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ORIGINAL INVESTIGATION

Memory and psychostimulants: modulation of Pavlovian fear conditioning by amphetamine in C57BL/6 mice

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Abstract
Rationale and objectives With the use of prescription stimulants on the rise, it is important to examine the cognitive effects of low and moderate doses of stimulants rather than only those typical of addicts.
Materials and methods The present study examined the effects a range of doses (0.005–8 mg/kg) of D-amphetamine sulfate on cued and contextual Pavlovian fear conditioning in mice.
Results In agreement with previous research, subjects administered with a moderately high dose of amphetamine (8 mg/kg) pre-training, typical of what addicts might take, displayed impaired conditioned freezing when tested off-drug. Alternately, subjects injected with a very low dose of amphetamine (0.005, 0.025, or 0.05 mg/kg) pre-training, similar to the therapeutic doses for attention deficit hyperactivity disorder, displayed enhanced memory when tested off-drug. A control study showed that these effects were not due to state-dependent learning.
Conclusions Thus, dose is a critical determinant of the cognitive effects of psychostimulants.

Keywords Amphetamine · Fear conditioning · Learning and memory · Mouse

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Introduction

Amphetamine can be highly addictive, leading individuals to exhibit behaviors ranging from relatively minor cognitive impairments to severe psychotic symptoms. Methamphetamine abusers display worse performance on tests of word recall, perceptual speed, vocabulary, and abstract reasoning, compared with controls (Simon et al. 2002). After prolonged amphetamine abuse, addicts may also exhibit symptoms similar to schizophrenia, commonly referred to as amphetamine or stimulant psychosis (Harris and Batki 2000).

Conversely, amphetamine is also used to treat ailments such as narcolepsy and attention deficit hyperactivity disorder (ADHD). Amphetamine (e.g., Adderall®) improves ADHD symptoms for most affected children (Ahmann et al. 2001), and several related stimulants are similarly effective (e.g., methamphetamine, methylphenidate, atomoxetine, modafinil; Leonard et al. 2004).

While prescription stimulants containing amphetamine have proven beneficial to many people suffering from ADHD, excessive daytime sleepiness, and narcolepsy, off-label use of these drugs is a growing problem. The life-time prevalence of non-medical use of prescription stimulants in college students has been recently reported to be 6.9% (McCabe et al. 2005) to 8.3% (Teter et al. 2006). These drugs tend to be regarded by the public as cognitive enhancers, presumably by promoting mental arousal or wakefulness. "Academic doping" is an emerging phenomenon in many educational settings (Butcher 2003). Stimulants also have documented benefits (and problems) in military use (Caldwell et al. 1995; Cornum et al. 1995; Cornum 1994). Indeed, a growing body of evidence demonstrates that healthy volunteers, not just those with ADHD, display cognitive benefits from low doses of stimulants (Barch and Carter 2005; Turner et al. 2003).

These trends illuminate the importance of studying the effects of a variety of acute doses of amphetamine rather than focusing solely on the higher, chronic doses typical of addicts. The current study examines the effects of a range of acute doses of D-amphetamine on a standard rodent learning and memory task, Pavlovian fear conditioning.

In Pavlovian fear conditioning, a discrete neutral stimulus (e.g., a tone) is paired with an aversive stimulus (e.g., a footshock). After training, when presented with the discrete stimulus, alone, the subject exhibits fear. In addition, upon returning to the environmental chamber in which it had been trained, the subject also exhibits fear, a phenomenon known as context conditioning. A common measure of conditioned fear in rodents is freezing, or the absence of all movements, excluding respiration (Fanselow 1980).

The neurobiology of Pavlovian fear conditioning has been studied extensively. The dorsal hippocampus is critically involved in encoding memory of the context in a time-graded manner (Anagnostaras et al. 1999, 2001). Because of the efficiency of contextual fear conditioning, it has become a leading model of explicit memory in rats and mice (Anagnostaras et al. 2000, 2001). The amygdala, and specifically the basolateral/lateral complex of the amygdala, is involved in encoding an aversive association with both the context and tone (Fanselow and Gale 2003; Fanselow and Poulos 2005). Amphetamine has been shown to alter amygdalar activity in a number of ways, including potentiating the synaptic transmission between the amygdala and nucleus accumbens (Kessal et al. 2005). The hippocampus has also been implicated in amphetamine-induced locomotor activity as lesions of the ventral hippocampus disrupt amphetamine-induced locomotion, and stimulation enhances it (White et al. 2006).

