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Enduring Effects of Tacrine on Cocaine-Reinforced Behavior: Analysis by Conditioned-Place Preference, Temporal Separation from Drug Reward, and Reinstatement

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Abstract

Previous work by our laboratory has shown that tacrine can produce long-lasting reductions in cocaine-reinforced behavior, when administered to rats as daily intravenous infusions over four days. Tacrine causes dose-related liver toxicity in different species, and its manufacture for human use was recently discontinued. This study was conducted to further characterize its actions on cocaine reward. Cocaine-experienced animals that had no contact with drug over one week resumed self-administration at levels similar to their initial baseline. When tacrine was administered over four days which were preceded and followed by washout periods to allow elimination of cocaine and tacrine respectively, subsequent cocaine self-administration was attenuated by more than one-half. Tacrine administered at 10 mg/kg-day as a chronic infusion by osmotic pump did not modify cocaine-induced increases in locomotor activity or conditioned-place preference. In rats that exhibited persistent attenuation of cocaine-self-administration after receiving tacrine, cocaine-induced reinstatement was also attenuated. No changes in plasma measures of renal or hepatic function were observed in rats receiving tacrine. In conclusion, pretreatment with tacrine can decrease cocaine-motivated behavior measured by self-administration or reinstatement, but not conditioned-place preference. Reductions in cocaine self-administration following pretreatment with tacrine do not require direct interaction with cocaine and are not secondary to either liver or kidney toxicity.

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Ethical Standards and Conflict of Interest

Experimental procedures were in compliance with all applicable regulations of the United States and the Department of Veterans Affairs. All authors declare that they have no conflict of interest pertaining to this manuscript.

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Graphical Abstract

Keywords

acetylcholine; acetylcholinesterase inhibitors; cocaine; liver toxicity; self-administration; reinforcement (Psychology); reward; and tacrine

1. Introduction

Behaviors motivated by psychostimulant reinforcement are known for their persistence over time (Weiss et al., 2001; Grimm et al., 2002). Within the first three months of initiating treatment for cocaine dependence, most patients will relapse to drug abuse (Schmitz et al., 2008). This persistence is paralleled by observations from animal models. Rats typically either continue a stable pattern of drug intake, or increase drug taking during chronic self-administration of either cocaine (Mantsch et al., 2001) or amphetamine (Gipson and Bardo, 2009). In the absence of drug treatments that interfere with stimulant reinforcement, animals in our laboratory typically maintain a consistent pattern of daily cocaine self-administration. Given the robust persistence psychostimulant-motivated behavior over time, alternative treatment approaches are needed.

Poor outcomes in substance abuse and other mental health disorders often stem from lack of compliance with medications prescribed as treatment. Such individuals often lack insight for the negative consequences of addictive behaviors (Moeller et al., 2010), and do not see the need to take prescribed medications. Recent clinical trials indicate that approximately one-third of medication doses being used to treatment psychostimulant abuse are not taken (Elkashef et al., 2012). This rate is almost certainly much higher in real-world patients who are not enrolled in a clinical trial, and are not receiving frequent coaching and monitoring. Despite many drug trials, no medications are either currently approved by the Food and Drug Administration or generally accepted as a means of preventing relapse in patients who abuse cocaine or other psychostimulants.

Although cholinesterase inhibitors have not been successful in modifying cocaine abuse in humans, only limited dose levels have been administered in laboratory (Grasing et al., 2010) or outpatient based studies (Winhusen et al., 2005). Administration of cholinesterase inhibitors in rats can produce two different effects on cocaine-reinforced behavior. Acute bolus dosing causes reductions in self-administration that resolve by the following day

(Grasing et al., 2008). In contrast, after delivery as a series of 21-hour infusions, cocaine self-administration is decreased in some animals over a period of weeks following tacrine treatment (Grasing et al., 2009). Doses of tacrine that produced reductions in drug self-administration at 1 to 3 days later, were more than three-fold more potent in modifying cocaine relative to food reinforcement. The long-lasting nature of tacrine-induced decreases in drug reinforcement, described as persistent attenuation, could offer a useful approach to the treatment of substance abuse disorders. If persistent attenuation could be produced in humans, continuous treatment with anti-addictive medications may not be needed, decreasing the importance of self-motivated compliant behavior.

Stimulants such as cocaine are known to produce both reinforcing and aversive effects. In addition to drug-induced euphoria, humans receiving intravenous cocaine in a laboratory setting also report 'bad' drug effects, dysphoria, and other negative somatic symptoms (Grasing et al., 2013). Taste aversions in response to flavors paired with addictive or aversive compounds are among the most enduring forms of learned behaviors (Bermudez-Rattoni et al., 2004). If tacrine and other cholinesterase inhibitors were to enhance cocaine's aversive properties, this could explain its long-lasting negative actions on drug reward.

