Published in final edited form as:

Neuropharmacology. 2013 January ; 64(1): 321–328. doi:10.1016/j.neuropharm.2012.07.005.

A Selective Dopamine Reuptake Inhibitor Improves Prefrontal Cortex-Dependent Cognitive Function: Potential Relevance to Attention Deficit Hyperactivity Disorder

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Abstract

Drugs used to treat attention deficit hyperactivity disorder (ADHD) improve prefrontal cortex (PFC)-dependent cognitive function. The majority of ADHD-related treatments act either as dual norepinephrine (NE) and dopamine (DA) reuptake inhibitors (psychostimulants) or selective NE reuptake inhibitors (SNRIs). Certain benzotropine analogs act as highly selective DA reuptake inhibitors while lacking the reinforcing actions, and thus abuse potential, of psychostimulants. To assess the potential use of these compounds in the treatment of ADHD, we examined the effects of a well-characterized benzotropine analog, AHN 2-005, on performance of rats in a PFC-dependent delayed-alternation task of spatial working memory. Similar to that seen with all drugs currently approved for ADHD, AHN 2-005 dose-dependently improved performance in this task. Clinically-relevant doses of psychostimulants and SNRIs elevate NE and DA preferentially in the PFC. Despite the selectivity of this compound for the DA transporter, additional microdialysis studies demonstrated that a cognition-enhancing dose of AHN 2-005 that lacked locomotor activating effects increased extracellular levels of both DA and NE in the PFC. AHN 2-005 produced a larger increase in extracellular DA in the nucleus accumbens, although the magnitude of this was well below that seen with motor activating doses of psychostimulants. Collectively, these observations suggest that benzotropine analogs may be efficacious in the treatment of ADHD or other disorders associated with PFC dysfunction. These studies provide a strong rationale for future research focused on the neural mechanisms contributing to the cognition-enhancing actions and the potential clinical utility of AHN 2-005 and related compounds.

1. Introduction

Attention-deficit hyperactivity disorder (ADHD) is conservatively estimated to affect 3%-5% of children and adults (Solanto, 2001; Wilens et al., 2004). Psychostimulants are currently the most effective treatment for ADHD (Greenhill, 2001). However, the abuse potential of these drugs raises significant concerns about their widespread use. Thus, there is

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Financial Disclosures: Dr. Berridge has received consulting fees from Phase 2 Discovery. P2D Bioscience is developing AHN2-005 for the treatment of attention deficit/hyperactivity disorder. FPZ is a full time employee of P2D Bioscience. Ms. Schmeichel has no financial disclosures to report.

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a need for new drug treatments for ADHD that display comparable efficacy while lacking the abuse potential of psychostimulants.

Extensive studies demonstrate that ADHD-approved medications improve cognitive processes dependent on the prefrontal cortex (PFC), including working memory, planning, response inhibition and regulation of impulsivity (Chamberlain et al., 2007; Diamond, 2005; Mehta et al., 2001; Turner et al., 2005). These observations are consistent with imaging data demonstrating ADHD is associated with PFC dysfunction (Castellanos and Tannock, 2002). Importantly, the cognition-enhancing actions of these drugs are not limited to ADHD, with similar effects observed in both normal human and animal subjects (Arnsten and Dudley, 2005; Berridge et al., 2006; Devilbiss and Berridge, 2008; Elliott et al., 1997; Gamo et al., 2010; Mehta et al., 2001; Rapoport and Inoff-Germain, 2002). Collectively, these observations suggest that the clinical efficacy of drugs used in the treatment of ADHD involves, at least in part, an ability to broadly improve PFC-dependent function.

Psychostimulants used in the treatment of ADHD (i.e. methylphenidate, amphetamine) act as non-selective catecholamine reuptake inhibitors (Berridge and Devilbiss, 2011). Additionally, selective norepinephrine reuptake inhibitors (SNRIs) are effective in the treatment of ADHD, though these drugs are typically viewed as less efficacious than psychostimulants (Berridge and Devilbiss, 2011). To date, selective DA reuptake inhibitors (SDRIs) have not been utilized in ADHD, largely due to a limited number of compounds that display selectivity for the DAT while lacking the abuse potential of psychostimulants. However, a series of benztropine analogs has been described that display high selectivity and affinity for the DAT while lacking reinforcing effects in rodents and monkeys (Hiranita et al., 2009; Li et al., 2005; Woolverton et al., 2001; Woolverton et al., 2000). The behavioral and pharmacological profiles of these compounds suggest they may be efficacious in the treatment of ADHD while lacking significant abuse potential.

The behavioral and neurochemical actions of the benztropine analog, N-allyl-3 α [[bis(4-fluorophenyl)methoxy]tropane (AHN 2-005), have been well-characterized. Prior work demonstrates that this compound displays high selectivity for the DAT relative to other transporters and receptors and lacks reinforcing effects as measured in conditioned place preference and self-administration paradigms at doses that produce robust increases in extracellular DA concentrations (Hiranita et al., 2009; Katz et al., 1999; Katz et al., 2004; Raje et al., 2005). To assess the potential use of AHN 2-005 in ADHD, we first examined the degree to which this compound improves PFC-dependent function of rats as measured in a delayed-response task of working memory. Importantly, the pharmacology of performance in this task aligns closely with the pharmacology of ADHD: all major classes of drugs used to treat ADHD (psychostimulants, SNRIs, α_2 -agonists) improve performance in this task (Arnsten, 2009; Berridge and Devilbiss, 2011). Thus this task is a useful preclinical screen for ADHD-related compounds. In the current studies, AHN 2-005 dose-dependently improved performance in this task, comparable to that seen with ADHD-related drugs.

Available evidence indicates that clinically-relevant, cognition-enhancing doses of psychostimulants and SNRIs simultaneously and preferentially elevate extracellular NE and DA within the PFC (Berridge et al., 2006; Bymaster et al., 2002). This has been posited to reflect, in part, a prominent role of the NET in the clearance of DA within the PFC (Berridge and Devilbiss, 2011; Carboni et al., 2006; Yamamoto and Novotney, 1998). These and other observations indicate a pivotal role of PFC catecholamines in the cognition-enhancing/therapeutic actions of ADHD-related drugs (Arnsten, 2009; Arnsten and Dudley, 2005; Devilbiss and Berridge, 2008; Spencer et al., 2011). If NE and DA binding at the NE transporter (NET) in the PFC is competitive, elevations in extracellular DA are expected to result in an elevation in extracellular NE levels. To test whether this occurs with AHN

2-005, additional microdialysis studies examined the degree to which a cognition-enhancing dose of AHN 2-005 (10 mg/kg) simultaneously impacts extracellular DA and NE within the PFC, the nucleus accumbens and the medial septal area. Similar to that seen with clinically-relevant doses of psychostimulants and SNRIs, AHN 2-005 elicited moderate increases in extracellular levels of *both* DA and NE within the PFC and medial septal area, and modestly higher increases in extracellular levels of DA in the nucleus accumbens. These neurochemical effects were observed in the absence of locomotor activating effects, consistent with results of earlier studies (Li et al., 2005).

