Imaging techniques, e.g., fMRI and PET, shed light on the regional and circuit level effects of stimulants in human participants (see Swanson et al., 2011). Model animal studies are required, however, to determine the molecular and cellular levels of action, as well as moderating factors related to genetic disposition. The utility of pharmacological studies of behavior in model species are inherently limited by the relevance of the animal testing paradigm to human behavior. Towards this end, a 5-choice CPT task (5C-CPT) has been developed for rodents which mirrors CPT paradigms developed for human testing (Young et al., 2009). The task elaborates upon the 5-choice serial reaction time task for rodents (5-CSRTT; Robbins, 2002) in which animals are trained to respond to the time-limited presentation of stimuli in 1 of 5 locations. The 5C-CPT retains this format for target trials but – consistent with human CPTs – additionally includes non-target trials to which subjects must inhibit from responding. Further, the task has been reverse-translated for human testing and validated in clinical populations that exhibit task deficits (Young et

al., 2013) comparable to those observed with traditional CPT variants (Bismark et al., In Press; Cornblatt and Keilp, 1994).

Demonstration of cross-species comparability of stimulant-induced improvement in 5C-CPT performance is needed to advance non-human animal studies of stimulant-induced cognitive enhancement. Mechanisms underlying such improvement could then be tested in animals to facilitate the development of more targeted therapeutics. Here, we hypothesized that D-amp would improve 5C-CPT performance in a similar manner for both healthy adult mouse and human participants, demonstrating pharmacological validity for the task.

2. Materials and Methods

2.1 Healthy Volunteer Study

2.1.1 Participants: Participants, aged 18–35 years, were recruited from the San Diego community and provided with a detailed description of the study. All participants were screened for capacity to provide informed consent, which was obtained in writing from willing participants in accordance with University of California San Diego (UCSD) institutional review board-approved procedures. After consent, participants were screened by a trained clinician for psychiatric illness using the Structured Clinical Interview for DSM-IV (SCID-CT; First et al., 2007). A full description of inclusion/exclusion is provided in MacQueen et al. (2017), where we report on D-amp effects across a broader range of tasks in a partially overlapping sample of participants recruited for the D-amp challenge. In brief, participants were excluded for psychiatric illness (including substance use disorders), pregnancy, and health conditions contraindicating D-amp administration. Suitability for D-amp challenge was determined by a study physician who evaluated medical history and electrocardiogram results to rule out any medical contraindications for administration of stimulants.

2.1.2 Procedures: A single-session, double-blind, placebo-controlled design was used for the D-amp challenge wherein 71 participants (47.9% male) completed the 5C-CPT and contributed data to the present report. After providing informed consent and being evaluated for inclusion/exclusion, participants were randomized to receive either placebo (n=29), 10 mg (n=17), or 20 mg D-amp (n=25) before behavioral testing, resulting doses of 0.15 and 0.28 mg/kg respectively. Oral doses were provided by the UCSD investigational pharmacy, which also conducted the randomization. The placebo and 20 mg conditions were oversampled during the latter phases of data collection as related efforts suggested the hypothesized contrasts would emerge from these groups. To mitigate potential effects related to participants' expectancies of the drug, participants were informed that they may receive one of several drugs (caffeine, amphetamine, modafinil) or a placebo. All participants were tested by research staff, who were blind to the participants' experimental condition, and began the 5C-CPT approximately 3.5 hours after ingesting their experimental dose. A between-subjects design was chosen to avoid potential pitfalls of repeated testing including; 1) participant drop-out resulting in selection bias; 2) development of practice effects from repeated behavioral testing; and 3) the development of drug expectancy effects for participants receiving an active dose during their initial testing session.

2.1.3 5-Choice Continuous Performance Test:

The 5C-CPT is a vigilance task that mirrors standardized human CPT procedures and has been adapted for use in both humans and rodents (Young et al., 2013). As in the Conners' CPT, participants were sequentially presented with visual stimuli and asked to provide a response after each target stimulus or withhold from responding to a less frequent non-target stimulus (Figure 1). A challenge to translating CPT designs for non-human testing stems from the tradition of using stimuli that carry inherent symbolic meaning to human participants (e.g., letters and numbers). In the 5C-CPT target/non-target status of each trial was conferred by the spatial position of stimuli. Participants were seated in front of a monitor presenting a black background with 5 straight white lines arranged in an arc. On target trials, a single white circle appeared above one of the 5 lines and participants were instructed to respond by moving a joystick in the direction of the circle. On non-target trials circles appeared simultaneously above all 5 lines and participants were instructed to withhold responding on the joystick. While stimuli only appear for 100 ms, participants were able to respond for up to 1 s after stimuli disappeared. Trials were separated by an intertrial interval lasting 0.5, 1, or 1.5 seconds, which was programmed in a quasi-random manner such that the same ITI never appeared in more than 3 consecutive trials. Each participant completed 12 practice trials (10 target, 2 non-target) to demonstrate understanding of task instructions before being tested on the full task (225 target and 45 non-target trials). Trials in the full task were quasi-randomly presented such that individual stimuli were never repeated on more than 3 consecutive trials.

Target trials were categorized as a **hit** when a correct response was detected. Target trials in which the participant moved the joystick in the wrong direction were labeled as **incorrect** while non-responses were registered as an **omission**. Non-target trials were categorized as a **correct rejection** (CR) when a response was withheld or a **false alarm** (FA) if a response was detected. Using these trial outcome designations signal detection analyses can be used to evaluate discrimination of target/non-target trials (i.e., d' or sensitivity index) and response bias (i.e., responsivity index). Description and calculation of these measures are presented in Table 1. In addition, the 5C-CPT yielded measures of response accuracy, omission rate, Hit RT (HRT), HRT Variability, and premature responses.