Although actual liver injury is rare, clinically significant increases in plasma liver enzymes occurred in approximately one-fourth of Alzheimer's-disease patients treated with tacrine (Madden et al., 1995). Based on this, monitoring of liver function has been recommended beginning at four weeks following initiation of tacrine treatment in humans. Because of the potential for liver toxicity, use of tacrine steadily declined and its manufacture was recently discontinued. It is possible that hepatic inflammation could have aversive properties which may contribute to behavioral actions of tacrine.

The present studies were conducted to better characterize tacrine's persistent effects on cocaine reward. If its long-term actions were caused by a direct interaction with cocaine, these should be prevented by administering cocaine and tacrine at different times. A related hypothesis is that intravenous cocaine becomes aversive in some animals receiving tacrine treatment. If so, combined administration of both drugs should exhibit aversive effects that can be detected using conditioned-place preference. After extinction, cocaine and other drugs of abuse can cause pronounced increases in non-reinforced responding, which serves as an animal model of human drug craving. Tacrine treatment may be effective by modifying these delayed actions of cocaine. Given its history of hepatic toxicity, it is possible that a subset of rats that exhibit tacrine-induced persistent attenuation have associated changes in liver function that underlie this effect. To differentiate these mechanisms, we determined effects of tacrine on cocaine-induced conditioned-place preference, reinstatement, and measured hepatic and renal function in rats that received tacrine and exhibited persistent attenuation.

2. Materials and Methods

2.1. Animals

For all studies, male Wistar rats (Charles River Laboratories, Raleigh, NC) were transferred to our Facility at 9 weeks of age. Animals were evaluated according to standards outlined in

the NIH Guide for Care and Use of Laboratory Animals (NIH publication no. 86-23, 1996), with procedures approved by the local Animal Care and Use Committee. To facilitate operant responding, rats were maintained under a reversed light-dark cycle (14 hours of darkness beginning at 9:00 AM, and 10 lighted hours). Starting at 10 weeks of age, animals were single-housed with drinking water available ad libitum and restriction of food as outlined below.

2.2. Experiment 1, Temporal Separation of Tacrine Treatment from Drug Reward

This experiment was designed to examine effects of tacrine that was separated by significant delays from prior and subsequent cocaine self-administration. As soon as rats exhibited a stable pattern of self-administration under fixed-ratio-5 (FR-5) with a 20-second time out (see below), sessions were discontinued over 24 hours and rats were left undisturbed in home cages, attached to a fluid swivel and steel-coil tether. This initial washout interval was assessed as more than adequate to allow clearance of plasma cocaine, which has a half-life of less than 20 minutes in rats (Carmona et al., 2005). Beginning on the following day, 10 mg/kg-day of tacrine or vehicle (saline) was administered as a chronic infusion over 4 days, delivered intravenously at 4.0 ml per day. After completion of these infusions, rats were then left undisturbed in home cages for an additional two days. This second washout period permitted complete clearance of tacrine, which has a half-life of less than two hours in rat brain (Telting-Diaz and Lunte, 1993). Cocaine self-administration was then re-initiated under FR-5 with a 20-second time-out period. To determine persistent effects of tacrine, the pattern of self-administration was monitored over six additional sessions. Afterwards, catheters were tested for patency in rats meeting criterion for persistent attenuation. This was accomplished by observing for rapid ataxia or loss of consciousness following intravenous injection of one or two 5.0 mg doses of pentobarbital.

2.2.1 Self-Administration Apparatus—The test boxes and computer interface utilized have previously been described in detail (Grasing et al., 2009). Food reinforcement (45 mg pellets) was provided by a pellet dispenser, with intravenous drug injections supplied by a pneumatic syringe that delivered a volume of 30 μ L within one second. Pneumatic syringes were connected to animals by a liquid swivel and metal-spring tether.

Presentation of food or drug reinforcers was accompanied by a two-second tone and continuous illumination of a cue light. After delivery of either reinforcer, responding had no consequence during a time-out period in which the cue light was continuously illuminated. A 5-second time-out period was used initially, with this interval gradually extended to 20 seconds as animals completed training, prior to tacrine treatment. After completion of the time-out period, the cue light was flashed for 0.75 seconds every 3 seconds, indicating contingent reinforcement was again available. Test boxes were equipped with two identical levers, with only responding on the left-hand (active) lever counted towards delivery of reinforcers. Responses on a right-hand (inactive) lever had no effect on reinforcement.