We previously found (Wood et al. 2007) that an acute dose of cocaine enhances learning on Pavlovian fear conditioning when given at a very low dose (0.1 mg/kg). Interestingly, we also found that a moderate dose of cocaine (15 mg/kg) disrupted conditioned freezing. These effects were general to both contextual and cued fear. These data reinforce the human findings that dosage is the critical determinant of whether a particular stimulant enhances or impairs memory. In particular, doses similar to those given for ADHD often improve attention, academic performance, and reduce impulsivity in humans. In contrast, high doses such as those taken by addicts are associated with unemployment, reduced executive function, anxiety, psychosis, and of course, addiction (Ellinwood et al. 1998). One potential confound of our prior study is that cocaine produces local anesthesia, which could directly reduce fear conditioning by reducing the painfulness of the footshock. Therefore, in the present study, we examined the effects of amphetamine on conditioned fear in order to extend the findings to a widely prescribed stimulant, as well as to control for local anesthesia. We also conducted a control study to address the issue of state-dependent effects on learning. We predicted that amphetamine would show the same pattern as cocaine, enhancing Pavlovian fear conditioning at low doses and interfering with fear conditioning at higher doses. The results of the current study are consistent with this hypothesis, showing enhanced learning during acquisition and recall of cued fear at low doses of amphetamine (0.005, 0.025, and 0.05 mg/kg) and deficits in conditioned freezing at higher doses (4 and 8 mg/kg). In addition, there was no evidence found to support the idea that state-dependent learning was a factor in the present results.

Materials and methods

Subjects

One-hundred thirty-six (68 male, 68 female) C57BL6/NCrl (B6; Charles River Laboratories, San Diego, CA, USA) mice were used for experiment 1, and 40 (19 male, 21 female) B6129SF1/J (H; Jackson Laboratory, West Sacramento, CA, USA) hybrid mice were used for experiment 2. Mice were weaned at 3 weeks of age and were group-housed (two to five mice per same sex cage), with continuous access to food and water. The animal colony was maintained on a 14:10 light/dark schedule, and all tests were performed during the light phase of the cycle. Mice were at least 8 weeks old before testing. All animal care and testing procedures were approved by the UCSD IACUC and were in accordance with the NIH "Principles of laboratory animal care." B6 and H mice were chosen because they display robust conditioned freezing and typical psychomotor reactivity to amphetamine (Anagnostaras et al. 2000; Yates et al. 2007). Finally, B6 and H mice are the most common background strains in studies using targeted mutations, and the present studies are part of a larger plan of study that involves several mutants (Crawley et al. 1997; Silva et al. 1997). H mice were used in experiment 2 due to availability but perform comparably on this task (Anagnostaras et al. 2003).

Apparatus

Conditioning context Testing was performed in a windowless room. Background noise (65 dB) was provided by a HEPA air cleaner, and white light was provided by two 100-W bulbs. The mice were continuously recorded by a wall-mounted color video camera that was connected to a computer and video equipment in an adjacent room. Three to four mice were tested concurrently in individual conditioning chambers. Each chamber (32 cm wide, 25 cm high, 25 cm deep) was equipped with a stainless

steel grid floor (36 rods, each rod 2 mm in diameter, 8 mm center to center) and a speaker in the side wall. The side walls were white acrylic, and the front wall was clear to allow for viewing (Med-Associates Inc., St. Albans, VT, USA). A stainless steel drop-pan, scented with 7% isopropyl alcohol to provide a background odor, was located beneath each chamber. Between tests, the conditioning contexts were cleaned with 7% isopropyl alcohol solution. Each chamber was connected to a solid-state scrambler, providing AC constant current shock, and an audio stimulus generator was located in an adjacent room, controlled via an interface connected to a Windows computer running Med-PC (Med-Associates Inc., St Albans, VT, USA). Automated assessment of freezing and activity was provided by custom-designed software adapted from NIH Image running on an Apple Macintosh G4 (automated algorithm validated elsewhere; Anagnostaras et al. 2000).

Alternate context Multiple (three to four) mice were tested concurrently for tone fear in a separate room in individual boxes measuring 30 cm wide, 25 cm high, and 24 cm deep, and equipped with a speaker in the side walls. A clear Plexiglas front wall allowed the mice to be continually observed, while the ceiling, floor, and three interior walls of the chamber were solid white. To create a space distinct from the training context, a white plastic, triangular tent was placed inside each box; each side of the triangle measured 23 cm. Between tests, the chambers were cleaned and scented with a 5% white vinegar solution. The room was lit with dim red light, and an infrared video camera, connected to the Macintosh G4 described above, was used to score freezing.

Drugs Drugs were administered intraperitoneally (i.p.) in a volume of 5 or 10 ml/kg. D-Amphetamine sulfate (Sigma-Aldrich Co., St. Louis, MO, USA) was dissolved in 0.9% physiological saline. Amphetamine injections (salt weight; 0.005, 0.025, 0.05, 0.5, 1, 2, 4, or 8 mg/kg) were given 15 min before introduction to the testing equipment.

Experiment 1: behavior measurement In order to measure the effects of amphetamine on exploratory locomotor activity, baseline activity in 2 min before the first tone–shock pairing on the training day was assessed by counting the number of cross-overs each subject performed (Maren et al. 1998). A single cross-over was defined as the movement of a subject's entire body from one half of the box to the other. Videotapes of the conditioning sessions were observed using a standard VCR and monitor. In addition to number of cross-overs, exploratory activity was also measured using an automated, video-based activity measure (Anagnostaras et al. 2000).

