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Long-lasting decreases in cocaine-reinforced behavior following treatment with the cholinesterase inhibitor tacrine in rats selectively bred for drug self-administration ☆

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ABSTRACT

Tacrine is a centrally acting, reversible cholinesterase inhibitor that increases synaptic levels of acetylcholine (ACh) and can potentiate the actions of dopamine (DA). The present study was conducted to evaluate effects of tacrine on cocaine-reinforced responding in *a* rat line selectively bred for high levels *of* drug self-administration (the HS line). HS rats self-administered different doses of cocaine under a fixed-ratio-5 (FR-5) schedule. Over a four-day period, vehicle or tacrine (1.0, 3.2, or 10 mg/kg-day) was infused when animals were maintained in home cages (21 h per day). Tacrine dose-dependently decreased cocaine self-administration. Actions of tacrine differed for self-administration that was initiated within 20 min of pretreatment (described as early sessions), and for self-administration that occurred between one and three days after administration of tacrine was discontinued (late sessions). Tacrine's potency for attenuating self-administration during late sessions was greater for cocaine- relative to food-reinforcement in HS rats, and for HS relative to outbred rats. In a subset of tacrine-treated HS rats, cocaine self-administration was persistently attenuated by more than 80% from pretreatment baseline levels over a one-week period during which no further tacrine was administered. In summary, pretreatment with tacrine can produce a long-lasting attenuation of cocaine-reinforced responding.
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1. Introduction

Reinforcing effects of cocaine are believed to arise from release of dopamine (DA) at terminal projections of midbrain dopaminergic neurons, including the nucleus accumbens, a brain region that plays a role in natural and drug reinforcement (Little et al., 1999; Di Chiara et al., 2004). In addition, acetylcholine (ACh) is involved in brain reward and learning functions, and contributes to psychostimulant- and opiate-motivated behaviors. Stimulation of nicotinic or muscarinic cholinergic inputs can enhance neurotransmitter release by mesolimbic DA neurons which project to the nucleus accumbens (Nisell et al., 1994; Westerink et al., 1996; Gronier and Rasmussen, 1998). Enhancement of cholinergic activity in the nucleus accumbens has been shown to attenuate either psychostimulant- or opiate-induced conditioned place preference (Hikida et al., 2003).

In mammalian brain, synaptic levels of ACh are regulated by two cholinesterases that inactivate ACh, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) (Giacobini, 2004; Ballard et al., 2005). Tacrine is a centrally acting, reversible inhibitor of AChE and BuChE (Wagstaff and McTavish, 1994). Because BuChE contributes to degradation of ACh (Ballard et al., 2005), inhibition of both AChE and BuChE may enhance effects on ACh relative to compounds that inhibit AChE alone. In addition to its effects on the cholinergic system, tacrine can potentiate the actions of DA, through blockade of reuptake (Jossan et al., 1992) and by inhibiting its metabolism by monoamine oxidase (Adem et al., 1989).

We have recently shown that pretreatment with tacrine can produce dose-related reductions in cocaine self-administration (Grasing et al., 2008). In this study, tacrine selectively attenuated self-administration of low-dose cocaine. Effects of tacrine on higher doses of cocaine were similar to its ability to attenuate self-administration of non-drug reinforcers. Moreover, effects of tacrine on drug- and food-reinforced behaviors did not persist beyond the day on which single bolus doses of tacrine were administered, which is consistent with its relatively short half-life (Telting-Diaz and Lunte, 1993).

Although family, twin, and adoption studies in humans indicate that genetic variation plays a significant role in the etiology of substance abuse disorders (Faraone et al., 2008), little is known about how genetic factors influence individual differences in the response to natural and drug reinforcers. Recently, a minor genetic variant for the gene that encodes the alpha-5 subunit of the nicotinic acetylcholine receptor has been shown to be associated with an increased risk for

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nicotine dependence and an opposite protective effect on cocaine dependence (Grucza et al., 2008). The bidirectional nature of this association may stem from excitatory and inhibitory modulation of nicotinic receptors on actions of DA. The findings support further study of genetic factors that influence cholinergic mechanisms underlying psychostimulant reward and dependence.

Selective breeding allows lines to be developed that can be less sensitive to environmental effects and may include multiple alleles that are causative of trait differences (Koch and Britton, 2005). Various rodent lines have been developed by differential selection for altered preference to oral self-administration of alcohol (Brush and Driscoll, 2002). In addition to strains selected for responses to alcohol, selective breeding has been utilized to develop rat lines that differ in locomotor activity after injections of cocaine (Marley et al., 1998), nicotine (Smolen et al., 1994), and methamphetamine (Palmer et al., 2005). Despite these advances, relatively little effort has been made to use selective breeding to characterize psychostimulant reinforcement.