2.2.2 Initial Food Reinforcement—To facilitate acquisition of cocaine self-administration, rats were initially trained to respond for food reinforcement, with food intake limited during these studies. Rats were deprived of food over 48 hours prior to their initial

food self-administration session. Afterwards, daily allowances of standard rat chow were set at 14.8 gm, which was continued until the completion of experiments. Initially, 45 mg food pellets were available under a fixed-ratio-1 (FR-1) schedule for 20 minutes, in which each lever press led to delivery of one pellet. Daily sessions were continued until 50 pellets were obtained under FR-1 during one session. Two additional 20-minute sessions were conducted in which food pellets were made available under a progressive-ratio schedule, to expose animals to an increased response requirement. A PR 9-4 progressive ratio schedule was utilized in these sessions with a time-out of five seconds (Li et al., 2003), which incremented response requirement in the following pattern: 1, 1, 1, 2, 2, 2, 3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 9, 9, 10, 11, 11, 12, 13,

2.2.3 Surgery for Catheter Placement—Rats were anesthetized with 50 mg/kg of intraperitoneal pentobarbital, and the internal jugular vein dissected free of surrounding connective tissue. After a 25-gauge needle was used to cut a small hole in the vein, and a silastic catheter (model S25, IITC, Inc., Woodland Hills, CA) was then inserted. It was fastened with silk suture and cyanoacrylic cement, and routed subcutaneously to a skull cap made of acrylic cement. At least three days of recovery was allowed prior to any behavioral studies.

2.2.4 Initial Acquisition of Drug Self-Administration—First, 0.32 mg/kg per injection of cocaine was made available under FR-1 during 3-hour sessions, with contingent reinforcement limited to 75 injections per session. After at least 20 cocaine injections were self-administered during three consecutive daily sessions under FR-1, rats were exposed to a single session in which 0.32 mg/kg per injection of cocaine was available under FR-3 (injections were provided after 3 lever presses). Afterwards, two additional sessions were conducted in which 0.32 mg/kg per injection of cocaine was available under FR-5.

2.2.5 Cocaine Dose-Effect Functions—Either 0.1, 0.2, or 0.4 mg/kg per injection of cocaine, was available during separate 40-minute components, with different doses presented in an ascending order (Grasing et al., 2008). These doses are referred to as low, intermediate, and high in a comparative sense. Each component was initiated by a single priming injection of cocaine at the dose that would be available for that component. Components were separated by two-minute time-out periods for which cue-lights were not illuminated and responding had no consequence. Post reinforcement time-out intervals were increased to 10 seconds following the second multiple-component session, and 20 seconds following the fourth multiple-component session. Self-administration was considered to be stable as soon as variance for the component with the highest response rate was less than 20% across three consecutive sessions.

2.2.6 Cholinergic Stimulation—As previously described (Grasing et al., 2009), a blinded observer scored behavior over 20 minutes on the first and last day of tacrine or vehicle infusions. Fine tremor (fasciculation) was scored at one-minute intervals as: 0-None, 1-Mild (intermittent, involving some digits), 2-Moderate (involving limbs and digits), and 3-Severe (involving the entire body). Numbers of events were counted for vertical jaw

deflections ('vacuous' movements not associated with chewing or yawning), yawning, and diarrhea.

2.3. Experiment 2, Extinction and Reinstatement in Rats Exhibiting Persistent Attenuation

A stable pattern of cocaine self-administration was established as outlined under Experiment 1. Animals then received treatment with vehicle or 10 mg/kg day of tacrine on four consecutive days as previously described (Grasing et al., 2009). Afterwards, cocaine self-administration sessions were continued for at least seven additional days to evaluate drug intake patterns following cholinesterase inhibition. Persistent attenuation was defined as a decrease of more than 80% in the number of self-administered cocaine injections, relative to an animal's pretreatment baseline, over at least three consecutive daily self-administration sessions. After their final self-administration session, rats were run during daily two-hour extinction sessions in which lever pressing had no consequences until active lever pressing declined to less than 5% of baseline cocaine-reinforced responding. During extinction, cue-lights were not illuminated and the tone used to signal completed ratios was also disabled. For reinstatement sessions, rats received intraperitoneal injections of 0.0, 3.2, or 10 mg/kg of cocaine hydrochloride. Active and inactive lever responding was then recorded over two hours. Cue-lights and tones continued to be disabled during reinstatement sessions. Individual rats received all three doses of cocaine in an ascending order during different reinstatement sessions, with these sessions separated by a single extinction session. Procedures for extinction and reinstatement were carried out in a total of 14 rats, 4 of which were evaluated under Experiment 1.