Collectively, these preclinical observations suggest that AHN 2-005 and other benztropine analogs may have utility in the treatment of ADHD and other conditions associated with PFC dysfunction.

2. Methods and Material

2.1. Animals and surgery

Male Sprague-Dawley rats (260-280 g, Charles River, Wilmington, MA) were housed in pairs with *ad lib* access to food and water on an 11:13 hour light:dark cycle (lights on 7:00 AM). For microdialysis studies, probes were surgically implanted under isoflurane anesthesia, as previously described (Berridge et al., 2006). All procedures were in accordance with NIH guidelines and were approved by the University of Wisconsin Institutional Animal Care and Use Committee.

2.2. Spatial delayed alternation/working memory testing

Training and testing were similar to that used previously (Berridge et al., 2006; Devilbiss and Berridge, 2008). Briefly, animals were pair housed and placed on a restricted feeding schedule in which they were allowed to eat 15-25 g of standard chow immediately after each training/testing session. The quantity of food/chow was titrated for each animal to maintain motivation for food rewards (chocolate chips) while avoiding weight loss. The testing apparatus was a T-maze consisting of a runway (91cm), and two arms (66 cm) perpendicular to the runway and placed at the end of the runway farthest away from the experimenter. The runway and arms were 10 cm in height and width. 20 cm from the end of the runway closest to the experimenter was an 18 cm tall removable gate that, when in position, created a start-box from which the animal could not enter the rest of the maze.

For this task, animals were rewarded (chocolate chip) when they entered the arm of the maze not chosen on the previous trial (10 trials per session, 1 session per day). Following each trial, the animal was placed in the start box for a delay period. Inter-trial delays were titrated for each animal to elicit performance accuracy in the range of 60-80%. If an animal exceeded this range, the delay was lengthened on the following testing day and baseline testing resumed. Stable performance was defined as two consecutive days in which performance did not differ by more than 10%. Accuracy of performance increases over time, necessitating periodic increases in delays to maintain performance level in the target range. Given the need for demonstrating stable baseline and the fact delays are periodically adjusted, animals received a treatment on average of once every two weeks. To ensure prominent PFC-dependency, delays were limited to 120-seconds. We have previously observed that with delays up to 80-120-seconds in length, temporary inactivation of the medial PFC of rats reduces performance to chance levels (unpublished observations, Spencer et al., 2011). Thus, even at 120-sec delays this task is highly PFC-dependent. This range of delays is identical that that used in our previous studies documenting cognition-enhancing effects of methylphenidate in this task (Berridge et al., 2006). Given the 120-second cut-off for delays, not every animal received all treatments prior to reaching the 120-second cut-off.

Spatial cues were minimized by black plastic draping that surrounded the maze. All training and testing were conducted by a single individual. The maze was cleaned with 5% ethanol between animals. For a given animal, fecal boli and urine were removed/absorbed by a dry tissue prior to the start of the next trial. Between trials, rats were placed in the start box located at the base of the T and were prevented from exiting by a removable acrylic glass gate. Intraperitoneal treatments were counter-balanced within and across animals and were administered 20-minutes prior to testing.

2.3. Microdialysis Studies

On the day prior to testing, a microdialysis probe was inserted into one or two of the following regions: PFC (A+3.2; L0.8; V-5.2), the nucleus accumbens (A+1.7; L1.4; V-7.85), or the medial septal area (A+0.25; L1.05; V-6.5 at 6° from vertical) as described previously (Berridge et al., 2006; Berridge and Stalnaker, 2002). The last 0.5-1.0 mm of a dialysis probe contained an epoxy plug (corresponding to the ventral-most portion of the probe when implanted). The length of functional membrane was 4 mm for PFC, 3 mm for the medial septal area and 2 mm for the nucleus accumbens. This active membrane began immediately above the epoxy plug. Animals were housed in a Plexiglas testing chamber (32×32×40cm) contained within a ventilated, sound-attenuating outer chamber for 1-2 days (see below). Artificial extracellular fluid (AECF: 147 mM NaCl, 1.3 mM CaCl₂, 0.9 mM MgCl₂, 2.5 mM KCl; pH 7.4) was perfused through the dialysis probe.

DA and NE were measured in dialysate samples using HPLC with electrochemical detection as previously described (Berridge and Stalnaker, 2002). Briefly, AECF was delivered at a rate of 1.5 µl/minute through dialysis membrane (MW cut-off 13,000, o.d. 250 µm; Spectrum Labs, Rancho Dominguez, CA). 30-minute samples were collected prior to and following vehicle or AHN 2-005 treatment. For the PFC and medial septal area, samples were split and analyzed for both DA and NE. 20 µl aliquots were injected onto an HPLC-EC system consisting of an ESA Model 582 pump set at 0.6 ml/min and an ESA 5100A Coulochem II detector with 2 electrodes in series: -.025V, +.220V (ESA Inc. Boston, MA). For DA, samples were injected onto a Velosep C18 100 × 3.2 mm column with a mobile phase consisting of: 200 mM sodium phosphate (pH 3.0-4.5), 0.1 mM EDTA, 0.3 mM sodium octyl-sulfate, and 5% v/v methanol. For NE, samples were injected onto an ion exchange column (ESA, MD-16, #70-7277) and the mobile phase consisted of 150 mM ammonium acetate (pH 6.0), 0.14 mM EDTA, 15% v/v methanol, and 5% acetonitrile. The quantitation limit for NE and DA (using a criterion of 3 times background noise) was approximately 0.3 pg. NE levels display robust elevations during quiet waking relative to sleep (Berridge and Stalnaker, 2002). To avoid potential arousal-state related increases in NE release, baseline samples were collected during periods when the animal was awake a majority of the time (this occasionally required gentle tapping on the chamber and/or leaving the outer chamber door ajar). An average baseline value was calculated from three 30-minute baseline samples displaying no greater than 10% variation from the average value. The mean baseline concentration of NE per sample was 1.50 ± 0.12 pg within the PFC (n = 14) and 1.15 ± 0.15 pg within the medial septal area (n = 8). The mean baseline concentration of DA was 0.83 ± 0.06 pg within the PFC (n = 14), 6.9 ± 0.60 pg within the nucleus accumbens (n = 15), and 1.06 ± 0.19 pg within the MSA (n = 8).

Following collection of at least three baseline samples, animals received an intraperitoneal (IP) injection of vehicle or a dose of AHN 2-005 demonstrated to improve PFC-dependent working memory performance (10 mg/kg; see Figure 1). IP injections were performed without picking up the animal by gently lifting a back leg when the animal faced away from the experimenter (which is typical), minimizing the stress/arousal associated with injection.