The high self-administration (HS) rat line was developed by selectively breeding individual outbred Wistar rats that exhibited increased self-administration of morphine or cocaine (He et al., 2008). The majority of rats were chosen for selective breeding based on cocaine self-administration score. This score is a composite measure used to quantitatively rank animals according to increasing tendency for cocaine self-administration. It encompasses measures of how rapidly self-administration was initially acquired and levels of self-administration under different schedules and doses of cocaine (see He et al., 2008 for details). Beginning with the third selected generation, HS rats began exhibiting significant increases in cocaine self-administration score. The purpose of the present study was to evaluate effects of tacrine in HS rats being selectively bred for increased drug self-administration with self-administration by outbred Wistar rats, the founder population for the HS line. Given that selective breeding had been conducted to enhance drug self-administration, our hypothesis was that HS rats would be less sensitive to the ability of tacrine to attenuate cocaine-reinforced responding. Unexpectedly, we observed greater and more prolonged effects of tacrine in HS rats.

2. Materials and methods

2.1. Animals

As outlined in Table 1, two experiments were conducted using animals from the HS rat line, with one additional experiment evaluating outbred Wistar rats purchased from Charles River Laboratories (Raleigh, NC). Standards outlined in the NIH Guide for Care and Use of Laboratory Animals (NIH Publication No. 86–23, 1996) were followed and experimental procedures approved by the local Animal Care and Use Committee. To facilitate operant responding during experimental sessions, animals were maintained under a reversed light–dark cycle (14 h of darkness beginning at 9:00 AM, and 10 lighted h). Beginning at 10 weeks of age, rats that were evaluated for self-administration were moved to individual housing without restriction of drinking water and food restriction as outlined below.

Table 1
Different experiments that evaluated the effects of tacrine on cocaine and food pellet reinforced responding.

Experiment	Strain	Reinforcer	Breeding generation (range, mean, SE)	Number of animals evaluated (male, female)	Number of treatments per individual animal
1	HS	Cocaine	3 to 7, 4.4 ± 0.22	15, 15	1 to 4
2	HS	Food pellets	3 to 7, 3.4 ± 0.19	2, 5	1 to 5
3	Outbred	Cocaine	–	15, –	1 to 5

2.2. Apparatus

Experiments were performed under dim lighting within sound-attenuated chambers using a commercially available interface and software that have previously been described in detail (Grasing et al., 2008). Food-reinforcement was supplied by a pellet dispenser, and intravenous injections of cocaine were provided by a pneumatic syringe set to deliver a volume of 0.030 ml. Availability of reinforcers was signaled by flashing of a cue light, which was alternately illuminated for 0.75 s and turned off for 3.0 s. Drug self-administration chambers were equipped with two identical levers (model ENV-110 M, Med Associates, Inc.). Responding on the active (left) lever was counted towards completion of ratios leading to contingent drug injections, with responding on the inactive (right-hand) lever having no consequence. Drug injections were delivered over approximately 1 s, which was accompanied by continuous illumination of the cue light and activation of a tone generator. Two seconds after completing the ratio, the tone and cue light were turned off, with neither signal presented during a time-out period. Responses during either the injection or time-out had no consequence. Following the time-out period, the cue light was flashed, and lever presses were again counted towards completion of ratios. Fluid lines for drug injections were routed to animals through a liquid swivel and steel-spring tether attached to a skull cap made of acrylic cement.

2.3. Initial food self-administration and food restriction

Initially, animals were allowed to lever-press for 45 mg food pellets (Bio-Serve, Frenchtown, NJ) available under FR-1 during daily 20-minute sessions. Before the initial session, animals were maintained without food for 48 h. Because chronic food restriction enhances drug self-administration (De Vry et al., 1989), food intake was limited after the initial session. Male and female rats received 14.8 and 13.8 g, respectively, of standard rat chow daily, with this level of food restriction maintained for the duration of experiments for all rats evaluated in this study. As soon as at least 50 food pellets were delivered during a single session under FR-1, that type of session was terminated and rats were advanced to two additional 20-minute sessions in which food pellets were made available under the PR 9–4 progressive ratio schedule with a time-out of 5 s. Under this progressive ratio schedule, response requirement was advanced according to 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, …, which served to gradually introduce rats to an increased response requirement (Li et al., 2003).