2.4 Experiment 3, Conditioned-Place Preference and Locomotor Activity

Conditioned-place preference testing was conducted using an unbiased design. Polycarbonate shuttle boxes for place preference conditioning were 60 cm long, 30 cm wide, and 30 cm tall. All sessions were conducted under conditions of dim illumination (1.0 lux) in the presence of white noise. Infrared beams were passed through eight holes along the sides of shuttle boxes, allowing computer monitoring of activity and location using Med Associates, Inc. software and instrumentation (ENV-253SD, ENV-258-8, and ENV-256C). To condition animals, a partition was inserted to divide shuttle boxes into two equal-sized compartments. For all sessions, one compartment was white with a white textured plexiglass floor. In contrast, the opposite side was colored black, and had a smooth black polycarbonate floor. Test sessions were conducted by giving rats free access to both sides of a shuttle box over 20 minutes. For test sessions, a 1.0 cm tall partition was inserted along the border between white and black compartments, which reduced the time spent at this location. Under baseline conditions, rats exhibited a slight preference for white compartments, spending an average of 75.4 seconds more time on that side over a 20-minute test session.

Firstly, two test sessions were conducted to determine time spent in white and black sides of shuttle boxes under baseline conditions prior to drug treatments. Afterwards, osmotic pumps (Alzet model 2ML1) were implanted, which delivered either vehicle or 10 mg/kg-day of tacrine as a 10 μ L per hour infusion. Beginning at Day 4 following implantation of osmotic pumps, twice daily conditioning sessions were then conducted over three days. Immediately

prior to conditioning sessions, rats were injected with saline or 7.5 mg/kg of cocaine hydrochloride, and confined to either the white or black compartment for 30 minutes. Cocaine injections were paired with non-preferred sides of shuttle boxes. After an additional injection with the opposing treatment (i.e. saline for rats that received cocaine in the morning), rats were confined to the opposite side of the shuttle box. Treatment compartment and presentation order were counterbalanced within the different treatment groups. During test sessions, time spent in cocaine- and saline- paired compartments were quantified by infrared beam interruptions. For conditioning sessions, locomotor activity was recorded by counting interruptions of infra-red beams. Linear movements were estimated by counting consecutive beam breaks for detectors at different locations, with repeated activity estimated by consecutive activation of a detector at the same location. One day after the final conditioning session, a single test session was conducted to determine cocaine preference.

2.5 Experiment 4, Tacrine Effects on Plasma Measures of Renal and Hepatic Function

Immediately after their final behavioral session, rats were deeply anesthetized with 100 mg/kg of pentobarbital delivered by intraperitoneal injection. They were then decapitated to allow collection of trunk blood. Samples were then centrifuged for collection of plasma and analyzed for measures of renal and hepatic function. Plasma measures were obtained from all 18 rats that underwent conditioned-place preference, and 3 additional animals that met criteria for persistent attenuation following cocaine self-administration.

2.6 Statistical Analysis

Analyses were performed using Systat software (version 13) with data presented as group means and standard error. Most comparisons were performed by either a t-test or analysis of variance (ANOVA), using drug treatment as a factor. Post hoc contrasts were made by t-tests after applying the correction by Sidak (Sankoh et al., 1997). Planned experiments made comparisons across three broad areas that included conditioned-place preference, drug self-administration, and non-reinforced responding during reinstatement. Estimating a 40% correlation between these interrelated areas, and applying a Sidak correction, yields a type I error (α) of 0.026.

Significant intersubject variability is typically observed during self-administration studies, with individual rats choosing to self-administer cocaine at relatively low or high levels. To allow efficient comparisons between animals that differed widely in their self-administration of cocaine, effects of tacrine were measured as change from pretreatment baseline, expressed as a percentage. For the three components in which low, intermediate, or high doses of cocaine were available; the number of self-administered cocaine injections was averaged across the three days prior to tacrine treatment. Subsequent values were then expressed as change from pretreatment baseline for a given component. To allow correlation with cholinergic signs and change in body weight, a single value for each animal was obtained by averaging change in self-administration across all three components over three days. These values were then correlated with change in body weight and signs of cholinergic stimulation using simple linear regression. Presentation in this manner also facilitated comparison with earlier reports on tacrine (Grasing et al., 2008; Grasing et al., 2009) and other cholinesterase inhibitors (Grasing et al., 2011).

Cocaine-induced conditioned-place preference score was calculated as the difference between times spent in the drug-paired compartment after conditioning (during test sessions) and before conditioning (prior to cocaine injections). For locomotor activity and place-preference testing, comparisons were made by repeated measures ANOVA and unpaired t-test, respectively. Discrete scores for cholinergic stimulation were compared by the Mann-Whitney U test.