To assess whether amphetamine disrupted shock reactivity, mouse activity burst displayed during the 2 s of shock exposure (unconditioned response to shock), as well as activity during the 2 s leading up to the shock, was measured as velocity (centimeter per second; Anagnostaras et al. 2000).

Experimental procedures

Experiment 1

Conditioning Mice were injected with saline or amphetamine 15 min prior to training. Training consisted of a 2-min baseline period, followed by three tone–shock pairings, each separated by 30 s. A tone–shock pairing consisted of a 30-s tone (2.8 kHz, 85 dB, A Scale), with a scrambled, constant current AC footshock (0.75 mA) administered during the last 2 s of the tone. Immediate post-shock freezing was measured for another 5 min. Thus, mice were inside the fear-conditioning chambers for a total of 10 min before being returned to their home cages.

Testing Mice were returned to the conditioning context without drug 24 h after training, and freezing was scored for 5 min. Mice were placed in the alternate context 48 h after training, also off-drug. Tone testing consisted of a 2-min baseline, followed by a continuous 3-min tone identical to the training tone. Freezing was scored for the entire 5-min period.

Experiment 2

Procedures for experiment 2 were identical to experiment 1, with the exception that amphetamine or saline injections were administered 15 min before testing, in addition to those administered before training.

Results

Experiment 1

Generalized activity Amphetamine produced a dose-dependent increase in activity during the 2-min baseline period of training, measured both by an automated computer scoring system and by hand-counted cage cross-overs (Fig. 1a). Group differences in cage cross-overs were found on an analysis of variance [ANOVA; $F(8, 127)=16.5$, $p<0.0001$]. Subjects administered with 4–8 mg/kg amphetamine pre-training displayed significantly more cross-overs during baseline than the saline control group [Fisher's protected least significant difference (PLSD) multiple post

hoc comparisons, p values <0.01]. No other doses differed significantly from saline controls (p values >0.05). An ANOVA demonstrated differences in the automated activity measure as well [$F(8, 127)=7.5$, $p<0.0001$]. As with the cross-over measures, only mice given 4–8 mg/kg of amphetamine showed a significant difference in activity from saline controls (Fisher's PLSD, p values <0.01).

Activity burst velocity A large difference in velocity was elicited by the first 2-s shock presentation [unconditioned response (UR)], compared with the preceding 2 s, during which no shock was present (Fig. 1b). A multivariate ANOVA revealed a significant effect of dose on velocity [$F(8, 127)=2.3$, $p<0.05$], a significant effect of the shock [UR versus baseline; $F(1, 127)=1687.5$, $p<0.0001$], along with a significant time period by dose interaction [$F(8, 127)=2.5$, $p<0.05$], so the baseline and UR were considered separately. There were significant differences in the baseline velocity [$F(8, 127)=5.8$, $p<0.0001$]. Post hoc comparisons revealed similar differences in velocity as those seen in measurements of baseline activity, with subjects administered with 1, 4, or 8 mg/kg amphetamine displaying increased velocity compared with subjects administered with saline (Fisher's PLSD, p values <0.05). No other doses differed significantly in terms of baseline activity from saline. There were also significant differences during the UR [$F(8, 127)=2.2$, $p<0.05$]. Only subjects given 0.025 or 8 mg/kg exhibited a larger UR to the shock compared to controls (p values <0.05), but these differences were small and unrelated to the memory effects. Thus, amphetamine did not dull shock reactivity and seemed to enhance reactivity in certain cases.