2.4. Catheter placement

Rats were anesthetized with 50 mg/kg of intraperitoneal pentobarbital, and the internal jugular vein was then exposed and dissected free of surrounding connective tissue. A small incision was made in the vein, and a commercially available silastic catheter (model S25, IITC, Inc., Woodland Hills, CA) inserted and fastened in place by silk suture and cyanoacrylic cement. The catheter was then passed subcutaneously and exited through a skull cap made of dental acrylic.

2.5. Initial cocaine self-administration

Cocaine hydrochloride was donated by the National Institute of Drug Abuse (Bethesda, MD) and dissolved in 0.9% saline. After initial food-reinforcement training followed by surgery for catheter implantation and at least three days of recovery, rats were allowed to self-administer 0.32 mg/kg per injection of cocaine under FR-1 during daily 3-hour sessions with a 5-second time-out period. After obtaining at

least 20 cocaine injections over three consecutive sessions under FR-1, rats were advanced to a single 3-hour session in which 0.32 mg/kg per injection of cocaine was available under FR-3 with a 5-second time-out period. During time-out periods, lever presses had no consequence and the cue light was turned off. Two additional 3-hour sessions were conducted in which 0.32 mg/kg per injection of cocaine was available under FR-5 using a 5-second time-out period, with animals advanced to multiple-component sessions on the following day. To prevent an overdose of cocaine, the maximum number of cocaine injections was limited to 75 when 0.32 mg/kg per injection was available under FR-1, FR-3, or FR-5.

2.6. Experiments 1 and 3, cocaine self-administration during multiple-component sessions

Multiple-component sessions consisted of three, 40-minute components separated by 2-minute time-out periods, with no limit on the number of available injections. Delivery of cocaine reinforcement during multiple-component sessions was followed by a 5-second time-out period, which was increased to 10 and 20 s on the third and fifth multiple-component sessions, respectively. Treatment with vehicle or tacrine was initiated as soon as rats exhibited a stable pattern of self-administration (see below) following their sixth multiple-component session.

Because reductions in basal levels of DA (which could potentially be ameliorated by treatment with tacrine) have only been reported following self-administration of unit doses of cocaine that are on the intermediate (Gerrits et al., 2002) or descending (Mateo et al., 2005) portions of the dose–effect relationship for cocaine self-administration (Mello and Negus, 1996), we chose similar dose levels for self-administration in the present study.

Cocaine dose–effect functions during multiple-component sessions were accomplished by administering 0.1, 0.2, and 0.4 mg/kg per injection of cocaine (low, intermediate, and high doses, all of which were on either the intermediate or descending portions of the dose–effect relationship for cocaine self-administration) during the first, second, and third components, respectively (Grasing et al., 2008). This was achieved with a cocaine solution that delivered 0.1 mg/kg per injection, with one injection administered contingently during the first component, two injections during the second component, and four injections during the third component. For the second and third components, contingent injections were administered at 5-second intervals. During multiple-component sessions, different doses of cocaine were always presented in an ascending order, with the number of injections to be available presented noncontingently at the start of the component (e.g. the second component is initiated with two noncontingent injections of cocaine).

2.7. Experiment 2, evaluation of drug effects on food pellet reinforced responding

After initial training for food pellet reinforced responding followed by surgery for catheter implantation and at least three days of recovery, 45 mg food pellets were again made available under FR-1 during daily 2-hour sessions with a 5-second time-out period. After at least 50 food pellets were delivered during a single session, rats were advanced to an FR-5 schedule with a 20-second time-out period. Animals were advanced to an FR-10 schedule after completing three sessions under FR-5, or at least 100 food pellets were received during a single session under FR-5 (whichever came first). Finally, rats were advanced to an FR-15 schedule after completing three sessions under FR-10, or at least 100 food pellets were received during a single session under FR-10 (whichever came first). Treatment with tacrine or vehicle was initiated as soon as rats exhibited a stable food pellet reinforced responding (see below) after their third session under FR-15.

2.8. Scoring of behavior for signs of cholinergic stimulation

Rats were observed and scored over 20 min immediately prior to the start of self-administration sessions by an observer who was blinded to drug treatments. For fasciculation, a fine, involuntary tremor of limbs and other body parts, intensity was rated at 1-minute intervals according to an adaptation of the scoring system described by Ogura et al. (2001): 0—None, 1—Mild (intermittent, involves some digits), 2—Moderate (involves limbs and digits), 3—Severe (involves the entire body). Vacuous jaw movements were counted as outlined by Mayorga et al. (1997), with individual movements defined as vertical jaw deflections excluding yawning, directed chewing, or grooming. Yawning, vacuous jaw movements, and diarrhea were scored as the number of events observed.