3. Results

3.1 Experiment 1, Temporal Separation from Drug Reward

Results for self-administration of different cocaine doses are graphed in Figure 1. Baseline responding was closely matched in the two treatment groups. After a one-week delay in which included administration of saline or tacrine preceded and followed by washout periods, vehicle-treated rats resumed similar levels of drug self-administration for each dose of cocaine. Infusion of tacrine over a four-day period when animals were not self-administering cocaine attenuated subsequent drug-reinforced behavior. For low and intermediate doses of cocaine, repeated measures ANOVA showed significant effects of tacrine treatment [$F(1,13) = 40.6$ and 18.2 ; $p < 0.01$ for both values], time [$F(8,104) = 9.42$ and 2.45 ; $p < 0.05$ for both values], and the interaction of time by treatment [$F(8,104) = 7.46$ and 4.71 ; $p < 0.001$ for either value]. A similar analysis of high-dose cocaine failed to show significance for cocaine self-administration expressed as change from baseline. Criteria for persistent attenuation was met by three of eight rats receiving tacrine, and none of the vehicle-treated animals.

Pretreatment with tacrine also modified absolute levels of cocaine self-administration during reacquisition, graphed in the upper right of Figure 1. For all three cocaine doses, repeated measures ANOVA showed a significant interaction of phase of study (pre- or post-treatment) and treatment with tacrine [$F(1,13) = 9.85$, 7.15 , and 9.07 ; $p < 0.05$ for all values]. Post hoc comparisons indicated that each dose of cocaine was self-administered at a lower level following treatment with tacrine.

Most rats exhibited low levels inactive lever responding during both baseline and post-treatment phases of study, with less than 10 lever presses per 40 minute component. Three rats exceeded this value during a minority of their baseline sessions (two that later received saline, and one randomized to tacrine). Only one animal exhibited more than 10 lever presses per 40 minute component during two sessions following tacrine treatment. No statistically significant effects on tacrine treatment on inactive lever responding were observed.

Body weight declined approximately one-half percent over four days of treatment with intravenous tacrine (Figure 2). Vehicle-treated rats tended to maintain a constant body weight over the same interval. The trend for decreased weight following administration of tacrine did not reach statistical significance [$t(16) = 1.70$, p not significant]. Mean and standard error values for absolute pretreatment body weight were 316 ± 25.0 and 292 ± 24.0 for vehicle- and tacrine- treated rats, respectively. Neither pre- nor post- treatment absolute body weight values differed with tacrine administration.

As shown in Figure 2, values for cholinergic stimulation after treatment with tacrine closely match those reported in our previous study of rats receiving this dose and pattern of intravenous administration (Grasing et al., 2009). Tacrine treatment increased total cholinergic score [$t(15) = 6.02, p < 0.001$], but no statistically significant changes in the frequency of individual cholinergic signs were observed.

None of the various individual cholinergic signs were correlated with declines in cocaine-reinforced behavior after tacrine treatment. In addition, change in self-administration was not correlated with either the total cholinergic score or change in body weight during tacrine treatment.

3.2 Experiment 2, Conditioned-Place Preference, Locomotor Activity, and Liver Function

Figure 3 shows effects of subcutaneous saline and tacrine on cocaine-induced preference and locomotor activity. As previously reported, on average, both active and sham-treated rats spent longer periods on the cocaine-paired sides of shuttle boxes. Review of preference scores for individual animals showed that three of nine tacrine-treated rats exhibited negative values, indicating avoidance of the cocaine-paired side, versus one of nine saline-treated animals. Nonetheless, group means for cocaine-induced conditioned-place preference in animals treated with tacrine did not differ from those receiving vehicle [$t(16) = 0.91, p$ not significant]. When collapsed across either osmotic pump treatment, acute cocaine increased both linear and repeated locomotor activity [$F(1,48) = 77.4$ and $12.9, p = 0.001$ for either value]. Delivery of tacrine by osmotic pump did not alter either linear- or repeated-cocaine-induced locomotor activity.

3.3 Experiment 3, Extinction and Reinstatement

Lever-pressing during extinction and cocaine-induced reinstatement for rats exhibiting persistent attenuation is shown in Figure 4. No significant effects of cholinesterase inhibitor treatment, persistent attenuation status, or cocaine dose on extinction responding were observed. During reinstatement, active lever responding varied significantly with persistent attenuation status [$F(1,33) = 12.2; p < 0.01$]; cocaine dose [$F(2,33) = 11.2; p < 0.001$], and the interaction of persistent attenuation and dose [$F(2,33) = 6.68; p < 0.01$]. There was no significant main effect or interaction with tacrine treatment on active lever responding during reinstatement. After pretreatment with 10 mg/kg of cocaine, rats exhibiting persistent attenuation had active lever responding that was more than five-fold lower than control animals.