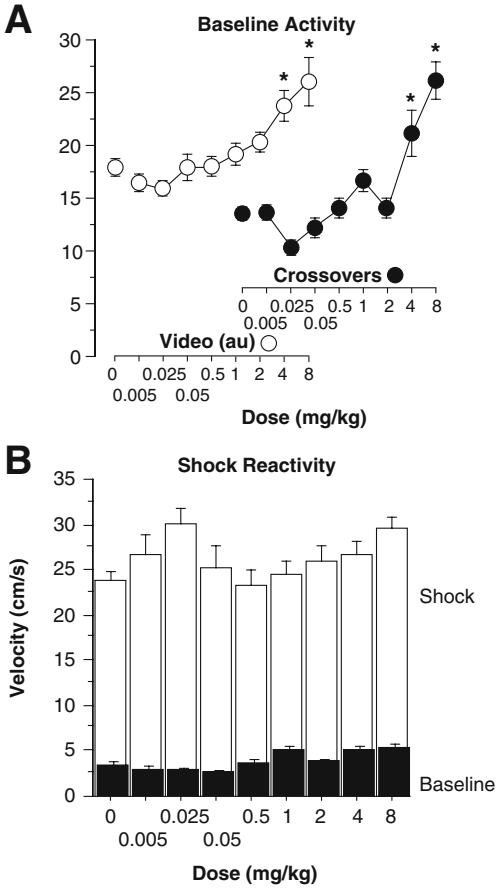

Fig. 1 **a** Baseline activity. A dose-dependent increase in activity was observed during the 2-min baseline period prior to the first tone–shock pairing, with subjects administered with 4 or 8 mg/kg amphetamine prior to training showing greater activity than saline controls (*left*, an automated video activity measure and *right*, full cage cross-overs; mean ± SEM). *Asterisks* indicate a significant difference from saline controls. **b** Shock reactivity. All groups showed a significant increase in velocity (centimeter per second, mean ± SEM) during the 2-s shock compared with the 2-s baseline period leading up to the shock

Training Subjects were inside the conditioning contexts on the training day for a total of 10 min. Figure 2a depicts the first 5 min of training, consisting of a 2-min baseline period, followed by three tone–shock pairings during 3 min. A main effect for dose was present within the 5-min period [$F(8, 127)=36.5$, $p<0.0001$], as was a main effect for minute [$F(4, 127)=248.1$, $p<0.0001$], along with a significant dose by minute interaction [$F(32, 127)=15.4$, $p<0.0001$]. Post hoc comparisons revealed a dose-dependent effect of amphetamine on freezing, with subjects that had received 1, 2, 4, or 8 mg/kg freezing less than saline controls (Fisher's PLSDs, p values <0.0001). Interestingly, subjects that had been administered with 0.005, 0.025, or 0.05 mg/kg amphetamine froze more than the saline control subjects (p values <0.01). Subjects that received 0.5 mg/kg were not significantly different from saline controls ($p>0.05$).

Post-shock freezing In minutes 6 through 10 of training, freezing was measured with no further presentation of tone or shock, as an index of post-shock freezing (Fig. 2b). Dose-dependent differences in freezing were apparent [$F(8, 127)=68.7$, $p<0.0001$]. Post hoc tests revealed that subjects that had received 1, 2, 4, or 8 mg/kg amphetamine displayed less freezing than saline controls (Fisher's PLSD, p values <0.001). As these subjects were still on-drug, it is important to note that amphetamine's locomotor effects could be influencing these results. Interestingly, however, subjects administered with 0.005, 0.025, or 0.05 pre-training again froze more than saline controls (p values <0.05). Subjects that received 0.5 mg/kg were not significantly different from saline controls ($p>0.05$).

Context fear Subjects were returned to the conditioning chambers 24 h after training. Freezing was measured for 5 min, with all subjects off-drug (Fig. 3a). Dose-dependent differences in freezing were apparent [$F(8, 127)=20.7$, $p<0.0001$]. Subjects that had previously received 4 or 8 mg/kg

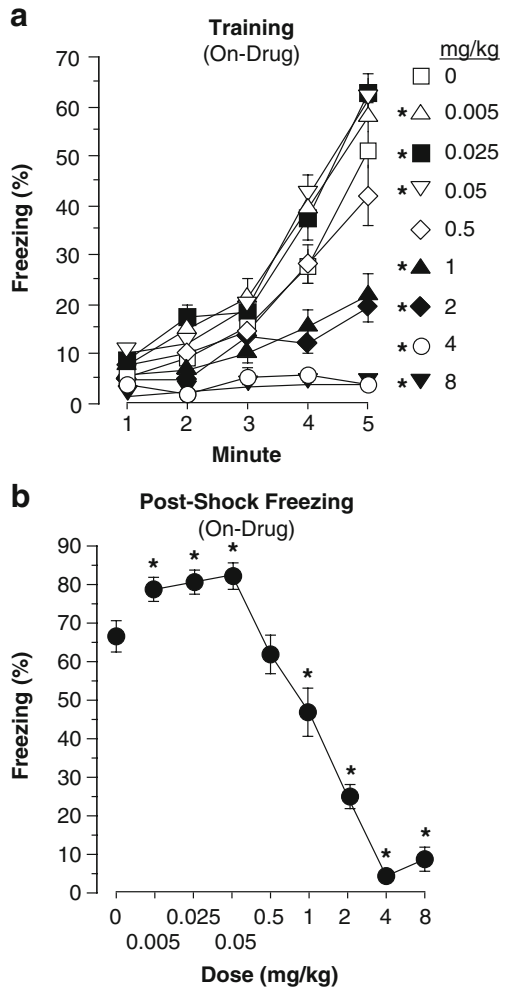

Fig. 2 **a** Training. Freezing (mean ± SEM) of subjects on 1, 2, 4, or 8 mg/kg amphetamine was less than saline control subjects, while freezing of subjects on 0.005, 0.025, or 0.05 mg/kg amphetamine was greater than controls. **b** Post-shock freezing. Dose-dependent, post-training freezing was observed, with subjects on 0.005, 0.025, or 0.05 mg/kg amphetamine freezing more than controls, while those on 1, 2, 4, or 8 mg/kg amphetamine displayed less freezing than controls