2.9. Procedures

Rats were allowed to lever-press for either cocaine or food pellet reinforcement under baseline conditions until a stable pattern of responding was established, defined as a variance of 20% or less for the number of ratios completed over the preceding three sessions (standard deviation divided by the mean for these three values). As previously described, the stability of self-administration during multiple-component sessions was assessed using the component under which the maximum number of reinforcers was received (Caine et al., 2002). Immediately following the first session in which a stable pattern of self-administration was achieved, pretreatment with vehicle (0.9% saline) or tacrine was initiated.

Reductions in DA release in the nucleus accumbens have been shown to precede daily self-administration of cocaine or heroin in rats (Gerrits et al., 2002; Mateo et al., 2005). Given its potential to ameliorate this change through cholinergic mechanisms and direct effects on DA, tacrine was administered chronically during the period preceding cocaine self-administration sessions. This pattern of dosing is also consistent with its clinical use in Alzheimer's disease, in which tacrine is administered chronically in divided daily doses. Vehicle or tacrine was administered intravenously by infusion pump (Model A, Razel, Stamford, CT), at a rate of 167 μL/h, over 21 h/day, as rats were maintained in home cages with fluid swivels and tethers, through the same catheter used for cocaine self-administration. Tacrine hydrochloride was purchased from Sigma Chemical Company (St. Louis, MO). Pretreatment with vehicle or tacrine was infused following self-administration sessions over 21 h on four consecutive days. Beginning on the following day, self-administration was monitored over three or more additional daily sessions in the absence of pretreatments. Scoring for signs of cholinergic stimulation was performed after the initial and final (fourth) infusions of vehicle or tacrine.

In the event that an animal exhibited a reduction in responding that persisted on the third day after treatment with tacrine or vehicle, catheter patency was tested by observing rats after an intravenous injection of 5 mg of pentobarbital. If needed, a second injection of 5 mg of pentobarbital was made. Rats that failed to rapidly show signs of sleep or ataxia following either injection were considered to have non-patent catheters. Animals that maintained patent catheters and exhibited a stable pattern of self-administration after recovering from four days of treatment received additional pretreatments with vehicle or tacrine.

2.10. Data analysis

Statistical analyses were performed using Systat software (version 5, Evanston, IL), with results presented as group means ± standard error. For each individual animal, baseline responding was averaged over the three sessions prior to treatment with vehicle or tacrine, with a separate baseline calculation made for each dose of cocaine evaluated. Results of self-administration were expressed as a percentage of each individual

rat's rate of responding during its baseline sessions, prior to treatment with vehicle or tacrine. Cocaine and food pellet reinforced responding were evaluated using repeated measures analysis of variance (ANOVA), using dose, session number, and gender as factors. Session number included three days of baseline responding, four days of treatment with vehicle or tacrine, and either three or seven days of responding during a recovery period. To provide a measure of non-reinforced, nonspecific behavior, the number of responses made on the inactive lever was also evaluated (neither active- or inactive- lever responding was assessed during time-out periods). Post-hoc comparisons were made by Bonferroni *t*-tests. Based on comparisons with three doses of tacrine, $p<0.017$ (0.05 divided by 3) was used as a criteria for statistical significance.

As shown below, dose–response relationships were evaluated by plotting the percent attenuation of cocaine- or food-reinforced responding against the common logarithm of tacrine dose. A curve-fitting routine (SigmaPlot Version 8, Systat Software, Chicago, IL) was then used to determine the second-order regression curve that best described attenuation by tacrine under different conditions, using the following equation:

$$\text{Percent Attenuation} = a + b^{*}log(\text{dose}) + c^{*}log(\text{dose})^{2}$$

In the above equation, dose refers to the dose of tacrine; and constants a, b, and c were determined by the curve-fitting routine. Because less than 50% attenuation occurred under some conditions, calculations were based on doses of tacrine required to attenuate self-administration by 40%, described as the 40% effective dose, or ED40. ED40 values were calculated for two different time intervals. Firstly, changes in cocaine or food pellet reinforced responding were averaged over the final three days of pretreatment with tacrine, described as early effects because these sessions were conducted within 20 min of completing tacrine infusions. Secondly, ED40 values were calculated for effects on sessions conducted between one and three days after discontinuation of pretreatment, described as late effects of tacrine.

After pretreatment with vehicle or tacrine, individual animals in which responding was decreased by more than 80% from their pretreatment baseline levels of reinforcement, averaged over one to three days after discontinuation of pretreatment, were considered to have a persistent attenuation of cocaine or food pellet reinforced responding. The likelihood ratio chi-square test was used to compare numbers of rats that exhibited persistent attenuation, using $p<0.017$ as a criteria for statistical significance.