A single animal that did not exhibit persistent attenuation made 20 inactive lever responses after receiving 10 mg/kg of cocaine. Otherwise, all other animals emitted two or fewer inactive lever presses over 120-minute reinstatement periods, with no effects of cocaine dose or persistent attenuation. Response latency did not vary significantly for rats with and without persistent attenuation.

3.4 Experiment 4, Plasma Measures of Renal and Hepatic Function

In one saline treated rat, plasma direct (conjugated) bilirubin was measured at 0.3 milligram/deciliter (mg/dL), with gamma-glutamyl transpeptidase (GGTP) present at 3.0 units/Liter

(U/L). Neither of these values reflect clinically significant elevations, and direct bilirubin and GGTP were not detectable in plasma from any other animal. Additional plasma measures are shown in Table 1. Group means for blood urea nitrogen were slightly elevated in all animals, independent of tacrine treatment. This elevation may have been a consequence of sample handling. Alkaline phosphatase varied significantly with treatment group [$F(2,18) = 3.60$; $p < 0.05$]. Post hoc comparisons indicated that rats self-administering cocaine had significantly lower alkaline phosphatase levels, relative to tacrine- but not saline- treated rats evaluated by conditioned-place preference. This difference is not clinically significant, as all individual values were within the normal range for alkaline phosphatase, and may also reflect specimen handling.

Otherwise, all measures were within their expected normal range. There were no significant effects of tacrine treatment on plasma measures of renal or hepatic function, for nine rats treated with tacrine by osmotic pump prior to conditioned-place preference, or an additional three animals that exhibited persistent attenuation after self-administration of intravenous cocaine.

4. Discussion

In summary, the present study replicates previous findings that pretreatment with tacrine can produce long-lasting reductions in cocaine-reinforced behavior. The current study extends this finding in several ways. We show that attenuation of drug-reinforced behavior does not require that tacrine and cocaine be administered coincidentally. Non-reinforced responding is greatly diminished in rats that meet criterion for persistent attenuation. Importantly, tacrine treatment that caused persistent reductions in cocaine self-administration in rats did not cause either hepatic or renal toxicity. Neither cocaine-induced locomotor activity nor conditioned-place preference were altered by tacrine pretreatment.

Intravenous drug self-administration is viewed as one of the most valid experimental models to evaluate the reinforcing properties of abused substances. Using this technique, drugs abused by humans engender consistent responding when contingent intravenous injections are made available in rodents and non-human primates (Collins et al., 1984). One limitation of the self-administration model is its inability to directly detect aversive properties of self-administered compounds. In previous studies, a subset of animals treated with cholinesterase inhibitors delivered by chronic infusion subsequently responded at very low levels for contingent injections of cocaine (Grasing et al., 2009; Grasing et al., 2011). This is in spite of animals receiving priming injections of cocaine at the start of each component. It is unclear whether this is due to either reductions in cocaine reinforcement or aversive properties of cocaine combined with cholinesterase inhibitors. The latter possibility would explain the long-lasting nature of persistent attenuation, with responding decreased for one or more weeks.

If it modifies drug reward by enhancing aversive properties, one would expect that temporal separation of tacrine and cocaine treatments would prevent this effect. Contrary to this expectation, separation of tacrine treatment by washout periods in the present study produced persistent attenuation at a similar frequency to that observed when rats were

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treated immediately before and after self-administration sessions (Grasing et al., 2009). Persistent reductions in self-administration were clearly evident in group means of tacrine-treated rats that self-administered low- or intermediate-doses of cocaine, and appeared comparable in magnitude to reductions when cholinesterase inhibitors were administered immediately prior to self-administration sessions. For high-dose cocaine, reductions caused by tacrine were less pronounced and resolved after two days of drug self-administration. Even so, the findings indicate that its anti-addictive properties do not require concomitant treatment with cocaine and tacrine. We speculate that tacrine treatment modifies brain neurochemistry to be less receptive to cocaine reward over a period of days to weeks after tacrine treatment.

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To separate outcomes of diminished reinforcement and aversive effects, we evaluated actions of tacrine through cocaine-induced conditioned-place preference. Tacrine pretreatment did not significantly modify cocaine preference. Therefore, it appears that tacrine-induced reductions in drug reward do not occur because of an increase in the aversive properties of cocaine. Because it does not require implantation of an intravenous catheter, conditioned-place preference is more easily performed than drug self-administration. Based on this, conditioned-place preference may allow actions of tacrine to be more easily studied. Nonetheless, our findings indicate that further studies of tacrine's persistent actions on cocaine reward should rely on intravenous drug self-administration.