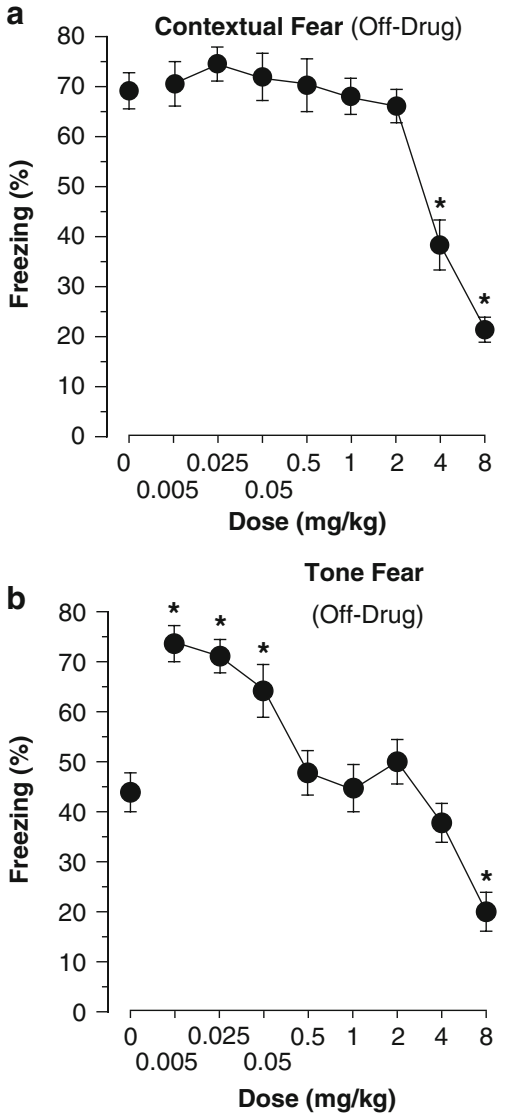

Fig. 3 **a** Contextual fear. When returned to the training context 24 h after training off-drug, subjects that had received 4 or 8 mg/kg pre-training amphetamine displayed less freezing (mean ± SEM) than all other groups. **b** Tone fear. Freezing (mean ± SEM) during the 3-min period during the test when the tone was presented is depicted. Those that had been injected with 8 mg/kg pre-training displayed less freezing than all other groups, while those that had been administered with 0.005, 0.025, or 0.05 mg/kg displayed more freezing than all other groups

amphetamine before training displayed less freezing off-drug when reintroduced to the training context compared with saline controls (Fisher's PLSD, p values <0.01). No other groups differed from saline controls (p values >0.05).

Tone fear Subjects were introduced to a new context 48 h after training off-drug. Freezing was measured for 5 min, consisting of a 2-min baseline period and a 3-min tone presentation (Fig. 3b). The tone was the same frequency and volume as that which had been paired with the shock during training. Dose-dependent differences in freezing during the tone presentation were apparent [$F(8, 127)= 17.1$, $p<0.0001$]. Only subjects that had received 8 mg/kg amphetamine pre-training showed decreased freezing during the tone test compared with saline controls (Fisher's PLSD, $p<0.0001$). Those that had been injected with 0.005, 0.025, or 0.05 mg/kg amphetamine pre-training again showed increased freezing compared with saline controls (p values <0.001). No other doses differed from saline controls (p values >0.05).

Experiment 1: summary Overall, contextual fear memory tested off-drug was sensitive to disruption by 4–8 mg/kg of amphetamine, whereas tone fear was sensitive only to 8 mg/kg. An enhancement in cued, but not contextual, memory was observed at 0.005, 0.025, and 0.05 mg/kg.

Experiment 2

Training and post-shock freezing The training protocol for the state-dependent control study was the same as the dose–response study. Fifteen minutes after drug or saline injection, subjects were exposed to the conditioning chambers for a total of 10 min, composed of a 2-min baseline period, 3 min during which three tone–shock pairings were presented, and a 5-min immediate post-shock freezing test (Fig. 4a). Drug-dependent effects on freezing were evident [$F(3, 36)=88.7$, $p<0.0001$], with mice injected with saline pre-training showing markedly more freezing throughout training than those injected with 8 mg/kg amphetamine (Fisher's PLSD, p values <0.0001). A main effect for training period was evident as well [$F(2, 36)=259.9$, $p<0.0001$], with average freezing increasing throughout training (p values <0.0001). A significant drug by training period interaction [$F(6, 36)=59.7$, $p<0.0001$], however, demonstrated that this effect was driven differentially by the two saline groups, as the two groups on amphetamine did not show an increase in freezing.