2.2 Mouse Studies

2.2.1 Subjects:

Male C57BL/6J mice (n=8 per dose; ~8 weeks at onset of testing; Jackson Laboratory, Bar Harbor, ME) were housed 2 per cage in a temperature- and humidity-controlled vivarium under a reverse 12-hour light/dark cycle (lights off 0800 hours) and were tested during the dark phase. All experimental procedures were performed in accordance with the National Institutes of Health Guide for care and Use of Laboratory Animals and were approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee.

2.2.2 Procedures:

2.2.2.1 Operant Apparatus.: The 5-Choice Continuous Performance Task (5C-CPT) was adapted from previously described methods (Young et al., 2009) for use in a touchscreen apparatus (Marquardt et al., 2017; Marquardt et al., 2014). Briefly, operant behavior was conducted in a chamber measuring 21.6 × 17.8 × 12.7 cm (model # ENV-307W, Med

Associates, St. Albans, VT) housed within a sound- and light-attenuating box (Med Associates, St. Albans, VT). The standard grid floor of the chamber was covered with a solid acrylic plate to facilitate ambulation. A pellet dispenser delivering 14 mg dustless pellets (#F05684, BioServ, Frenchtown, NJ) into a magazine, a house-light, tone generator and an ultra-sensitive lever was located at one end of the chamber. At the opposite end of the chamber there was a touch-sensitive screen (Conclusive Solutions, Sawbridgeworth, U.K.) covered by a black acrylic aperture plate allowing five active touch areas measuring 2.5 × 2.5 cm separated by 0.6 cm and located at a height of 1.6 cm from the floor of the chamber. Stimulus presentation in the response windows and touches were controlled and recorded by the K-Limbic Software Package (Conclusive Solutions, Sawbridgeworth, U.K.).

2.2.1.2 Pretraining.: Mice were first food restricted and maintained at 85% free-feeding body weight. Prior to training, mice were acclimated to a 14 mg pellet food reward by provision of ~10 pellets/mouse in the home cage for 1–3 days. Mice were then habituated to the operant chamber and to eating out of the pellet magazine by being placed in the chamber for 30 min with 10 pellets available in the magazine. Mice that retrieved 10 pellets within 30 min were moved to touch training. A session during training consisted of 60 total trials or one hour, whichever was achieved first. In touch training, a nose poke into the magazine initiated stimulus presentation in one square of the 5 response windows. The stimulus remained on the screen until a response was made to the illuminated square. Touches in the non-illuminated squares had no consequence. Touches in the illuminated square resulted in reward delivery, a 1 sec tone and illumination of the magazine. Following retrieval of the reward, there was a 5 sec inter-trial interval (ITI) before the magazine was re-illuminated indicating that the next trial could be initiated. Mice initiating, touching and retrieving 30 pellets within 30 min were moved to 5-Choice Serial Reaction Time Task (5-CSRTT).

2.2.1.3 5-CSRTT Training.: During testing, initiation led to presentation of a stimulus in 1 of 5 locations for 20 seconds. Mice touching the stimulus during presentation or during a 2 sec limited hold period received reward as above. Responses at a blank window during stimulus presentation was measured as an **incorrect** error, failure to respond to a stimulus was measured as an **omission**, and responses before stimulus presentation were labeled **premature responses**. Each non-correct response produced a 4 sec timeout, signaled by illumination of the house light, to discourage indiscriminate responding. Stimulus duration was decreased to 10 sec, 8 sec, 4 sec, 2 sec, 1.5 sec as criterion was met or six sessions were completed. Criterion was ≥33% correct responses over a 60-trial session and a mean HRT of half the time of the stimulus duration for two consecutive days. HRT was defined as the time between when stimulus was presented to when response was made to illuminated stimulus.

2.2.1.4 5C-CPT Training.: Following 5-CSRTT acquisition, mice were tested on a 60-trial paradigm as described above with the addition of non-target (hold) trials (Figure 1). As in training, target trials were defined as stimulus presentation of 1.5 sec. Non-target trials consisted of illumination of all 5 stimuli windows and withholding of response was measured as a **correct rejection (CR)** and a reward was delivered. Response during a non-target trial was measured as a **false alarm (FA)** and resulted in a 4 sec time-out period. Mice initially started on a 2:1 ratio, which consisted of 2 target trials to 1 non-target trial and a

variable ITI of 3–7 sec, for a minimum of 10 sessions. Mice were then moved to a 5:1 ratio. Mice stayed on the 5:1 non-variable stage until obtaining a sensitivity index (SI) of >0.50 for at least 3 consecutive sessions. Once criterion was obtained for the 5:1 non-variable stage, mice were randomly assigned to different doses of amphetamine (0.10 mg/kg, 0.30 mg/kg, 1.0 mg/kg, vehicle; i.p.). Ten min after injection with drug, mice were tested on 5C-CPT paradigm and returned to their home cage after completion. Target and non-target trials were categorized as described in the human data analysis. Each dependent variable was calculated and analyzed for the mouse data in an identical fashion to those described for the human study. All mice (n=32) completed each stage of pretraining, 5-CSRTT and 5C-CPT training prior to D-amphetamine dosing (Table S1).