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Results of the present report differ from previous studies in which cholinesterase inhibitors other than tacrine attenuated cocaine-induced conditioned-place preference (Hikida et al., 2003; Takamatsu et al., 2006). Besides cholinesterase inhibition, tacrine has effects on neurotransmitters other than acetylcholine (Wagstaff and McTavish, 1994). Tacrine can inhibit MAO-A and to a lesser extent MAO-B (Adem et al., 1989). Uptake of dopamine, norepinephrine, and serotonin are also inhibited by micromolar concentrations of tacrine (Drukarch et al., 1988; Jossan et al., 1992). These effects appear to increase brain levels of monoamines and contribute to therapeutic actions of tacrine. In Alzheimer's disease patients receiving tacrine chronically, increases in cerebrospinal fluid concentrations of homovanillic acid and 5-hydroxyindoleacetic acid (HVA and 5-HIAA, metabolites of dopamine and serotonin, respectively) have been reported, with 5-HIAA levels correlated with improvements in cognition and daily living activities (Alhainen et al., 1993). These alternate mechanisms may explain why cocaine-induced conditioned-place preference is modified by other cholinesterase inhibitors but not tacrine.

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As we have previously reported (Grasing et al., 2009; Grasing et al., 2011), cholinesterase inhibition causing persistent reductions in cocaine self-administration did not modify body weight in the present study. Signs of cholinergic stimulation were again not correlated with later reductions in cocaine-reinforced behavior. The implication of this finding is that effects on drug reward are not simply due to a nonspecific disruption of behavior by excessive cholinergic activity or other toxic effects. Consistent with this, single bolus doses of tacrine produce dose-related reductions in cocaine self-administration and much higher levels of cholinergic stimulation than observed after treatment as a chronic infusion, but do not cause persistent attenuation (Grasing et al., 2008).

Even in the absence of contingent drug injections, exposure to appropriate cues, stress, or noncontingent cocaine can cause high levels of non-reinforced responding. Reinstatement may reflect a valid model of relapse in humans, and may parallel the role of specific places, stressors, or drug use in the deterioration of cocaine addicts (Katz and Higgins, 2003). Of the different forms of reinstatement, psychostimulant-induced responding may be more resistant to treatment. For example, indirect serotonergic agonists can attenuate cue- but not cocaine-induced reinstatement (Burmeister et al., 2003). In rats exhibiting persistent attenuation, we found that cocaine-induced reinstatement was greatly attenuated, again pointing towards the potential value of cholinesterase inhibition in the treatment of substance abuse disorders.

Dose-related hepatotoxicity occurs in mice, rats, and humans that receive tacrine, and can also be shown using in vitro methods (Pan et al., 2011). Increases in plasma transaminases (alanine transaminase and aspartate transaminase) occur after rats receive single 40 mg oral doses of tacrine, and are accompanied by hepatocyte swelling without either steatosis or necrosis (Ma et al., 2003). These changes were not modified by pretreatment with the muscarinic blocker atropine. Based on this, and the absence of liver toxicity associated with various other inhibitors, tacrine-induced hepatotoxicity is believed to be mediated by immunologic mechanisms or mitochondrial injury that is unrelated to cholinesterase inhibition (Alfirevic et al., 2007). Because absorption of oral tacrine is about one-third of that following intravenous delivery (Goh et al., 2011), bioavailability of the dose chosen for our study is only slightly below that used by Ma et al. (2003). We found no evidence of hepatic toxicity, which may have been mitigated by administering of tacrine as a slow infusion rather than bolus dosing. In addition, parenteral dosing would be expected to decrease hepatic exposure by avoiding first-pass metabolism.

There are several important limitations of our findings. As cocaine pharmacokinetics was not determined, we cannot ensure that tacrine did not cause persistent changes in its metabolism or excretion. This seems unlikely, given that no other hepatic or renal parameters were modified by tacrine treatment. Cocaine doses evaluated in this study were chosen to be on the ascending and falling limbs of the cocaine dose-response relationship, and also matched previous evaluations of tacrine. It is acknowledged that cocaine reward can be observed at either higher or lower doses. While relatively few doses of cocaine were evaluated, these supported significant levels of cocaine self-administration and conditioned-place preference. Although only one tacrine dose was used, it was chosen based on previous studies that evaluated a larger dose range and showed its effectiveness in causing persistent attenuation (Grasing et al., 2009). In general, previous studies of tacrine have shown only limited selectivity for effects on cocaine-relative to food-reward, with two exceptions. Firstly, when self-administration of low-dose cocaine was compared with responding reinforced by small amounts of liquid food, single bolus doses of tacrine were more than seven-fold more potent in attenuating cocaine reward (Grasing et al., 2008). Late effects of tacrine that occur 1 to 3 days after its administration are a second exception. When late effects of tacrine were compared in food- and cocaine-reinforced rats, tacrine was approximately three-fold more potent in suppressing drug-reinforced responding (Grasing et al., 2009). Based on this earlier work, the present studies did not examine an alternative, non-drug reinforcer. Because reductions in cocaine-reinforced behavior occurred more than

two days after drug treatment, they would be considered to be late effects of tacrine, which have been associated with good selectivity for cocaine self-administration in the past.