Context fear Using the same protocol as experiment 1, subjects were returned to the conditioning chambers 24 h after training to measure contextual fear memory (Fig. 4b). Drug treatment had significant effects on contextual fear expression [$F(3, 36)=66.5$, $p<0.0001$], with both groups injected with 8 mg/kg amphetamine pre-testing displaying less freezing than those injected with saline (Fisher's PLSD, p values <0.0001). Mice that received amphetamine during testing exhibited very little freezing and did not differ from one another ($p>0.05$). Consistent with experiment 1, mice that had been injected with 8 mg/kg amphetamine pre-training but then injected with saline pre-testing displayed less contextual freezing than those that had been injected with saline both pre-training and pre-testing ($p<0.0001$). Overall, no state-dependent learning effects were evident. Consistent with experiment 1, amphetamine was able to disrupt performance of freezing when animals were trained on-drug, as well as contextual freezing when those animals were tested 24 h later off-drug.

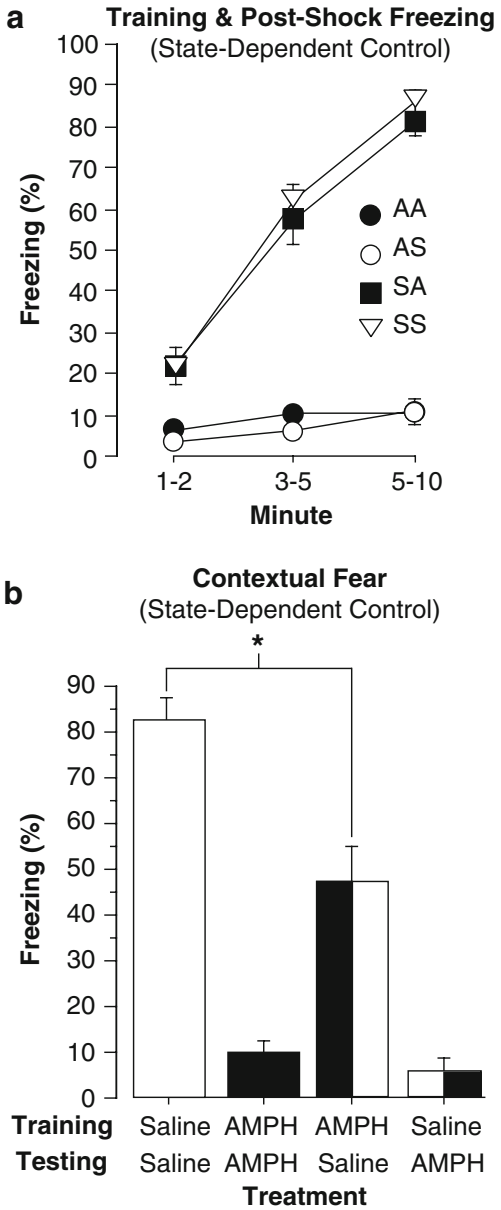

Fig. 4 **a** State-dependent control—training and post-shock freezing. While subjects administered with saline before training showed a steady increase in freezing during training, subjects administered with 8 mg/kg amphetamine showed no increase in freezing. **b** State-dependent control—contextual fear. Regardless of treatment before training, subjects administered with 8 mg/kg amphetamine before contextual testing displayed very little freezing. In contrast, mice injected with saline before contextual testing showed some freezing if injected with 8 mg/kg before training and greater levels of freezing if injected with saline before training (*asterisk* indicates significant difference between these two groups). There was no evidence of state-dependent memory

Discussion

We predicted that amphetamine would disrupt Pavlovian fear conditioning at moderate to high doses, similar to those that addicts take, and enhance fear conditioning at low doses, similar to those used to treat ADHD. As expected, moderate doses of amphetamine administered pre-training inhibited contextual and cue-elicited freezing off-drug, while very low doses enhanced cued fear memory. These data are in agreement with the results of our previous work with cocaine, which induced a similar pattern of enhancements and deficits in fear conditioning (Wood et al. 2007). A low dose of cocaine (0.1 mg/kg) produced an enhancement in contextual and cued fear, whereas a high dose

(15 mg/kg) produced an impairment in both measures. The combined results of these studies lead us to theorize that it is perhaps not the addictive properties of the drugs or the clinical profile of the subjects taking the drugs but, rather, the dose of drugs that lead to the divergent cognitive effects associated with psychostimulants.