2.3 Statistical Analysis

Demographic characteristics of human participants were compared across groups with analysis of variance (ANOVA) for continuous variables (age and years of education) and Chi Squared tests of independence for categorical variables (gender, race, and ethnicity). Outcome measures of the 5C-CPT from the human trial were aggregated into three time blocks. Human trial data was first submitted to a three-way (dose by block by gender) ANOVA; however, the gender variable was dropped for analyses in which gender did not produce significant main effect or interaction. Subsequently, measures were submitted to two-way (dose by block) ANOVAs. Mauchly's test was conducted for within-subject analyses, and a Greenhouse-Geisser correction was applied when significant deviations from sphericity were detected. Significant effects of block were followed with Tukey's post-hoc analysis. Significant dose x block interactions were characterized with one-way repeated measures ANOVAs of block at each of the three doses. Outcome measures from the rodent trial were submitted to a one-way between subjects ANOVA of dose. Given the limited number of trials in the touchscreen 5CCPT used with rodents (60), performance was not assessed by trial block. For analyses of both human and rodent data, significant effects of dose were characterized by post-hoc comparisons of each dose of D-amp with placebo. Data were analyzed using SPSS (v24; Chicago, IL).

3. Results

3.1 Amphetamine effects on human 5C-CPT

Participant demographics are reported by group in Table 2. Significant group differences were not detected for age [$F_{(2,71)}$=0.472, p=0.626], years of education [$F_{(2,71)}$=1.243, p=0.295], gender [$\chi^2_{(2)}$=0.478, p=0.787], race [$\chi^2_{(2)}$=5.956, p=0.428], or ethnicity [$\chi^2_{(2)}$=0.168, p=0.919]. Additionally, significant main or interaction effects involving gender were not detected on any 5C-CPT measure (all ps>0.142). As such, this variable was not included in subsequent analyses.

As depicted in Figure 2A, D-amp significantly improved signal detection as indicated by improved d' [$F_{(2,68)}$=5.427, p=0.007] at both the 10 and 20 mg doses, relative to placebo (*d=0.821 & 0.758; p*<0.05). This effect was driven by increased hit rate [$F_{(2,136)}$=7.628, p=0.001; d=0.938 & 0.903] and concurrent reduced percent omissions [$F_{(2,68)}$=7.350, p=0.001; d=0.897 & 0.807] at both doses (Figure 2D&E). Response accuracy was also

improved [$F_{(2,136)}$=12.037, p<0.001; d=1.115 & 1.076] by both doses of D-amp (Figure 2C). Neither false alarm rate [$F_{(2,68)}$=0.747, p=0.478] nor HRT [$F_{(2,68)}$=2.112, p=0.129] were significantly impacted by D-amp (Figure 2F&G). HRT variability (Figure 2H) was, however, reduced by both doses [$F_{(2,68)}$=12.964, p<0.001; d=1.001 & 1.338]. A main effect of D-amp on responsivity did not reach significance [Figure 2B; $F_{(2,68)}$=3.007, p=0.056] but, a significant D-amp by trial block interaction was detected [$F_{(4,136)}$=7.628, p=0.001]. The interaction was characterized by evaluating the effect of trial block at each dose. Responsivity was not significantly impacted by trial block at placebo [$F_{(2,56)}$=2.420, p=0.098] or the 10mg dose [$F_{(2,32)}$=2.093, p=0.140]. At the 20 mg dose a significant effect of trial block was observed [$F_{(2,68)}$=3.892, p<0.05] in which responsivity was increased in the second trial block relative to the first (d=0.679). Nonetheless, responsivity was negative at all trial blocks for each dose indicating a generally more conservative (non-responding) response strategy. A trend towards reduced premature responses produced by D-amp did not reach significance [Figure 2I; $F_{(2,68)}$=10.444, p=0.051]. No other significant effects of dose, trial block, or a trial block x dose interaction were detected for any measure (all ps > 0.264). Full statistics for the analysis of each variable are presented in Table S2.

3.2 Amphetamine effects on mouse 5C-CPT

Matched-pair random assignment was used to avoid *a priori* differences in pre-training performance on the 5-CSRTT or 5C-CPT prior to D-amp delivery. Analysis of pre-training revealed that there were no significant differences between dose groups on the number of sessions required to learn lever-press initiation and responding to stimuli on the touchscreen for reward [$F_{(3,28)}$=0.410, p=0.747]. Similarly, analysis of training on the 5-CSRTT revealed that all mice required more sessions to reach criteria as the duration of stimulus presentation was sequentially reduced (e.g. 20, 10, 8, 4, 2 & 1.5 sec.) [main effect of session: $F_{(5,140)}$=100.09, p<0.001]. Importantly, there was no significant difference in sessions to acquire each stimulus duration of the 5-CSRTT learning by future dose groups [treatment: $F_{(3,140)}$=1.52, p=0.244] or interaction [$F_{(18,140)}$=1.25, p=0.246]. When non-target trials were introduced for the 5C-CPT, all mice required more sessions to reach criterion as the number of non-target trials was reduced from 2:1 to 5:1 [main effect of session: $F_{(1,28)}$=51.21, p=0.001]. Again, no significant differences were present between future dose groups [main effect of dose: $F_{(3,28)}$=0.21, p=0.887] and there was no interaction [$F_{(3,28)}$=1.07, p=0.376] with training.

As depicted in Figure 3A, 0.3 mg/kg D-amp significantly improved signal detection as indicated by improved d' [$F_{(3,28)}$=3.438, p=0.016] at 0.3 mg/kg relative to saline (d=1.138). This effect was primarily driven by significantly increased hit rate [$F_{(3,28)}$=4.015, p=0.017] at the 0.3 mg/kg dose (d=1.197; Figure 3D). Percent omissions was also reduced at the moderate dose (Figure 3E), although the reduction did not reach statistical significance [$F_{(3,28)}$=2.852, p=0.055]. Response accuracy was improved by D-amp [$F_{(3,28)}$=6.012, p=0.002; Figure 3C) with significant improvements at the 0.3 mg/kg dose of D-amp relative to saline (d=1.676) while 1.0 mg/kg reduced accuracy (d=0.845). D-amp had no significant effects on false alarm rate [F(3,28)=0.844, p=0.481; Figure 3F], HRT [$F_{(3,28)}$=1.577, p=0.218; Figure 3G], HRT variability [$F_{(3,28)}$=0.252, p=0.859; Figure 3H], premature

responses [$F(3,28)=1.911$, $p=0.151$; Figure 3I], or responsivity [$F_{(3,28)}=1.355$, $p=0.278$; Figure 3B]. Full statistics for the analysis of each variable are presented in Table S3.