2.4. Measures of Locomotor Activity, Feeding, Drinking and Sleep/Arousal

To better compare the behavioral actions of cognition-enhancing doses of AHN 2-005 to clinically-relevant doses of psychostimulants and other ADHD-related drugs (see Berridge et al., 2006; Devilbiss and Berridge, 2008), spontaneous behavior was scored from videotaped records in a subset of microdialysis animals, as described previously (see Berridge and Foote, 1996). For these analyses, behavior was scored in the one 30-minute epoch immediately preceding and two 30-minute epochs immediately following treatment. The following behaviors were scored: 1) the number of quadrant entries (a measure of horizontal locomotion defined by hind legs crossing into a new quadrant of the testing chamber); 2) the number of rears (both free and wall); 3) time spent eating; 4) time spent drinking; 5) time spent asleep (body resting on floor, head resting on floor); 6) time spent in quiet waking (head raised off of floor, body resting on floor); 7) time spent in active waking (all waking behavior other than quiet waking). In earlier studies we demonstrated that these behavioral measures of sleep-wake state align closely with EEG/EMG measures of sleep-wake state (Berridge and Foote, 1996; Berridge et al., 1999; Berridge and Wifler, 2000).

2.5. Drug Treatment

One goal of the current study was to compare the neurochemical and behavioral actions of AHN 2-005 with previously described actions of psychostimulants and selective NE reuptake inhibitors. Given virtually all previous work with these drugs in rats has been conducted during the light phase of the circadian cycle, the current studies tested animals between the hours of 0900 and 1800. Additionally, a majority of work with psychostimulants and selective NE reuptake blockers in animals used IP administration. Moreover, we previously demonstrated that methylphenidate exerts similar cognition-enhancing and neurochemical actions when administered IP and orally, provided dose is adjusted to yield similar peak and clinically-relevant plasma concentrations (Berridge et al., 2006; Devilbiss and Berridge, 2008). Thus, for these studies animals received IP treatment with vehicle (0.9% saline) or AHN 2-005 dissolved in vehicle.

2.6. Statistical Analyses

Given increasing delays are needed to maintain a set performance level and we limited delay length to 120 seconds, it was not possible that every animal receive every dose of AHN 2-005. Thus, the dose-dependent effects of AHN 2-005 on working memory performance were analyzed with a between-subject one-way ANOVA. Post-hoc analyses were conducted by the Dunnett's test, comparing drug-treatment with vehicle-treatment. The neurochemical effects of 10 mg/kg AHN 2-005 were analyzed using a mixed-design two-way ANOVA with treatment as a between-subjects (2 levels) and time as a within-subjects factor (11 levels). In the case of medial septal area, effects of 10 mg/kg AHN 2-005 were analyzed using a one-way ANOVA with time as a within-subjects factor (11 levels). For the neurochemical data, matched-pair t-tests were used to determine whether, within a given treatment group, post-treatment measures differed significantly from the baseline epoch that immediately preceded AHN 2-005 administration. Effects of 10 mg/kg AHN 2-005 on spontaneous behavior (locomotor, eating, drinking and sleep-wake) during the first two 30-minute post-treatment epochs were analyzed using a mixed-design two-way ANOVA with treatment as a between-subjects and time as a within-subjects factor. When statistical significance ($p < 0.05$) was indicated, *post-hoc* analyses were conducted using independent t-tests.

2.7. Histological analyses and data selection

Placement of microdialysis probes was verified in 40- μ m thick coronal sections stained with Neutral Red dye. Neurochemical data were included only when histological analyses

verified accurate placement of microdialysis probes and NE or DA concentrations were stable (<10% variability) throughout baseline.

3. Results

3.1. Effects of AHN 2-005 on working memory performance

To assess the effects of AHN-2005 on working memory performance, animals were treated with vehicle (n=8), 1.0 mg/kg AHN 2-005 (n=7) or 10.0 mg/kg AHN 2-005 (n=8) 20-minutes prior to testing in the T-maze. This dose range was based on: 1) published observations indicating behavioral and neurochemical actions of AHN 2-005 (Hiranita et al., 2009; Li et al., 2005; Raje et al., 2005); 2) limited pilot studies; 3) limited observations indicating that at 30 mg/kg locomotor-activating effects may begin to emerge (unpublished observations, Frank Zemlan, Ph.D.). The delay interval was adjusted to produce moderate baseline performance levels. For this study, mean baseline accuracy was: vehicle, $76\% \pm 2\%$ (SEM); 1.0 mg/kg AHN 2-005, $78\% \pm 1\%$; 10 mg/kg AHN 2-005, $76\% \pm 2\%$. Delays ranged between 10-120-seconds with an average delay of 82 ± 9 seconds. As shown in Figure 1, AHN 2-005 dose-dependently improved performance from baseline, with a significant improvement at 10 mg/kg ($F_{2,20} = 5.81$, $P = 0.01$). The magnitude of this improvement is similar to that seen previously in our laboratory with clinically-relevant doses of methylphenidate tested under identical conditions (Berridge et al., 2006; Devilbiss and Berridge, 2008).

3.2. Effects of a cognition-enhancing dose of AHN 2-005 on NE and/or DA efflux in the PFC, nucleus accumbens, and medial septal area

To better understand the neurochemical actions that may contribute to the cognition-enhancing effects of AHN 2-005, additional studies examined the effects of a cognition-enhancing dose of AHN 2-005 (10 mg/kg) on NE and DA efflux in the PFC, nucleus accumbens (DA only), and the medial septal area, a subcortical region that, like the PFC, receives only a moderate innervation of both DA and NE (see Figures 2-4). Additionally, in a subset of microdialysis animals we examined the locomotor, wake-promoting, feeding and drinking effects of this dose of AHN 2-005.

3.2.1. PFC DA and NE—Within the PFC, 10 mg/kg AHN 2-005 modestly elevated extracellular DA levels with increases of 70%-85% above baseline levels between the 2nd and 5th 30-minute post-treatment sample (Figures 2-4; vehicle n=7; AHN 2-005 n = 8; treatment $F_{1,13} = 22.6$, $P < 0.001$, time $F_{10,130} = 8.5$, $P < 0.001$, treatment $\times$ time $F_{10,130} = 5.4$, $P < 0.001$). AHN 2-005 also significantly elevated extracellular NE levels in the PFC by 75%-90% in post-treatment samples 2-5, comparable to that seen with PFC DA (Figures 2-4; vehicle n = 6; AHN 2-005 n = 8; treatment $F_{1,12} = 12.5$, $P < 0.001$, time $F_{10,120} = 3.8$, $P < 0.001$, treatment $\times$ time $F_{10,120} = 4.5$, $P < 0.001$). The sustained neurochemical action of AHN 2-005 is consistent with the relatively long half-life of this compound (Raje et al., 2005).