A single score for signs of cholinergic stimulation was calculated as the sum of all scored behaviors over a 20-minute period, prior to self-administration sessions. Because the discrete values of this score did not vary continuously, statistical comparisons were made by Kruskal–Wallis ANOVA, with post-hoc comparisons made by the Mann–Whitney test, again using $p<0.017$ as a criteria for statistical significance.

3. Results

3.1. Absolute rates of reinforcement

Table 2 shows absolute rates of baseline, pretreatment responding for Experiments 1 to 3. For self-administration of cocaine by HS and outbred rats, repeated measures ANOVA showed significant main effects of strain [$F(1,73)=5.13$, $p<0.05$] and dose [$F(2,146)=67.3$, $p<0.001$], but the interaction of these terms was not significant. Food pellet reinforced responding under FR-15 supported a similar hourly rate of reinforcement to self-administration of low-dose cocaine under FR-5.

Table 2
Absolute rates of pretreatment baseline responding for Experiments 1 to 3.

Experiment	Strain	Food pellets	Cocaine (mg/kg per injection)		
			0.1	0.2	0.4
1	HS	–	31.6 ± 1.9	28.3 ± 2.0	16.0 ± 1.3
2	HS	35.1 ± 2.2	–	–	–
3	Outbred	–	24.6 ± 2.1	23.4 ± 2.1	13.3 ± 1.2

Data shows the hourly rate at which different reinforcers were delivered.

3.2. Experiment 1: pretreatment with tacrine in cocaine-reinforced HS rats

Treatment of HS rats with tacrine produced a dose-related attenuation of cocaine-reinforced responding (Fig. 1). For all three cocaine doses, self-administration varied significantly with tacrine dose [$F(3,39)=12.0$, 11.8, and 10.1; $p<0.001$ for all values; for self-administration of low, intermediate, and high doses of cocaine, respectively], session number [$F(9,351)=11.3$, 12.8, and 7.72; $p<0.001$ for all values], and the interaction of tacrine dose and session number [$F(27,351)=5.74$, 6.45, and 4.11; $p<0.001$ for all values].

Post-hoc comparisons indicated that treatment of HS rats with either 3.2 or 10 mg/kg-day of tacrine attenuated self-administration during most sessions, for sessions conducted up to 3 days after tacrine treatment. This effect persisted for longer than expected, with significant decreases in cocaine self-administration occurring over the one to three days following discontinuation of tacrine.

For tacrine-treated HS rats, there were no significant main effect of gender on cocaine self-administration, and no significant interaction between gender and tacrine dose. Inactive lever responding did not vary significantly with tacrine dose, session number, or the interaction of tacrine dose and session number.

3.3. Experiment 2: pretreatment with tacrine in food pellet reinforced HS rats

To compare effects of tacrine on reinforcement provided by a natural non-drug reinforcer, 45 mg food pellets were made available under FR-15 (Fig. 2). The number of food pellets delivered varied significantly with tacrine dose [$F(3,19)=12.0$, $p<0.001$], session number [$F(9,171)=2.28$, $p<0.05$], and the interaction of dose and session number [$F(27,171)=3.28$, $p<0.001$].

Post-hoc comparisons indicated that self-administration of food pellets was attenuated after pretreatment with the initial two infusions of 3.2 mg/kg-day of tacrine. Food pellet reinforced responding was also decreased after all sessions that were immediately preceded by a 10 mg/kg-day infusion of tacrine, with this effect persisting during two additional sessions that occurred one and two days after discontinuation of treatment.

3.4. Experiment 3: pretreatment with tacrine in cocaine-reinforced outbred rats

To evaluate the effects of tacrine in animals not genetically selected for altered self-administration, an additional experiment was performed with male outbred Wistar rats (Fig. 3). Cocaine self-administration varied significantly with session number [$F(9,252)=3.85$, 6.73, and 5.89; $p<0.001$ for all values; for self-administration of low, intermediate, and high doses of cocaine, respectively]. For low- but not intermediate- or high-dose cocaine, there was a significant main effect of tacrine dose [$F(3,28)=4.18$, $p<0.05$]. The interaction of tacrine dose and session number was significant for low- and intermediate- dose cocaine [$F(27,252)=1.76$ and 1.66; $p<0.05$ for either value], but not high-dose cocaine. Inactive lever responding did not vary significantly with tacrine dose, session number, or the interaction of tacrine dose and session number.