4. Discussion

The present study demonstrates that D-amp improves healthy human performance on the 5CCPT, consistent with traditional CPT variants such as the Conners' (MacQueen et al., 2017), and consistent with D-amp effects on mouse performance in the 5C-CPT. Overall, performance was improved by D-amp and this effect was largely driven by enhanced hit rate and, reduced omissions. These effects were detected in humans at both the 10 mg and 20 mg dose of D-amp, suggesting that the 5C-CPT may be more sensitive to stimulant effects when compared with the Conners' CPT with which significant effects were only detected at 20 mg (MacQueen et al., 2017). The profile of effects was consistent with prior studies on stimulant effects in healthy adults, which have reported improved signal detection (Strauss et al., 1984), reduced omission errors (Coons et al., 1981; Cooper et al., 2005; Hermens et al., 2007; Strauss et al., 1984) and faster HRT (Camp-Bruno and Herting, 1994; Cooper et al., 2005; Hermens et al., 2007) with MPD. Thus, D-amp and MPD can be considered to produce comparable enhancement of attention/vigilance in healthy human adults.

In a prior study of human performance, Young et al. (2013) demonstrated that patients with schizophrenia exhibit a pronounced vigilance deficit on the 5C-CPT, evidenced by progressive increases in omission rate. This effect was observed in healthy control participants as well, although the effect was rather subtle, with a slight elevation observed in trial block 3. In the present study, we observed no main effects of trial block on performance. Amongst participants receiving placebo, omission rates were higher at trial block 3 (3.01%) relative to block 1 (2.21%) however, this difference did not reach statistical significance. Thus, this 8 min 5C-CPT may be less sensitive to vigilance decrement in healthy participants relative to other CPT paradigms, such as the Conners'. For example, a vigilance deficit *was* observed on the 14 min Conners' CPT-II in healthy participants receiving placebo; increases in HRT variability and reductions in d' with time on task. Given the lack of a vigilance decrement in the present study, we did not observe a protection of vigilance with D-amp as was demonstrated on the Conners' (MacQueen et al., 2017) or with MPD on other CPT paradigms (Coons et al., 1981; Strauss et al., 1984). As a whole, 5C-CPT performance was comparable to that produced by alternative CPT procedures and may be more sensitive to stimulant effects although less sensitive for detecting general vigilance decrements.

The primary advantage of the 5C-CPT is that the procedure has been validated for use in rodents, enabling cross-species testing. Comparable drug-induced changes of performance across species is an important facet of cross-species task validation given that a primary utility of such tasks relates to screening potential therapeutic compounds and determining the neural substrates of human performance (pharmacological predictive validity; Young and Geyer, 2015). Importantly, here we demonstrate pharmacological predictive validity of the 5C-CPT given that D-amp improved mouse 5C-CPT performance similarly to humans. The moderate D-amp dose (0.3 mg/kg) enhanced d'. This effect was unlikely a result of hyperactivity as 1.4 mg/kg did not increase activity of C57BL/6 mice (Minassian et al.,

2016). The 0.3 mg/kg dose also lowered omission rates in mice (18.8%) relative to placebo (28.75%) but, in contrast to the human data, this effect was not significant. The difference in magnitude of D-amp effects on omission rate across species could be, in part, due to the frequency of incorrect responses (responding in the wrong location) observed in mice, which were less frequent with human participants. D-amp improved response accuracy in mice and humans with large effect sizes (1.3–1.4), indicating similar effects despite different baselines. Similarly, both species emitted very few premature responses, with D-amp not significantly affecting either species, despite modest increases observed in mice, but reductions in humans. In contrast with human performance, there was no evidence of reduced HRT variability at the effective dose of D-amp in mice. This contrast may be due to differences in the manner by which responses are provided across species. While humans provide a joystick response rapidly (300 – 500 ms), mice were required to move to the appropriate aperture and press a touchscreen, resulting in longer latencies. As a result, the HRT variability measure in mice is likely less sensitive and may reflect processes not required of the human joystick response. Overall, the main effects of D-amp in mouse and human 5C-CPT was an increase in target detection (hit rate) driving improved overall attention (d').

The performance of C57BL/6J mice on the novel touchscreen variant of 5C-CPT was consistent with 5C-CPT performance assessed in 5-choice chambers. As with the traditional variant, omission rate centered around 30%, relatively few premature responses were observed, and responsivity was negative, suggesting a more conservative response strategy (for comparison see Young et al., 2009), consistent with other laboratories (Porter et al., 2016). Response accuracy was diminished (75.39%) relative to performance of this strain on the traditional 5-choice chamber procedure (>90%). Notably, hit rate, FA rate, and d' values were all consistent with rates achieved with a similar touchscreen task of attention, the rodent CPT (rCPT; Kim et al., 2015), which utilizes more non-target to target stimuli. The present data further demonstrate the feasibility of 5C-CPT testing with a touchscreen and bolsters the case for inclusion of the task in touchscreen test batteries. Alternative measures of attention, such as the 5-choice serial reaction time task (5-CSRTT) are available in humans, rats, and mice. Although D-amp treatment has not been reported in humans, early studies in rats primarily pointed toward it elevating premature responses (see Robbins, 2002). More recently, D-amp improved rat performance but only in prefrontal cortical lesioned rats (Chudasama et al 2005) and poorly performing mice (Caballero-Puntiverio et al, 2017), not in overall performance as seen here in mice and humans. Studies still predominantly record D-amp-induced increases in premature responses in the 5-CSRTT, however, (Paterson et al, 2011, Fitzpatrick et al, 2018; Cole et al, 1987; Loos et al, 2010, and Yan et al, 2011), an effect not observed in humans here, nor in mice (although levels were elevated). Hence, the combined target and non-target trials of the 5CCPT (consistent with human CPT paradigms) may be important for detecting the attention-enhancing effects of D-amp and understanding its mechanism of action.