The magnitude of the AHN 2-005-induced increase in PFC DA is comparable to that seen with clinically-relevant doses of methylphenidate and the SNRI, atomoxetine (Berridge et al., 2006; Bymaster et al., 2002). The magnitude of the AHN 2-005-induced increase in PFC NE is comparable to that seen with atomoxetine and somewhat less than that seen with methylphenidate (Berridge et al., 2006; Bymaster et al., 2002).

3.2.2. Nucleus Accumbens DA—Compared to the PFC, AHN 2-005 produced a more robust increase in extracellular levels of DA in the nucleus accumbens, consistent with the high density of DA fibers and DAT in this region. Specifically, in all samples collected after

the first 30-minutes post-treatment, we observed an increase in accumbens DA ranged between approximately 170%-200% above baseline levels following AHN 2-005 (Figures 2-4; vehicle $n=6$; AHN 2-005 $n=8$; treatment $F_{1,12}=140.2$, $P<0.001$, time $F_{10,120}=11.7$, $P<0.001$, treatment $\times$ time $F_{10,120}=11.0$, $P<0.001$). There were no obvious differences between baseline DA levels or drug-induced alterations in DA levels when probes were estimated to have been located within the core subregion vs. the shell subregion of the nucleus accumbens. Thus, averaging across post-treatment samples 2-5, AHN 2-005 increased DA levels in the shell accumbens by $175\% \pm 41\%$ and increased core accumbens DA levels by $197\% \pm 68\%$.

3.2.3. Medial Septal Area DA and NE—To assess the degree to which AHN 2-005 alters NE and DA efflux in a subcortical region that, like the PFC, receives a moderate innervation by both NE and DA fibers, additional studies examined the effects of AHN 2-005 on DA ($n=8$) and NE ($n=8$) efflux within the medial septal area (Figures 3, 4). Vehicle treatment had minimal effects on extracellular DA and NE in the PFC and accumbens in the current studies as well as our previously published studies measuring NE within the medial septal area (Berridge, 2006). Thus, we did not include vehicle-treated controls in the studies examining the effects of AHN 2-005 on medial septal NE and DA. As in the PFC, AHN 2-005 significantly increased extracellular levels of both DA and NE in the medial septal area (DA: time $F_{10,70} = 6.4$, $P<0.001$; NE: time $F_{10,70} = 11.1$, $P<0.001$). The magnitude of AHN 2-005-induced increases in NE and DA above baseline in this region was comparable to that seen in the PFC (post treatment samples 2-5, DA = 55%-82%; NE = 50%-100%).

3.3. Effects of AHN 2-005 on locomotor activity, eating/drinking and sleep-wake

To better compare the behavioral effects of a cognition-enhancing dose AHN 2-005 to the well-characterized behavioral effects of psychostimulants, in a subset of randomly selected microdialysis animals, a broad array of behavioral effects of AHN 2-005 ($n=7$) and vehicle treatment ($n=7$) were examined during the first two 30-minute post-treatment epochs. AHN 2-005 lacked pronounced locomotor-activating actions (Table 1; Quadrant Entries, treatment $F_{1,12}=1.6$, $P=0.24$, time $F_{1,12}=5.2$, $P=0.04$, treatment $\times$ time $F_{1,12}=2.8$, $P=0.12$; Rears, treatment $F_{1,12}=2.0$, $P=0.18$, time $F_{1,12}=2.8$, $P=0.12$, treatment $\times$ time $F_{1,12}=0.3$, $P=0.63$). The minimal level of locomotor activity observed following AHN 2-005 and vehicle treatment is comparable to that seen in spontaneous waking (Berridge and Foote, 1996; Berridge and O'Neill, 2001) and well below that seen with moderate doses of psychostimulants (Kuczenski et al., 1997). Similarly, AHN 2-005 lacked significant effects on time spent eating and drinking (Eating, treatment $F_{1,12}=0.6$, $P=0.47$, time $F_{1,12}=0.01$, $P=0.93$, treatment $\times$ time $F_{1,12}=0.3$, $P=0.87$; Drinking, treatment $F_{1,12}=0.2$, $P=0.69$, time $F_{1,12}=0.2$, $P=0.69$, treatment $\times$ time $F_{1,12}=1.8$, $P=0.20$). Finally, AHN 2-005 lacked robust wake-promoting effects, particularly after the first post-injection 30-minute epoch (Sleep, treatment $F_{1,12}=1.0$, $P=0.34$, time $F_{1,12}=5.4$, $P=0.04$, treatment $\times$ time $F_{1,12}=1.2$, $P=0.30$; Quiet Wake, treatment $F_{1,12}=9.5$, $P=0.01$, time $F_{1,12}=8.08$, $P=0.015$, treatment $\times$ time $F_{1,12}=3.8$, $P=0.07$; Active Wake, treatment $F_{1,12}=0.03$, $P=0.86$, time $F_{1,12}=3.3$, $P=0.10$, treatment $\times$ time $F_{1,12}=0.5$, $P=0.51$).

4. Discussion

Drugs used to treat ADHD have been demonstrated to improve an array of PFC-dependent processes (Chamberlain et al., 2007; Diamond, 2005; Mehta et al., 2001; Turner et al., 2005). The current studies demonstrate that the selective DA reuptake inhibitor, AHN 2-005, improves PFC-dependent cognitive function as measured in this working memory task while lacking locomotor-activating or arousal-promoting actions. This behavioral profile is similar to all drugs currently approved for use in ADHD, including psychostimulants, SNRIs and

α_2 -agonists (see Arnsten, 2009; Berridge and Devilbiss, 2011). Moreover, at a cognition-enhancing dose AHN 2-005 modestly elevated extracellular levels of DA and NE within the PFC (e.g. 75%-100%) similar to that seen with cognition-enhancing doses of psychostimulants and SNRIs (Berridge et al., 2006; Bymaster et al., 2002). These latter observations are consistent with recent studies demonstrating that methylphenidate acts directly within the medial PFC to improve working memory performance (Spencer et al., 2011). Collectively, these observations suggest a potential use of this or related benztropine analogs in the treatment of ADHD and other conditions associated with prefrontal dysfunction.