In conclusion, pretreatment with tacrine can cause long-lasting decreases in drug taking and non-reinforced responding that are not explained by hepatic toxicity. Reductions in drug reward occur despite a 48-hour delay between tacrine and cocaine dosing. Cocaine addicts often lack insight for a personnel need for treatment, have poor compliance with medications, and are resistant to abstinence. Most anti-addictive medications require frequent dosing, such as use of methadone or buprenorphine in opiate dependence. In contrast, effects of tacrine do not require either repeated dosing or combined treatment with cocaine. These properties may simplify further study and potential clinical implementation of tacrine's anti-addictive properties.

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Figure 1.

Changes in cocaine self-administration following pretreatment with tacrine, over a period in which rats were not self-administering cocaine. Results for components in which low-, intermediate-, and high- doses of cocaine were available, are shown in panels A, B, and C, respectively. In these panels, vertical axes indicate self-administration of cocaine, expressed as percent of responding during the last three days of self-administration prior to treatment with vehicle or tacrine. The gray hatched areas show periods over which animals received chronic intravenous infusions of 10 mg/kg of tacrine or vehicle over four days. Aside from a break during infusion periods, horizontal axes are drawn to scale, showing the duration of both washout periods. Resumption of drug self-administration after tacrine or vehicle treatment is shown on right-hand sides of panels A, B, and C; beginning two days after infusion periods. Vehicle and tacrine were evaluated in 7 and 8 animals, respectively. Panels D and E show absolute levels of cocaine self-administration averaged over 3-day baseline periods and the initial two days after self-administration had resumed, respectively. * indicates significant differences for post-hoc comparisons to vehicle-treated rats, with one and two symbols corresponding to $p < 0.026$ and $p < 0.01$ respectively.

Figure 2. Body weight and cholinergic signs after tacrine treatment for the animals shown in Figure 1. Panel A shows group means for change in body weight over four-day intravenous infusions. The line graph for Panel B displays increases in total cholinergic score during treatment with saline or tacrine, with the frequency of individual signs plotted on the bar graphs below. * corresponds to $p < 0.001$, relative to vehicle-treated animals.

Figure 3.

Effects of chronic tacrine treatment on cocaine-induced conditioned-place preference and locomotor activity. Expression of place preference is shown in Panel A, with scores of individual saline- and tacrine-treated rats shown by open and closed symbols, respectively. Panels B and D show linear movements, with repeated movements graphed in panels C and E. Activity following injections of cocaine is shown in the upper panels (B and C), with values following injection of saline shown in the lower panels (D and E). Vehicle and tacrine were both evaluated in 9 animals per group.

Figure 4.

Active lever responding during reinstatement for rats exhibiting persistent attenuation. Cocaine dose is shown on horizontal axes, with response number during extinction and reinstatement graphed vertically on the left- and right-hand sides, respectively. In the legend, persistent attenuation is abbreviated as PA. Values for rats that did or did not exhibit persistent attenuation are shown with shaded and open symbols, respectively. Data is shown for 5 to 8 animals per condition. * corresponds to $p < 0.026$, relative to rats receiving the same dose of cocaine but not meeting criterion for persistent attenuation.

Table 1

Plasma Measures of Hepatic and Renal Function. Data shows group means and standard error for rats evaluated by conditioned-place preference (CPP) after pretreatment with either saline or tacrine by osmotic pump (nine animals in each group), with results shown for an additional three rats that exhibited persistent attenuation of cocaine self-administration (SFA).

Measure (units, normal range)	CPP		SFA
	Saline	Tacrine	Tacrine
Blood Urea Nitrogen (BUN, mg/dl, 12 – 22)	24.8 (1.6)	25.1 (1.1)	23.7 (2.7)
Creatinine (mg/dl, 0.38 – 0.8)	0.32 (0.03)	0.36 (0.03)	0.33 (0.07)
Total Bilirubin (mg/dl, 0.23 – 0.48)	0.39 (0.12)	0.28 (0.02)	0.30 (0.05)
Alanine transaminase (ALT, U/L, 17 – 50)	45.4 (3.6)	49.3 (4.6)	41.0 (1.7)
Alkaline phosphatase (ALP, U/L, 39 – 216)	137.0 (14.0)	122.9 (9.9)	71.7 (3.6)*

* indicates significantly different from rats evaluated by conditioned-place preference and treated with tacrine, $p < 0.026$.