Having demonstrated comparable D-amp effects across species on the 5C-CPT, the task can be utilized to evaluate the neural substrates underlying the attention enhancing effects of stimulants. In humans, modern imaging techniques such as PET and fMRI enable the evaluation of neural activity in participants completing tasks of attention. Recently, analyses

have been developed that can identify patterns of interconnected regional activity associated with resting function (default mode network; DMN) or task engagement (task mode network; TMN). During tasks of attention, DMN and TMN are negatively correlated suggesting distinct opposing states. Critical differences in the strength of and interaction of DMN and attention-related TMNs have been observed in individuals with ADHD and this contrast is lessened or absent amongst patients taking stimulant medications (for a review see Swanson et al., 2011). Methods for functional network identification in rodents have undergone rapid development (Liska et al., 2015). As such, it may be possible to validate the role of DMN and TMN interactions in attentional performance in animals using the 5C-CPT. In humans, the 5C-CPT is already in use for human fMRI testing, wherein the neural correlates of performance have been consistent with those observed with alternative CPT variants (Eyler et al., 2011; McKenna et al., 2013). EEG analysis of performance can also be assessed in the human 5C-CPT (Young et al., 2017), with efforts to-date to develop a comparable mouse EEG-based task.

Critically, consistency of performance across humans and mice enable research with model organisms for molecular, cellular, and circuit level analyses, which can be used to delineate the neural substrates of attention and potential pathological processes (such as in ADHD or schizophrenia). In mice, 5C-CPT performance was impaired by scopolamine, a non-specific mAChR antagonist (Young et al., 2013), and improved in both mouse and human by D-amp (present data) and by modafinil in humans, a dopamine transporter (DAT) and norepinephrine transporter (NET) inhibitor (Cope et al., 2017). These data corroborate the importance of DAT/NET inhibition in improving attentional functioning. Performance deficits in mice have also been observed as a result of genetic manipulation, e.g., mice with reduced transcription factor Sp4 expression, also implicated in schizophrenia, depression and bipolar disorder, exhibit pronounced 5C-CPT deficits that were reversed by a glycine transporter inhibitor (Young et al., 2015). These data highlight the importance of the Sp4 protein in the development of attentional dysfunction as well as a role for NMDA receptor function in remediating these targeted deficits. Interestingly in relation to ADHD, 5C-CPT performance was also disrupted in mice with reduced dopamine receptor 4 (Drd4) expression (reduced signal detection driven by increased false alarms), but not impaired with regard to measures of exploratory activity (behavioral pattern monitor) or sensorimotor gating (prepulse inhibition; Young et al., 2011). This effect is of particular interest in that DRD4 polymorphisms likely reduce receptor function (Asghari et al., 1995) and/or expression (D'Souza et al., 2004; Schoots and Van Tol, 2003) and have been associated with ADHD (Barr et al., 2000; Kereszturi et al., 2007; McCracken et al., 2000; Sunohara et al., 2000; Swanson et al., 2007). Furthermore, activation of the Drd4 in rats improved 5C-CPT performance (Hayward et al., 2016), highlighting the importance of this receptor as a potential target for therapeutics. Similarly, activation of dopamine D_1 receptors can improve rat 5C-CPT performance both alone and in an animal model of schizophrenia (Barnes et al., 2016; Barnes et al., 2012). Given the consistency of the attentional effects of drugs which act on DAT and the implication of DAT polymorphisms in ADHD pathology (McHugh and Buckley, 2015), DAT mutant mice may also represent a relevant model that can be studied using the 5C-CPT.

Such cross-species measures may also be utilized to dissociate mechanisms underlying the attention enhancing effects of D-amp from the direct rewarding effects of the drug, which play a role in the human abuse liability of the drug. The doses used in the present study result in euphoric properties in human participants as reflected by increased subjective ratings of “drug-liking” and feeling “high” (Brauer & de Wit, 1996; Zawertailo, Busto, Kaplan, & Sellers, 1995). In mice, higher D-amp doses than those used in the present study are required to produce drug-liking behavior in adult – but not adolescent – animals on conditioned-place preference (CPP) paradigms (Adriani & Laviola, 2003; Laviola, Dell’Omo, Chiarotti, & Bignami, 1994). Limited data is available on CPP effects in mice at the lower dose ranges of d-amp used presently. Notably, in the present study attention enhancing effects were observed in mice at the 0.3 mg/kg dose but not at 1.0 mg/kg at which CPP effects have been reported (Laviola et al., 1994). In a similar approach to the current manuscript a CPP paradigm was also developed for adult human participants, whom demonstrated drug preference at a 20 mg oral dose (Childs & de Wit, 2009). Both doses of D-amp used presently (10 and 20 mg) enhanced attention in human participants however. It is therefore conceivable that lower doses of D-amp may improve attention performance without drug-liking effects. In future studies, a combination of 5C-CPT and CPP testing could be utilized to determine if attention-enhancing effects of D-amp emergeat low doses which do not engender drug-liking effects.