4.1. What PFC-Dependent Processes are Targeted by AHN 2-005?

These preclinical studies were designed to initially assess the potential use of benztropine analogs in the treatment of ADHD. Pharmacological and lesion studies demonstrate that the delayed-response test of spatial working memory used in these studies is highly dependent on the PFC (Kesner et al., 1989; Spencer et al., 2011). Additionally, the pharmacological sensitivity of performance in this task closely aligns with the pharmacology of ADHD (Arnsten, 2009; Berridge et al., 2012; Gamo et al., 2010). This is in contrast with other animal-based tests of PFC-dependent function, including sustained attention and attentional set shifting, which display a pharmacological sensitivity distinct from both delayed alternation testing and ADHD. Specifically, working memory performance is improved by stimulation of PFC α_2 -receptors and impaired by α_1 -receptor activation, while attention set shifting and sustained attention are improved by α_1 -receptors and, at least where examined, are insensitive to α_2 -receptor activation (Berridge et al., 2012; Lapid and Morilak, 2006). Moreover, although working memory performance is maximally improved by doses of methylphenidate that produce clinically-relevant plasma concentrations, attention set shifting and sustained attention are maximally improved by doses that yield plasma concentrations above the range typically associated with clinical efficacy (Berridge et al., 2012; Berridge et al., 2006). The close alignment between performance in this delayed-alternation task of spatial working memory and ADHD may reflect the fact that performance in this task is simultaneously dependent on a variety of cognitive and behavioral processes affected in ADHD, including attention, planning, resistance to distractors, and working memory. The multiplicity of behavioral and cognitive processes involved in performance of this task currently precludes definitive identification of the precise subset of cognitive processes affected by AHN 2-005.

Nonetheless, in both human and animal subjects, psychostimulants and other drugs used to treat ADHD have been demonstrated to facilitate a variety of PFC-dependent processes, including planning, sustained attention, working memory, response inhibition, and the regulation of impulsivity (Berridge et al., 2012; Berridge et al., 2006; Mehta et al., 2004; Mehta et al., 2001; Robbins and Arnsten, 2009). These observations are consistent with imaging data demonstrating ADHD-associated hypofrontality is reversed by clinically-relevant doses of psychostimulants (Bush et al., 2008; Vaidya et al., 1998). Based on these and other observations, it has been proposed that the treatment of ADHD involves, at least in part, drug-induced general improvement in PFC-dependent function which is manifested across an array of PFC-dependent tasks (Arnsten and Pliszka, 2011). From this perspective, the ability of AHN 2-005 to improve performance in this well-validated test of PFC-dependent function suggests it would likely facilitate performance on other tests of PFC-dependent function.

4.2. Potential neurocircuitry and receptor mechanisms underlying the cognition-enhancing actions of AHN 2-005

Currently, the receptor mechanisms involved in the cognition-enhancing effects of AHN 2-005 are unknown. However, postsynaptic DA D1 and NE α_2 receptors located within the PFC have been documented to facilitate PFC-dependent function as measured in delayed-response tasks of working memory (Arnsten, 2007). Evidence further suggests a prominent role of these receptors in the cognition-enhancing actions of drugs used to treat ADHD. This includes the fact that clinically-relevant doses of psychostimulants and SNRIs elevate extracellular NE and DA preferentially within the PFC (Berridge et al., 2006; Bymaster et al., 2002), while systemic α_2 and D1 receptor antagonists block the cognition-enhancing actions of these drugs (Arnsten and Dudley, 2005; Gamo et al., 2010). Moreover, recent studies demonstrate that psychostimulant infusion directly into the PFC of rats improves working memory performance (Spencer et al., 2011) similar to that seen with intra-PFC infusion of clinically efficacious α_2 -agonists (Arnsten, 2009). These observations suggest the hypothesis that the cognition-enhancing actions of AHN 2-005 involve PFC D1 and/or α_2 -receptors.

Of course this hypothesis does not imply that the cognitive effects of AHN 2-005 (or drugs used to treat ADHD) are *solely* dependent on actions within the PFC. For example, substantial evidence implicates frontostriatal circuitry in ADHD (Castellanos and Tannock, 2002). AHN 2-005 and ADHD-related treatments may alter frontostriatal signaling through actions within the PFC. However, given both AHN2-005 and psychostimulants increase DA signaling within the striatum (Berridge et al., 2006), the cognition-enhancing effects of these compounds may also involve drug action within the striatum. Such a mechanism would differ from the SNRIs, which have minimal effects on extracellular DA within the striatum/core nucleus accumbens (Bymaster et al., 2002). Given psychostimulants are viewed as more effective in the treatment of ADHD than SNRIs (Greenhill, 2001), elevations in striatal DA signaling may be necessary to achieve maximal efficacy. Nonetheless, in recent studies we observed that, in contrast to that seen with intra-PFC infusions, methylphenidate infusion into the dorsomedial striatum had no effect on working memory performance even though performance in this task is highly dependent on this region (Spencer et al., 2011). This latter observation suggests the AHN 2-005 likely does not act within the dorsomedial striatum to facilitate frontostriatal function as measured by working memory performance.

Finally, AHN 2-005 also produced moderate elevations in extracellular NE and DA outside the PFC and striatum (i.e. within the medial septal area) similar to that seen with cognition-enhancing doses of psychostimulants (see Results 3.2.3; Berridge et al., 2006). Thus, actions on NE/DA signaling outside frontostriatal circuitry may contribute to the cognition-enhancing actions of AHN 2-005 and ADHD-related drugs. Indeed, the medial septum is implicated in delayed-response tasks of spatial working memory (Pang et al., 2011; Smith and Pang, 2005). Future studies will need to identify the neurocircuitry underlying the cognition-enhancing effects of AHN 2-005 and ADHD-related drugs.

4.3. Non-psychostimulant-like behavioral actions of AHN 2-005

AHN 2-005 lacked prominent wake-promoting, locomotor-activating and feeding/drinking effects. This behavioral profile is similar to clinically-relevant doses of psychostimulants and other drugs used in the treatment of ADHD (Berridge et al., 2006). In part, this appears to reflect relatively mild increases in extracellular NE/DA elicited by AHN 2-005, including in regions associated with psychostimulant-induced arousal (e.g. medial septal area; Berridge et al., 1999). Previous studies demonstrated AHN 2-005 and related benzotropine analogs lack reinforcing effects Hiranita et al., 2009; Li et al., 2005; Woolverton et al., 2001; Woolverton et al., 2000). This is unexpected, given the effects of this compound on

extracellular DA in the nucleus accumbens observed here and elsewhere (see above and Raje et al., 2005). However, it should be noted that although AHN 2-005 increased accumbens DA in the current studies, the magnitude of this (200%) was substantially less than that typically associated with motor-activating doses of commonly abused psychostimulants, including amphetamine, methamphetamine and cocaine (600%–<1000%; Florin et al., 1994,1995; Kuczenski et al., 1995). The reasons for the reduced action of AHN 2-005 on locomotion, reinforcement and extracellular DA are not fully understood. However, available evidence suggests that benztropine analogs physically interact with the DAT differently than cocaine-like DAT inhibitors, leading to a relatively slow DAT association rate (Desai et al., 2005; Loland et al., 2008). This is posited to result in behavioral and neurochemical profiles that are distinct from the cocaine and other psychostimulants. Despite our incomplete understanding of the pharmacology of AHN 2-005, the available evidence indicates that its ability to selectively target the DAT is not associated with reinforcing actions and thus is not a contraindicator for clinical use.