Post-hoc comparisons indicated that self-administration of low-dose cocaine in outbred rats was attenuated during some sessions

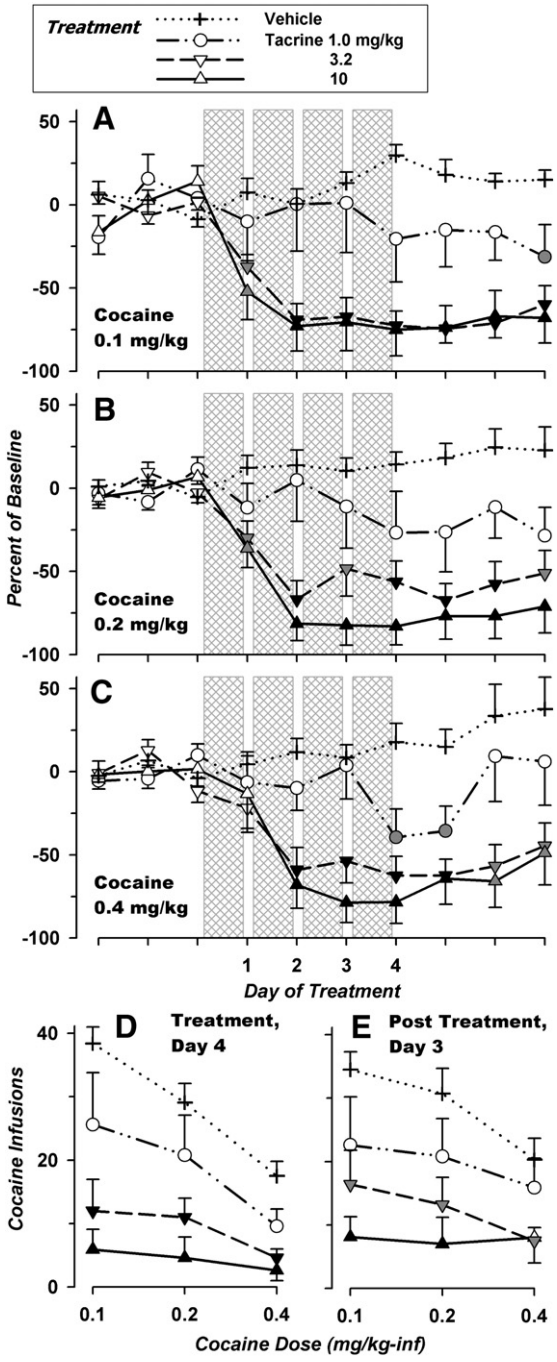

Fig. 1. Effects of pretreatment with tacrine on self-administration of cocaine by HS rats. Vertical axes show self-administration of cocaine, expressed as a percent of responding during the three days of self-administration prior to tacrine treatment. For panels A to C, time in days is plotted on the horizontal axis. Over four consecutive days, after completion of daily cocaine self-administration sessions, infusions of vehicle or tacrine were administered during the 21 h per day when rats were maintained in home cages (shown by gray-hatched bars). Vehicle or each dose of tacrine was evaluated in 8 to 15 animals. Results for components in which low-, intermediate-, and high- doses of cocaine were available, are shown in panels A, B, and C, respectively. Panels D and E show data for the same experiment, with cocaine dose plotted against the number of self-administered injections on the final day of treatment, and after 3 days of recovery from treatment, respectively. In all panels, filled symbols indicate significant differences for post-hoc comparisons to vehicle-treated rats, with gray and black corresponding to $p<0.017$ and $p<0.001$ respectively.

conducted immediately following treatment with 3.2 or 10 mg/kg-day of tacrine, as well as sessions conducted two and three days after discontinuation of 10 mg/kg-day of tacrine (Fig. 3, panel A). For intermediate-dose cocaine, effects of tacrine were limited to one of

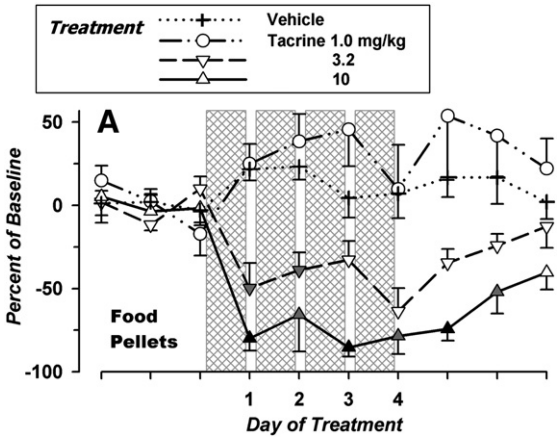

Fig. 2. Food pellet reinforced responding by HS rats during treatment with tacrine. Data presentation is similar to that shown for Fig. 1, except that 45 mg food pellets were available under FR-15 for the entire session, without the use of different components. Vehicle or each dose of tacrine was evaluated in 4 to 8 animals. Gray- and black- symbols correspond to $p<0.017$, and $p<0.001$, respectively, relative to vehicle-treated animals.

the four sessions immediately following each of the three active doses of tacrine (Fig. 3, panel B).