5. Conclusions

In summary, we report D-amp improves attentional performance in both humans and mice as measured by the cross-species 5C-CPT. Importantly, this effect was consistent with the standard Conners’ CPT, with larger effect sizes seen in the current study. These findings also highlight the cross-platform versatility of the 5C-CPT as it can be assessed using a joystick (humans), in 5-choice operant chambers (rats and mice), and touchscreens (mice). The limited number of trials in the present task for mice (60 trials/solid food reinforcement) precluded an analysis of performance across trial blocks and as such, the presence of a vigilance decrement could not be evaluated (unlike 250 trials available when using liquid reinforcement). As with the human task, a lengthening of task trials/duration may be necessary to evaluate this aspect of performance. Nonetheless, D-amp improved touchscreen 5C-CPT performance in a manner consistent with effects observed in humans (improved signal detection driven by increased hit rate). The consistency of both attentional performance and stimulant effects across species bolsters the utility of the 5C-CPT for translational investigation of attentional deficits in psychiatric illness and/or cognitive therapeutic development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The 5C-CPT can be conducted in humans and mice
- Mouse touchscreen performance is consistent with 5-hole operant chambers
- D-amphetamine (D-amp) treatment improved 5C-CPT performance across species
- D-amp similarly affected hit rate and d prime without increasing false alarms

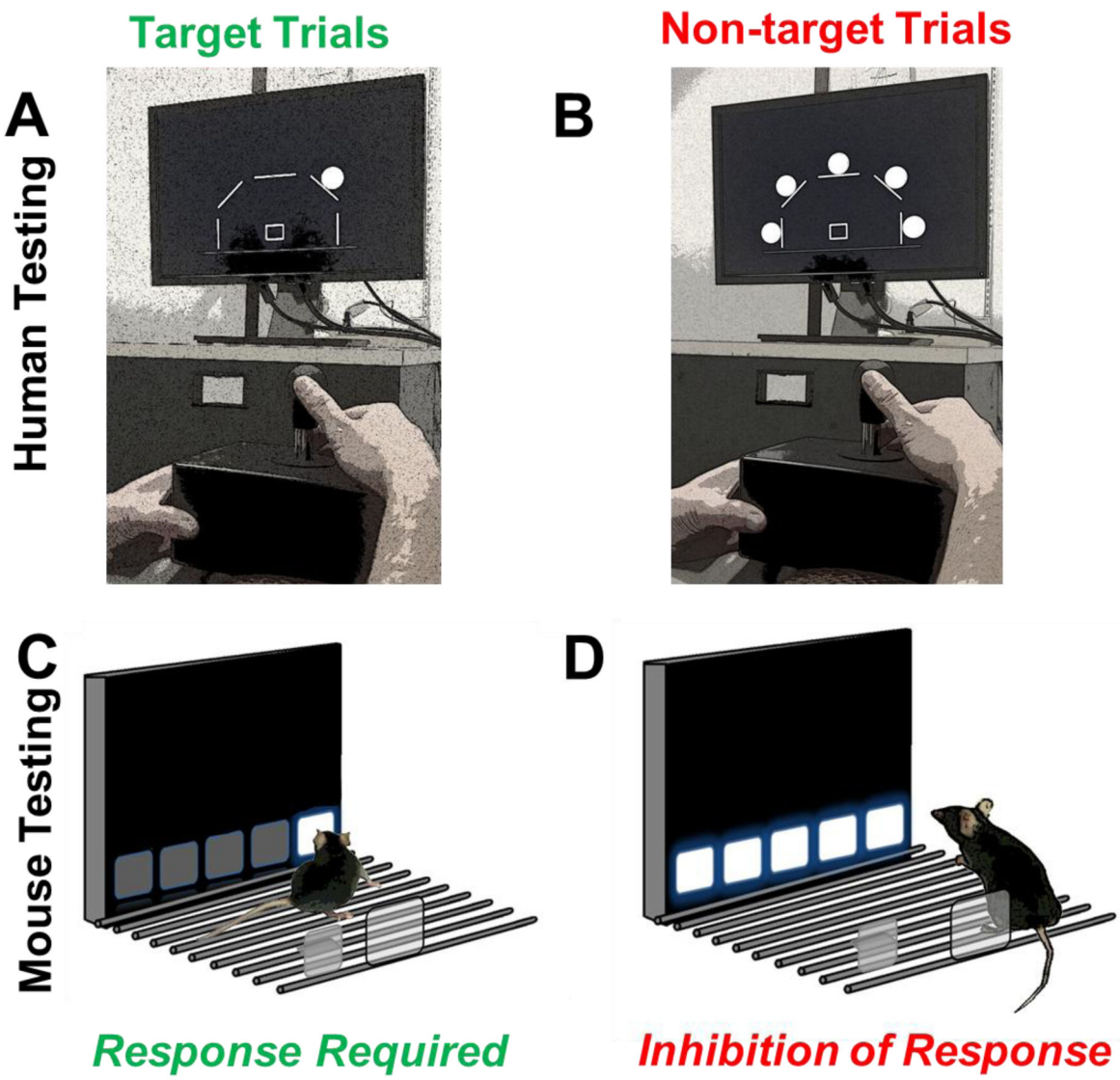

Figure 1: Cartoons of 5C-CPT testing in humans and mice.

During the 5-choice continuous performance test (5C-CPT), humans were required to respond to target stimuli (**A**) by moving the joystick in the direction of the single circle. The appearance of the non-target stimuli (all 5 circles), required the inhibition of responding however, maintaining the location of the joystick (**B**). In the mouse touchscreen 5C-CPT, for target trials mice are required to respond wherever a single target circle appears (**C**). Like the human 5C-CPT however, mice are required to inhibit from responding to non-target stimuli (all 5 lights appear; **D**). The responses/non-responses to each stimuli result in outcome measures related to vigilance and cognitive control (see Table 1).