One difference in the results of the present study and our previous research with cocaine (Wood et al. 2007) is that a range of low doses of amphetamine studied did not enhance contextual fear 24 h after training. It is unclear if the memory enhancement provided by a low dose of amphetamine is specific to cued fear as immediate post-shock freezing is believed to be a form of contextual fear (Fanselow 1986). Indeed, while low doses of amphetamine induced small but significantly higher levels of freezing during the immediate post-shock freezing test compared to saline controls, levels of freezing off-drug during the tone test were almost double that of controls. This evidence suggests the effects on locomotor activity and freezing are dissociable, especially for the low doses. It may be the case that some other, untested low dose of amphetamine would reveal enhanced memory on both contextual and cued tests off-drug, but it seems likely that a very low dose of amphetamine enhances cued memory, exclusively, since we tried a wide range of low doses (0.005–0.5 mg/kg) and induced large enhancements of tone memory. Another possibility is that the enhancement was not observed in contextual fear because of a "ceiling effect"; about 70% freezing is potentially the maximum that B6 mice may freeze under these training parameters (Anagnostaras et al. 2000, 2003). Clearly, a moderate or high dose of amphetamine disrupts conditioned freezing; there were some differences in terms of the deficits, in that 4 and 8 mg/kg produced significant deficits in contextual freezing, where only 8 mg/kg was effective in disrupting tone freezing. However, overall, the deficits in context and tone conditioning seem similar at high doses.

Research with a variety of human populations has produced mixed findings about the cognitive effects of stimulant use in humans. One study found cognitive benefits with amphetamine administration (0.25 mg/kg, orally) to people with schizophrenia already treated with antipsychotics (Barch and Carter 2005) despite the common notion that amphetamine exacerbates schizophrenia. The non-schizophrenic control subjects of the study, as well, exhibited improved language production and reaction time but had no benefit in working memory accuracy. In addition, it has been reported that low-dose amphetamine (10 mg, p.o., D-amphetamine, approximately 0.12 mg/kg for an 80-kg person) given during speech therapy leads to improved recovery from aphasia (Walker-Batson et al. 2001). These findings demonstrate the ability of stimulants to improve certain cognitive functions in a healthy population. Many studies have found cognitive deficits in amphetamine and cocaine addicts (Rogers and Robbins 2001). Currently using methamphetamine abusers were found to have worse performance on word recall, perceptual speed, an abbreviated IQ test, and the Wisconsin Card Sorting Task, compared with controls (Simon et al. 2002). In addition, methamphetamine addicts were found to perform more poorly on a decision-making task than their matched controls while displaying less activation in a number of frontal cortical areas, as measured by functional magnetic resonance imaging (fMRI; Paulus et al. 2002).

Previous research on amphetamine administration and associative learning in rodents has also yielded mixed findings. Rats administered with D-amphetamine (5 mg/kg, i.p.) or cocaine (40 mg/kg, i.p.) during extinction of fear-potentiated startle continued to show high startle amplitudes after 120 presentations of the nonreinforced CS (Borowski and Kokkinidis 1998). These results indicate that amphetamine and cocaine impaired the extinction of fear-potentiated startle. Extinction is generally conceived as the acquisition of new, inhibitory learning (Barad 2006). These doses of amphetamine and cocaine would produce impairments if given during training on our conditioned freezing task. Post-training injections of D-amphetamine at 1.0 mg/kg (i.p.), but not 0.25 or 4.0 mg/kg, have been shown to enhance inhibitory avoidance learning (Martinez et al. 1980). It is unclear if pre- versus post-training injections would share the same dose–response curve, so it is difficult to directly compare the results of the Martinez study with the current results. In addition, in order to examine amphetamine's effects on experience-dependent plasticity in rats, researchers examined fear conditioning in rats housed in a complex environment for 3 months (Briand et al. 2005). While rats showed enhanced learning after living in the complex environment, rats treated with D-amphetamine (4.0 mg/kg) for 21 days, then housed in the enriched environment for 3 months, did not show the same enhancement. The authors hypothesized that this moderate dose of amphetamine limited the experience-dependent structural plasticity normally engaged by a complex environment.

As with cocaine, several possible reasons exist as to why conditioned freezing was disrupted at high doses (Wood et al. 2007). While amphetamine does not have anesthetic properties, it could have induced a positive hedonic state in the mice. Those on higher doses may have perceived the shocks as less aversive than those on lower doses because of a drug-induced feeling of well being. While this experiment is not designed to fully explore this possibility, there is little evidence that this explanation accounts for the results. We measured the shock reactivity of subjects (Fig. 1b) and found that all subjects showed a large increase in velocity during the first shock presentation, in comparison to baseline velocity. Mice on 8 mg/kg

amphetamine seemed to perceive the shock at least as aversive as those on lower doses. In addition, this explanation of altered perception would imply that amphetamine produces a positive hedonic state disruptive to fear conditioning, whereas much research has shown that amphetamine is anxiogenic, enhancing most defensive behaviors in rodents (Markham et al. 2006).