3.5. ED40 values for effects of tacrine

Fig. 4 shows effects of tacrine on different strains and reinforcers. Note that attenuation of responding did not vary in a linear pattern with tacrine dose plotted on a logarithmic scale. Especially for early effects, doses of 3.2 and 10 mg/kg-day often showed similar effectiveness in attenuating self-administration of cocaine (see Panels D,E,F, and G). Accordingly, curvilinear analysis using second-order regression was utilized. Because none of the doses of tacrine evaluated attenuated self-administration of high-dose cocaine by more than 40% in outbred rats (Panel F), 40% effective doses were estimated, rather than the more often used 50% effective dose.

Actions of tacrine differed for self-administration that was initiated within 20 min of pretreatment (described as early sessions following pretreatment) and for self-administration that occurred between one and three days after administration of tacrine was discontinued (described as late sessions). For HS rats, cocaine-reinforced responding was decreased to a similar extent for each of the three cocaine doses evaluated, with similar results obtained during both early and late periods (Fig. 4, panel B). During early sessions in HS rats, the ED40 value for food pellet reinforced responding was increased by approximately 60% over values for cocaine-reinforced responding (compare panels B and C). In contrast, the ED40 value for late effects of tacrine on food pellet reinforced responding exceeded that observed for cocaine self-administration by approximately 3-fold (300%).

Tacrine attenuated self-administration of low- and intermediate-dose cocaine to a similar degree in HS and outbred rats during early sessions (panels A and B). However, ED40 values for high-dose cocaine during early sessions indicated that tacrine was approximately 2-fold more potent in HS rats, relative to outbred animals. During late sessions, for all cocaine doses, tacrine was approximately 3- to 5-fold more potent in HS rats.

3.6. Persistent attenuation of reinforced behavior following tacrine treatment

Some individual rats continued to self-administer at very low levels after discontinuation of tacrine treatment. Persistent attenuation was defined as cocaine self-administration by individual animals that was decreased by more than 80% of the animal's pretreatment baseline level,

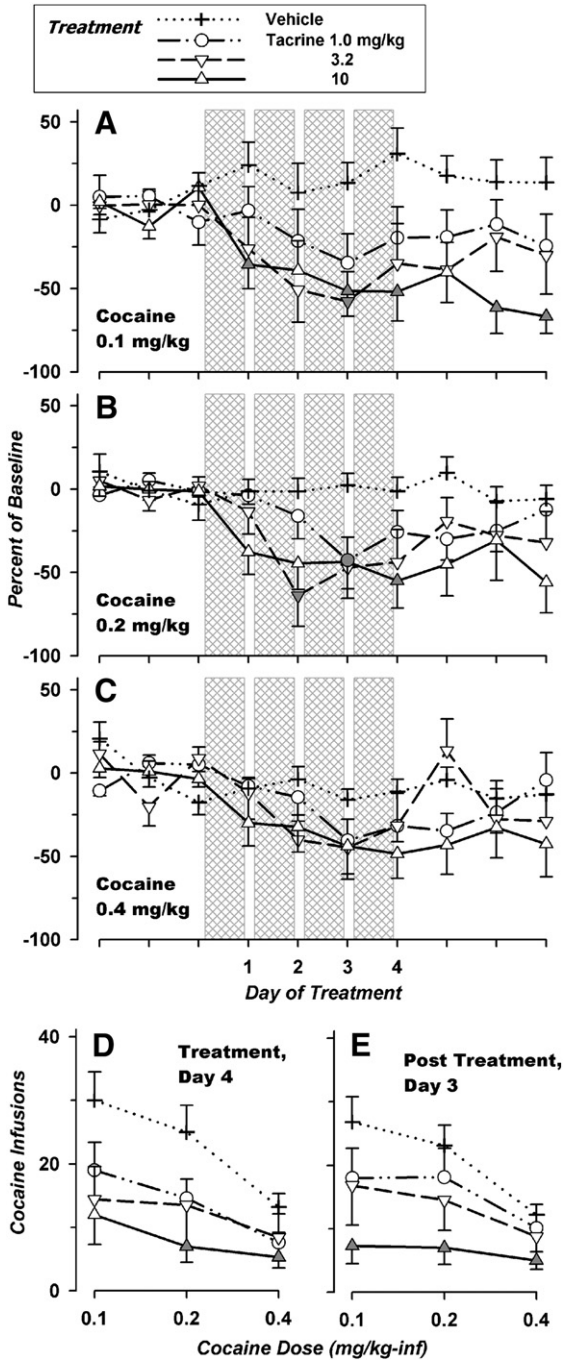

Fig. 3. Cocaine self-administration by outbred Wistar rats during treatment with tacrine. Vehicle or each dose of tacrine was evaluated in 7 to 9 animals. Gray- and black- symbols correspond to $p<0.017$, and $p<0.001$, respectively, relative to vehicle-treated animals.