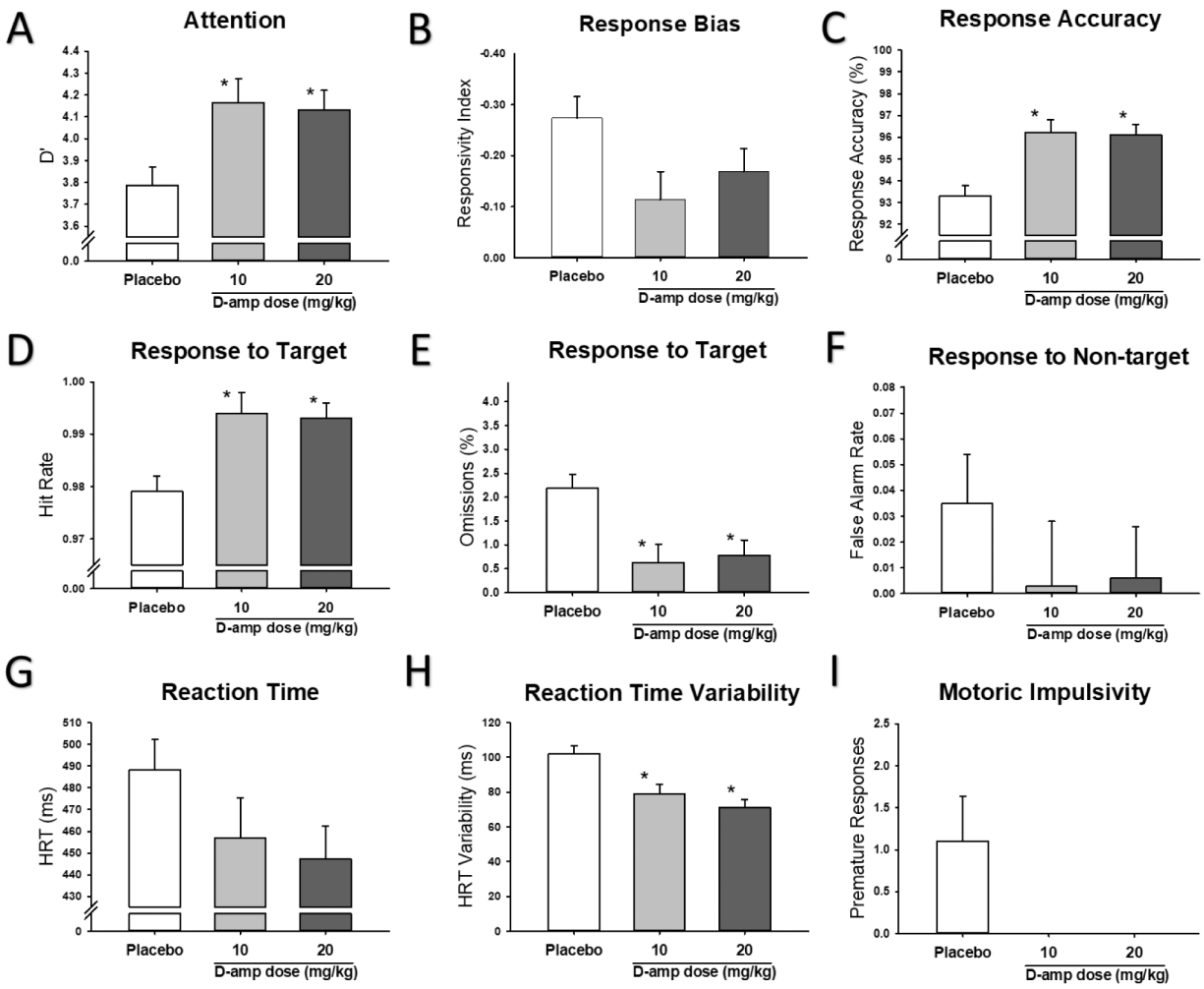

Figure 2: Amphetamine improved human 5C-CPT performance by increasing target responding.

Outcome measures of the 5C-CPT in human participants receiving placebo or one of two doses of amphetamine (D-amp; 10 or 20 mg) are depicted. (**A**) D-amp improved attentional performance measured in d', (**B**) but exerted no effect on response bias measured as responsivity index (**C**). D-amp improved accuracy (moving the joystick in the target direction) across all trials. (**D**) D-amp-induced improvement in vigilance was driven by an increased hit rate, as exemplified by (**E**) reduced % omissions. (**F**) D-amp did not reduce false alarms, as measured by percent of responses to non-target trials, nor affect (**G**) hit reaction-time (HRT) to target trials. (**H**) D-amp did reduce the variability of HRT however. * denotes significant difference ($p<0.05$) relative to placebo. Data presented as mean + S.E.M.