4.4. Potential mechanisms involved in the simultaneous elevation of DA and NE levels within the PFC

Earlier work demonstrates that blockade of the NET leads to an increase in extracellular DA in the PFC and other regions that display moderate NE and DA innervations and moderate DAT density (Carboni et al., 2006; Carboni et al., 1990; Sesack et al., 1998; Yamamoto and Novotney, 1998). Our neurochemical observations further demonstrate a close relationship between extracellular DA and NE levels, with a highly selective DAT inhibitor (AHN 2-005) elevating both DA and NE in the PFC and medial septal area (see Results 3.2). Given extracellular concentrations of DA and NE are similar in these regions (see Methods 2.3 and Results 3.2), the ability of AHN 2-005 to increase extracellular NE levels may involve drug-induced increases in competition between DA and NE at the NET. Alternatively or additionally, AHN 2-005-induced increases in DA signaling may directly or indirectly activate noradrenergic neurons that project to the PFC (Foote et al., 1983) and medial septal area (Espana and Berridge, 2006).

4.5. Summary

These studies demonstrate that the selective dopamine reuptake inhibitor, AHN 2-005, improves PFC-dependent cognitive function while simultaneously elevating extracellular NE and DA in the PFC. These actions are similar to those observed with low-dose psychostimulants and SNRIs used in the treatment of ADHD, suggesting that AHN 2-005 or other benztropine analogs may be useful in treating this disorder.

Acknowledgments

This work was supported by PHS grants, MH081843, DA000389, and MH08138, the Wisconsin Institutes of Discovery and the University of Wisconsin Graduate School.

References

- Arnsten AF. Catecholamine and second messenger influences on prefrontal cortical networks of “representational knowledge”: a rational bridge between genetics and the symptoms of mental illness. *Cereb Cortex*. 2007; 17(1):i6–15. [PubMed: 17434919]
- Arnsten AF. The Emerging Neurobiology of Attention Deficit Hyperactivity Disorder: The Key Role of the Prefrontal Association Cortex. *J Pediatr*. 2009; 154:1, S43. [PubMed: 20596295]
- Arnsten AF, Dudley AG. Methylphenidate improves prefrontal cortical cognitive function through alpha2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. *Behav Brain Funct*. 2005; 1:2. [PubMed: 15916700]

- Arnsten AF, Pliszka SR. Catecholamine influences on prefrontal cortical function: relevance to treatment of attention deficit/hyperactivity disorder and related disorders. *Pharmacology, biochemistry, and behavior*. 2011; 99:211–216.
- Berridge C, Shumsky JS, Andrzejewski ME, McGaughy J, Spencer RC, Devilbiss D, Waterhouse B. Differential sensitivity to psychostimulants across prefrontal cognitive tasks: differential involvement of Noradrenergic $\alpha 1$ - vs. $\alpha 2$ -Receptors. *Biol Psychiatry*. 2012; 71:467–473. [PubMed: 21890109]
- Berridge CW. Neural substrates of psychostimulant-induced arousal. *Neuropsychopharmacology*. 2006; 31:2332–2340. [PubMed: 16855535]
- Berridge CW, Devilbiss DM. Psychostimulants as Cognitive Enhancers: The Prefrontal Cortex, Catecholamines, and Attention-Deficit/Hyperactivity Disorder. *Biol Psychiatry*. 2011; 69:e101–e111. [PubMed: 20875636]
- Berridge CW, Devilbiss DM, Andrzejewski ME, Arnsten AF, Kelley AE, Schmeichel B, Hamilton C, Spencer RC. Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biol Psychiatry*. 2006; 60:1111–1120. [PubMed: 16806100]
- Berridge CW, Foote SL. Enhancement of behavioral and electroencephalographic indices of waking following stimulation of noradrenergic beta-receptors within the medial septal region of the basal forebrain. *J Neurosci*. 1996; 16:6999–7009. [PubMed: 8824336]
- Berridge CW, O'Neil J, Wifler K. Amphetamine acts within the medial basal forebrain to initiate and maintain alert waking. *Neuroscience*. 1999; 93:885–896. [PubMed: 10473254]
- Berridge CW, O'Neill J. Differential sensitivity to the wake-promoting actions of norepinephrine within the medial preoptic area and the substantia innominata. *Behav Neurosci*. 2001; 115:165–174. [PubMed: 11256440]
- Berridge CW, Stalnaker TA. Relationship between low-dose amphetamine-induced arousal and extracellular norepinephrine and dopamine levels within prefrontal cortex. *Synapse*. 2002; 46:140–149. [PubMed: 12325041]
- Berridge CW, Wifler K. Contrasting effects of noradrenergic beta-receptor blockade within the medial septal area on forebrain electroencephalographic and behavioral activity state in anesthetized and unanesthetized rat. *Neuroscience*. 2000; 97:543–552. [PubMed: 10828536]
- Bush G, Spencer TJ, Holmes J, Shin LM, Valera EM, Seidman LJ, Makris N, Surman C, Aleardi M, Mick E, Biederman J. Functional magnetic resonance imaging of methylphenidate and placebo in attention-deficit/hyperactivity disorder during the multi-source interference task. *Arch Gen Psychiatry*. 2008; 65:102–114. [PubMed: 18180434]
- Bymaster FP, Katner JS, Nelson DL, Hemrick-Luecke SK, Threlkeld PG, Heiligenstein JH, Morin SM, Gehlert DR, Perry KW. Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: A potential mechanism for efficacy in Attention Deficit/Hyperactivity Disorder. *Neuropsychopharmacology*. 2002; 27:699–711. [PubMed: 12431845]
- Carboni E, Silvagni A, Vacca C, Di CG. Cumulative effect of norepinephrine and dopamine carrier blockade on extracellular dopamine increase in the nucleus accumbens shell, bed nucleus of stria terminalis and prefrontal cortex. *J Neurochem*. 2006; 96:473–481. [PubMed: 16336224]
- Carboni E, Tanda GL, Frau R, Di CG. Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: evidence that dopamine is taken up in vivo by noradrenergic terminals. *J Neurochem*. 1990; 55:1067–1070. [PubMed: 2117046]
- Castellanos FX, Tannock R. Neuroscience of attention-deficit/hyperactivity disorder: The search for endophenotypes. *Nat Rev Neurosci*. 2002; 3:617–628. [PubMed: 12154363]
- Chamberlain SR, Del CN, Dowson J, Muller U, Clark L, Robbins TW, Sahakian BJ. Atomoxetine improved response inhibition in adults with attention deficit/hyperactivity disorder. *Biol Psychiatry*. 2007; 62:977–984. [PubMed: 17644072]
- Desai RI, Kopajtic TA, Koffarnus M, Newman AH, Katz JL. Identification of a dopamine transporter ligand that blocks the stimulant effects of cocaine. *J Neurosci*. 2005; 25:1889–1893. [PubMed: 15728828]