Previous research on a range of doses of amphetamine supports an interpretation of attentional deficits disrupting performance. Rats showed impairment on a delayed matching-to-sample task at low doses of 0.6 and 1.0 mg/kg amphetamine, i.p. (Harper et al. 2005). At these doses, considered low doses for the present study on mice, the responses for a given trial were greatly influenced by responses from the preceding trial. These results were interpreted to stem from poor attention or confusion, as opposed to a pure associative memory deficit. Evidence for attentional deficits due to chronic amphetamine (5.0 mg/kg, i.p.) use was also found in extinction deficits of active and passive avoidance responses in mice (Kokkinidis 1983). Human studies, utilizing techniques such as fMRI (Paulus et al. 2002), have revealed frontal lobe dysfunction in amphetamine-dependent individuals, resulting in poor attention and decision making. Taken together, there is evidence from previous work that amphetamine could be hindering performance on fear conditioning due to attentional rather than learning deficits.

An additional, potential confound to be addressed is state-dependent learning. According to this theory, subjects on-drug during training would remember what they learned better if also tested on-drug. A discrepancy in drug state between training and testing has been shown to be detrimental to learning, for certain drugs. For example, one representative study examined the effects of benzodiazepine [chlordiazepoxide (CDP)] administration during extinction of fear conditioning (Bouton et al. 1990). They found that extinction was state dependent when done on CDP. However, in the present study using amphetamine, we found no evidence of state-dependent learning (Fig. 4). In the present study, subjects that received 0.005, 0.025, or 0.05 mg/kg amphetamine pre-training displayed higher levels of freezing than controls when tested off-drug. This finding contradicts the prediction of state-dependent learning. In addition, the immediate post-shock freezing test, performed on-drug, elicited a greater deficit in freezing than when subjects were tested off-drug. While an immediate memory test for a study of this design is clearly confounded with the motor stimulant properties of amphetamine, it is worthy to note that animals administered with a high dose of amphetamine during both testing and training showed no increase in freezing over those that had received drug only during testing in experiment 2. Indeed, subjects given amphetamine during both training and testing exhibited almost no evidence of learning, suggesting that there was no state-dependent learning. Rather, amphetamine appears to have disrupted performance of the freezing response when given during testing and not training and disrupted the acquisition of fear conditioning when given during training and not testing. Interpretation of this data, thus, is somewhat problematic since high-dose amphetamine may have had two separate actions: disrupting memory formation and/or disrupting freezing by producing hyperactivity. Overall, however, amphetamine and other stimulants have not been shown to produce a high level of state-dependent learning compared with other addictive drugs (Overton 1972).

Studies on the effects of amphetamine on long-term potentiation (LTP), an experience-dependent increase in synaptic efficacy thought to be associated with learning, have produced conflicting results. Broadly, some research on methamphetamine users has shown a decreased density of dopamine transporter in the striatum (Lundqvist 2005). As activation of DA receptors can modulate the expression of glutamate receptors, DA receptor activity can, in turn, alter synaptic plasticity (Sun et al. 2005). LTP in the dentate gyrus was also found to be enhanced by amphetamine in a dose-dependent manner (Gold et al. 1984). Increased potentiation of population spike amplitude was found in rats that had been administered with 0.01, 0.1, 1.0, or 3.0 mg/kg amphetamine, i.p., while doses higher (10.0 mg/kg) and lower (0.001 mg/kg) produced no differences compared to controls. In other research, however, acute amphetamine administration (5.0 mg/kg, i.p.) did not alter perforant path LTP of EPSP slope but caused a small reduction in LTP of the population spike amplitude (Morimoto et al. 1987). The authors interpreted these results as showing amphetamine reduces cellular excitability. LTP was blocked in nucleus accumbens neurons when slices were bathed in 2.5 μM amphetamine solution (Li and Kauer 2004). Interestingly, this effect was attenuated when rats were administered with amphetamine (2.5 mg/kg, i.p.) in vivo for 6 days, and nucleus accumbens slices were prepared 8–10 days after the last injection. Additionally, a higher AMPA-receptor/NMDA-receptor ratio at the glutamatergic synapses in the ventral tegmental area (VTA) was associated with higher behavioral sensitization to a single dose of amphetamine in young rats (Faleiro et al. 2004). These results indicate that LTP occurred rapidly in the VTA and with a single amphetamine exposure. Overall, studies of amphetamine and synaptic plasticity have yielded mixed results but demonstrate that low doses of amphetamine appear to enhance cell excitability and synaptic plasticity.

In general, research in humans and rodents on memory and synaptic plasticity is in agreement with the current study for acute use of amphetamine, suggesting that very

low doses are beneficial to cognition while moderate or high doses are detrimental. We have previously found similar results with cocaine (Wood et al. 2007). The implications of this research are broad, ranging from addicts' use to students using stimulants for academic doping and those prescribed stimulants for learning disabilities. An enhanced understanding of when these drugs produce improvements in cognition, as opposed to deficits and addiction, is needed. The present study suggests that dosing is a crucial determinant of stimulant effects on cognition. At very low doses, cognition is enhanced, whereas high doses promote addiction and disrupt cognition. This hypothesis seems more parsimonious, given the sum of the data, than other theories that posit that stimulants act differently in children versus adults or in subjects with or without ADHD (Marx 1999).

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