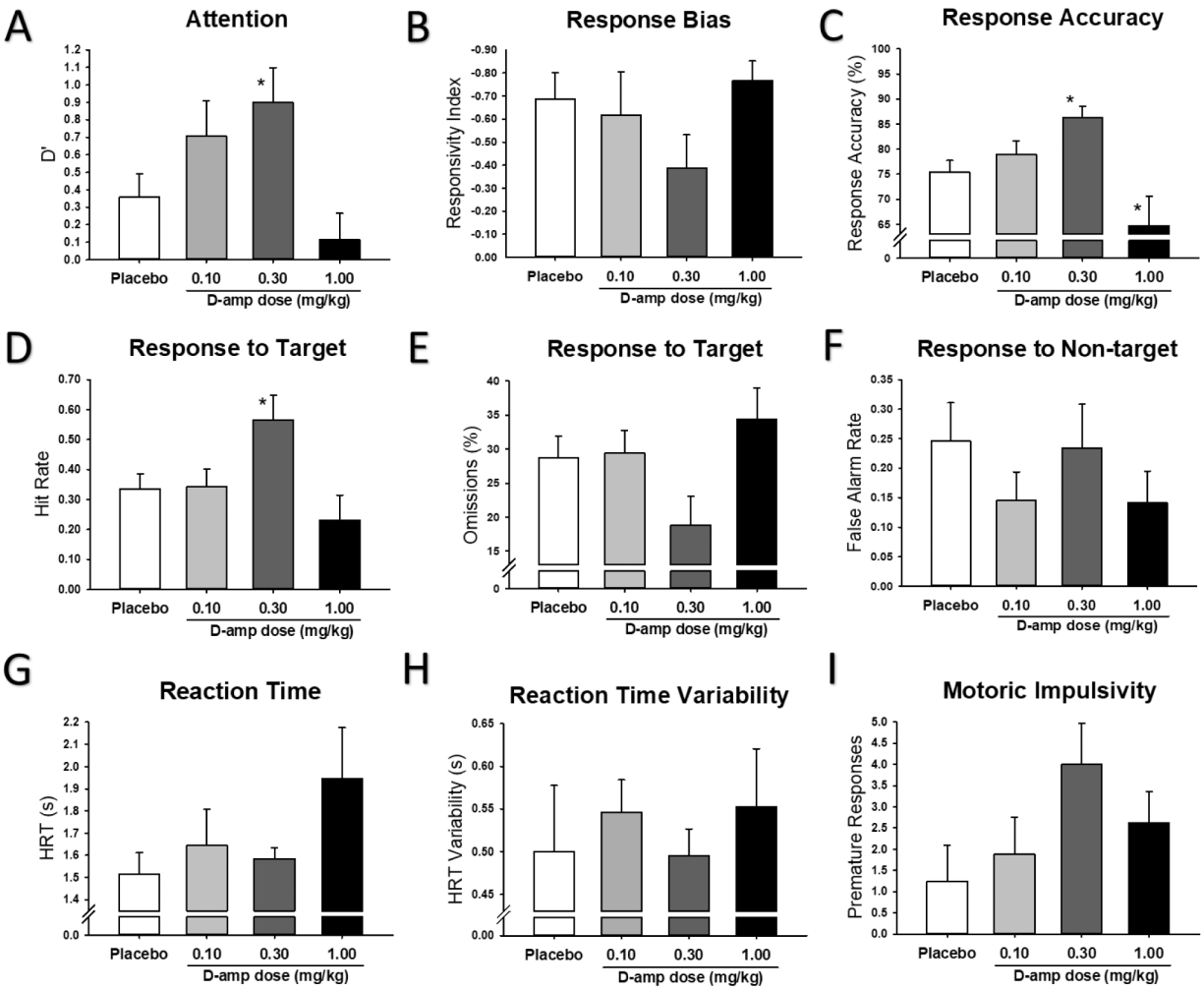

Figure 3: Amphetamine improved mouse 5C-CPT performance by increasing target responding.

Outcome measures of the 5C-CPT in C57BL/6J mice receiving placebo or one of three doses of amphetamine (D-amp; 0.1, 0.3, and 1.0 mg/kg) are depicted. (**A**) D-amp improved attentional performance as measured by d' (**B**) but did not affect response bias measured as responsivity index (**C**). D-amp also improved accuracy at 0.3 but worsened at 1 mg/kg. (**D**) D-amp-induced improvement in vigilance was driven by an increased hit rate, although (**E**) did not significantly reduce % omissions. (**F**) D-amp did not affect false alarms, as measured by percent of responses to non-target trials, (**G**) hit reaction-time (HRT) to target trials, or (**H**) variability of HRT (**I**). D-amp did not significantly increase responses occurring before stimulus presentation (premature responses). * denotes significant difference ($p<0.05$) relative to vehicle (Placebo). Data presented as mean + S.E.M..

Table 1

Measures computed from 5C-CPT performance

Measure	Description	Calculation
Hit rate (HR)	Ratio of correct responses during target trials (vs. omissions)	$\frac{Hits}{(Hits + Omissions)}$
False Alarm Rate (FAR)	Ratio of responding during non-target trials	$\frac{False\ Alarms}{(Non-target\ Trials)}$
Percent Omissions	Ratio of non-responding during target trials	$\frac{Omissions}{(Target\ trials)}$
Response Accuracy	Accuracy of correct responses during target trials (vs. incorrect)	$\frac{Hits}{(Hits + Incorrect)}$
d prime (d')	Parametric measure of trial-type discrimination	$z(HR)$ - $z(FAR)$
Responsivity Index (Rl)	Non-parametric measure of response bias	$\frac{HR - FAR - 1}{1 - (FAR + HR)^2}$

Table 2

Demographic Characteristics

	Placebo	10mg D-amp	20mg D-amp	Statistic	*P*
Gender					
Male	15	7	12	$\chi^2_{(2)}$= 0.478	0.79
Female	14	10	13		
Ethnicity (%)					
Hispanic or Latino	27.6	29.4	24.0	$\chi^2_{(2)}$ = 0.168	0.92
Not Hispanic or Latino	72.4	70.6	76.0		
Race (%)					
Caucasian	62.1	52.9	52.0		
African American	10.3		8.0	$\chi^2_{(2)}$ = 5.956	0.43
Asian	27.6	41.2	40.0		
Multiracial		5.9			
Age	23.7	22.5	23.0	$F_{(2,71)}$ = 0.47	0.63
Years of Education	14.8	15.7	15.2	$F_{(2,71)}$ = 1.24	0.30