- Devilbiss DM, Berridge CW. Cognition-enhancing doses of methylphenidate preferentially increase prefrontal cortex neuronal responsiveness. *Biol Psychiatry*. 2008; 64:626–635. [PubMed: 18585681]
- Diamond A. Attention-deficit disorder (attention-deficit/ hyperactivity disorder without hyperactivity): a neurobiologically and behaviorally distinct disorder from attention-deficit/hyperactivity disorder (with hyperactivity). *Dev Psychopathol*. 2005; 17:807–825. [PubMed: 16262993]
- Elliott R, Sahakian BJ, Matthews K, Bannerjea A, Rimmer J, Robbins TW. Effects of methylphenidate on spatial working memory and planning in healthy young adults. *Psychopharmacology (Berl)*. 1997; 131:196–206. [PubMed: 9201809]
- España RA, Berridge CW. Organization of noradrenergic efferents to arousal-related basal forebrain structures. *J Comp Neurol*. 2006; 496:668–683. [PubMed: 16615125]
- Florin SM, Kuczenski R, Segal DS. Regional extracellular norepinephrine responses to amphetamine and cocaine and effects of clonidine pretreatment. *Brain Res*. 1994; 654:53–62. [PubMed: 7982098]
- Florin SM, Kuczenski R, Segal DS. Effects of reserpine on extracellular caudate dopamine and hippocampus norepinephrine responses to amphetamine and cocaine: mechanistic and behavioral considerations. *J Pharmacol Exp Ther*. 1995; 274:231–241. [PubMed: 7616403]
- Foote SL, Bloom FE, Aston-Jones G. Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. *Physiol Rev*. 1983; 63:844–914. [PubMed: 6308694]
- Gamo NJ, Wang M, Arnsten AF. Methylphenidate and atomoxetine enhance prefrontal function through alpha2-adrenergic and dopamine D1 receptors. *J Am Acad Child Adolesc Psychiatry*. 2010; 49:1011–1023. [PubMed: 20855046]
- Greenhill, LL. Clinical effects of stimulant medication in ADHD. In: Solanto, MV.; Arnsten, AFT.; Castellanos, FX., editors. *Stimulant Drugs and ADHD: Basic and Clinical Neuroscience*. Oxford University Press; New York: 2001. p. 31-71.
- Hiranita T, Soto PL, Newman AH, Katz JL. Assessment of reinforcing effects of benztrapine analogs and their effects on cocaine self-administration in rats: comparisons with monoamine uptake inhibitors. *J Pharmacol Exp Ther*. 2009; 329:677–686. [PubMed: 19228996]
- Katz JL, Izenwasser S, Kline RH, Allen AC, Newman AH. Novel 3alpha-diphenylmethoxytropine analogs: selective dopamine uptake inhibitors with behavioral effects distinct from those of cocaine. *J Pharmacol Exp Ther*. 1999; 288:302–315. [PubMed: 9862785]
- Katz JL, Kopajtic TA, Agoston GE, Newman AH. Effects of N-substituted analogs of benztrapine: diminished cocaine-like effects in dopamine transporter ligands. *J Pharmacol Exp Ther*. 2004; 309:650–660. [PubMed: 14755006]
- Kesner RP, Farnsworth G, DiMattia BV. Double dissociation of egocentric and allocentric space following medial prefrontal and parietal cortex lesions in the rat. *Behav Neurosci*. 1989; 103:956–961. [PubMed: 2803562]
- Kuczenski R, Melega WP, Cho AK, Segal DS. Extracellular dopamine and amphetamine after systemic amphetamine administration: comparison to the behavioral response. *J Pharmacol Exp Ther*. 1997; 282:591–596. [PubMed: 9262319]
- Kuczenski R, Segal DS, Cho AK, Melega W. Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *J Neurosci*. 1995; 15:1308–1317. [PubMed: 7869099]
- Lapiz MD, Morilak DA. Noradrenergic modulation of cognitive function in rat medial prefrontal cortex as measured by attentional set shifting capability. *Neuroscience*. 2006; 137:1039–1049. [PubMed: 16298081]
- Li SM, Newman AH, Katz JL. Place conditioning and locomotor effects of N-substituted, 4',4''-difluorobenztrapine analogs in rats. *J Pharmacol Exp Ther*. 2005; 313:1223–1230. [PubMed: 15743929]
- Loland CJ, Desai RI, Zou MF, Cao J, Grundt P, Gerstbrein K, Sitte HH, Newman AH, Katz JL, Gether U. Relationship between conformational changes in the dopamine transporter and cocaine-like subjective effects of uptake inhibitors. *Mol Pharmacol*. 2008; 73:813–823. [PubMed: 17978168]

- Mehta MA, Goodyer IM, Sahakian BJ. Methylphenidate improves working memory and set-shifting in AD/HD: relationships to baseline memory capacity. *J Child Psychol Psychiatry*. 2004; 45:293–305. [PubMed: 14982243]
- Mehta, MA.; Sahakian, BJ.; Robbins, TW. Comparative psychopharmacology of methylphenidate and related drugs in human volunteers, patients with ADHD, and experimental animals. In: Solanto, MV.; Arnsten, AFT.; Castellanos, FX., editors. *Stimulant Drugs and ADHD: Basic and Clinical Neuroscience*. Oxford University Press; New York: 2001. p. 303-331.
- Pang KC, Jiao X, Sinha S, Beck KD, Servatius RJ. Damage of GABAergic neurons in the medial septum impairs spatial working memory and extinction of active avoidance: effects on proactive interference. *Hippocampus*. 2011; 21:835–846. [PubMed: 20865731]
- Raje S, Cornish J, Newman AH, Cao J, Katz JL, Eddington ND. Pharmacodynamic assessment of the benztropine analogues AHN-1055 and AHN-2005 using intracerebral microdialysis to evaluate brain dopamine levels and pharmacokinetic/pharmacodynamic modeling. *Pharm Res*. 2005; 22:603–612. [PubMed: 15846468]
- Rapoport JL, Inoff-Germain G. Responses to methylphenidate in Attention-Deficit/Hyperactivity Disorder and normal children: update 2002. *J Atten Disord*. 2002; 6:S57–S60. [PubMed: 12685519]
- Robbins TW, Arnsten AF. The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. *Annu Rev Neurosci*. 2009; 32:267–287. [PubMed: 19555290]
- Sesack SR, Hawrylak VA, Matus C, Guido MA, Levey AI. Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J Neurosci*. 1998; 18:2697–2708. [PubMed: 9502827]
- Smith HR, Pang KC. Orexin-saporin lesions of the medial septum impair spatial memory. *Neuroscience*. 2005; 132:261–271. [PubMed: 15802181]
- Solanto, MV. Attention-Deficit/Hyperactivity Disorder. In: Solanto, MV.; Arnsten, AF.; Castellanos, FX., editors. *Stimulant Drugs and ADHD: Basic and Clinical Neuroscience*. Oxford University Press; New York: 2001. -3.p. 30
- Spencer RC, Klein R, Berridge CW. Psychostimulants act within the prefrontal cortex to improve cognition function. *Biol Psychiatry*. 2011; 10.1016/j.biopsych.2011.12.002.
- Turner DC, Blackwell AD, Dowson JH, McLean A, Sahakian BJ. Neurocognitive effects of methylphenidate in adult attention-deficit/hyperactivity disorder. *Psychopharmacology (Berl)*. 2005; 178:286–295. [PubMed: 15338103]
- Vaidya CJ, Austin G, Kirkorian G, Ridlehuber HW, Desmond JE, Glover GH, Gabrieli JDE. Selective effects of methylphenidate in attention deficit hyperactivity disorder: A functional magnetic resonance study. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95:14494–14499. [PubMed: 9826728]
- Wilens TE, Faraone SV, Biederman J. Attention-deficit/hyperactivity disorder in adults. *JAMA*. 2004; 292:619–623. [PubMed: 15292088]
- Woolverton WL, Hecht GS, Agoston GE, Katz JL, Newman AH. Further studies of the reinforcing effects of benztropine analogs in rhesus monkeys. *Psychopharmacology (Berl)*. 2001; 154:375–382. [PubMed: 11349390]
- Woolverton WL, Rowlett JK, Wilcox KM, Paul IA, Kline RH, Newman AH, Katz JL. 3'- and 4'-chloro-substituted analogs of benztropine: intravenous self-administration and in vitro radioligand binding studies in rhesus monkeys. *Psychopharmacology (Berl)*. 2000; 147:426–435. [PubMed: 10672637]
- Yamamoto BK, Novotney S. Regulation of extracellular dopamine by the norepinephrine transporter. *J Neurochem*. 1998; 71:274–280. [PubMed: 9648875]

Highlights

- AHN 2-005 is a selective dopamine reuptake inhibitor lacking reinforcing effects.
- AHN 2-005 improved prefrontal-dependent cognition.
- AHN 2-005 increased extracellular dopamine and norepinephrine in prefrontal cortex.
- These actions are similar to those produced by ADHD-related drugs.

Figure 1.

AHN 2-005 improves PFC-dependent working memory performance. Shown are the mean ($\pm$ SEM) percent change correct from baseline in animals treated with vehicle or varying doses of AHN 2-005. AHN 2-005 produced a dose-dependent improvement in performance in this task with significant improvement occurring at the 10 mg/kg dose. The magnitude of AHN 2-005-induced improvement in performance is comparable to that seen with systemic administration of clinically-relevant doses of psychostimulants. * $P < 0.05$ vs. vehicle-treatment.

Figure 2.

Photomicrographs depicting placement of dialysis probes within the prefrontal cortex (PFC, Panel A), nucleus accumbens (NAc, Panel B), and the medial septal area (MSA, Panel C). For PFC, probes were placed within the medial subdivision of this region. In Panel B, dashed line indicates approximate boundary of the nucleus accumbens. For MSA, probe placement spanned the medial septum and diagonal band of Broca. Dashed line in Panel C indicates dorsal border of the medial septum. 40- μ m coronal sections were stained with Neutral Red. Arrows indicate probe track. AC = anterior commissure; CC = corpus callosum; LV = lateral ventricle; M = midline.

Figure 3.

Chromatograms of AHN 2-005-induced increases in DA and NE levels. Shown are chromatograms from a pre-treatment (PRE) and post-treatment (POST) sample for PFC DA (Top Panel), PFC NE (Middle Panel) and nucleus accumbens DA (NAc DA, Bottom Panel). Numbers adjacent to peaks indicate retention time and quantity (pg), respectively. 10 mg/kg AHN 2-005 increased the height of the NE and DA peaks (scale is the same in PRE vs. POST).

Figure 4.

Effects of a cognition-enhancing dose of AHN 2-005 on extracellular levels of NE and DA within the PFC (Panel A), nucleus accumbens (NAc, DA only, Panel B), and medial septal area (MSA, Panel C). Shown are the mean ($\pm$ SEM) DA and NE levels (per 20 μ l sample) expressed as a percentage of baseline in 30-minute samples collected prior to (negative numbers) and following (positive numbers) injection of vehicle or 10 mg/kg AHN 2-005. AHN 2-005 elicited significant but restrained increases in extracellular DA and NE in the PFC and MSA ($\sim$ 75%-100% above baseline) and larger increases in extracellular DA levels in the nucleus accumbens ($\sim$ 200% above baseline). $^+P < 0.05$, $^{++}P < 0.01$ compared to sample immediately preceding drug administration (Sample -1); $*P < 0.05$, $**P < 0.01$ compared to vehicle-treated animals.

Table 1

Behavioral Actions of AHN 2-005.

	Quad Entries	Rears	Eat	Drink	Sleep	Quiet Wake	Active Wake
Post1							
Veh	4 ± 2	2 ± 1	72 ± 68	0 ± 0	1213 ± 219	121 ± 31	213 ± 114
AHN	16 ± 8	1 ± 1	0 ± 0	6 ± 6	812 ± 194	377 ± 60*	490 ± 136
Post2							
Veh	2 ± 2	1 ± 1	95 ± 95	11 ± 11	1448 ± 224	82 ± 22	61 ± 57
AHN	1 ± 1	0 ± 0	9 ± 9	0 ± 0	1450 ± 130	167 ± 70	73 ± 52

Shown are the effects of a cognition-enhancing dose of AHN 2-005 (AHN; 10 mg/kg) on locomotor activity as measured by the number of quadrant entries and rears as well as time (seconds) spent eating (Eat), drinking (Drink), asleep (Sleep), in quiet waking (Quiet Wake) and in active waking (Active Wake). Data are expressed as the mean (± SEM) for vehicle-treated (Veh) and AHN 2-005-treated animals for the two 30-minute epochs immediately following treatment (Post1 and Post2). AHN 2-005 had no significant effects on any of these behavioral measures relative to vehicle treatment, except for a modest increase in quiet waking during the first 30-minute post-treatment epoch. The amount of time spent in quiet waking during this epoch in AHN 2-005-treated animals represents only 20% of this epoch. * $P < 0.01$ vs. vehicle-treatment.