between one and three days following discontinuation of pretreatment. Persistent attenuation of reinforced behavior was not observed after pretreatment with vehicle. However, significant numbers of HS but not outbred rats met criteria for persistent attenuation after receiving treatment with 3.2 or 10 mg/kg-day of tacrine (Fig. 5). A subset of these HS rats that maintained functional intravenous catheters was followed over a one-week period after treatment with tacrine (Fig. 6). Excluding the one animal treated with 1.0 mg/kg-day of tacrine, there were significant interactions of tacrine dose and session number for each of the three cocaine doses [$F(18,90)=8.95$, 19.0, and 8.18; $p<0.001$ for all values]. In all cases, these rats continued to self-administer at very low levels. Over the week following tacrine treatment, group means for cocaine self-administration in animals meeting criteria for persistent attenuation continued to be reduced by more than 80% from pretreatment baseline levels.

3.7. Effects of tacrine on body weight and signs of cholinergic stimulation

Signs of cholinergic stimulation and changes in body weight during tacrine treatment are shown in Fig. 7. For each of the experiments in which tacrine was administered, total score for cholinergic stimulation did not vary significantly with whether behavior was scored after one or four days of treatment. Based on this finding, results were analyzed independently of the time point at which behavior was scored. Kruskal–Wallis ANOVA showed that score for cholinergic stimulation varied significantly with dose for tacrine-treated cocaine-reinforced HS rats [$H(3)=18.7$, $p<0.001$] and tacrine-treated cocaine-reinforced outbred rats [$H(3)=15.4$, $p<0.01$], but not tacrine-treated food-reinforced HS rats. Score for cholinergic stimulation also did not vary significantly with gender for cocaine-reinforced HS rats.

Post-hoc comparisons indicated that cholinergic score was increased under both of the experiments in which 10 mg/kg-day of tacrine was administered to cocaine-reinforced rats, and also in cocaine-reinforced HS rats that received 3.2 mg/kg-day of tacrine. Cholinergic score was decreased for food- relative to cocaine-reinforced HS rats treated with 1.0 but not 3.2 mg/kg-day of tacrine.

In each of the three experiments, body weight did not change significantly over the four days during which tacrine infusions were administered (Fig. 7, panel D). For each of the experiments conducted, neither early or late attenuation of cocaine-reinforced responding was correlated with signs of cholinergic stimulation (see Fig. 8, panels A and B, respectively, for individual data from HS rats that self-administered cocaine). Changes in cocaine self-administration were also not correlated with either change in body weight during treatment or HS breeding generation.

4. Discussion

The main finding of this report is that long-lasting reductions in cocaine self-administration occur in rats genetically selected for increased drug self-administration treated with tacrine. Firstly, group means for cocaine self-administration were decreased in HS rats evaluated one to three days after discontinuation of tacrine treatment. Secondly, while many animals reacquired cocaine self-administration within three days of discontinuing tacrine treatment, a subset of HS rats failed to reacquire cocaine self-administration after receiving pretreatment with tacrine. In these rats, cocaine self-administration was reduced by more than 80% from pretreatment baseline levels over a one-week period during which no further tacrine was administered. Although limitations on the duration of catheter patency prevented monitoring of self-administration behavior over a longer period, there was no trend for increased self-administration in non-reacquiring HS rats over the seven-day period evaluated. Failure of levels of cocaine self-administration to return to baseline in these animals shows that persistent attenuation is stable over time. Similar results were observed for each of the three components under which drug was self-administered, indicating that persistent attenuation can occur across a four-fold range of cocaine doses. So far as we aware, this is the first report of a drug treatment that can produce long-lasting reductions in cocaine self-administration after drug treatment has been terminated.

Decreased DA levels in brain regions that receive terminal projections of mesolimbic DA neurons have been demonstrated prior to (Gerrits et al., 2002; Mateo et al., 2005) and following (Parsons et al., 1995) cocaine self-administration sessions. DA depletion or self-medication models suggest that initial exposures to drugs of abuse decreases DA transmission, which is perceived as a negative emotional state (Cryan et al., 2003); negative reinforcement

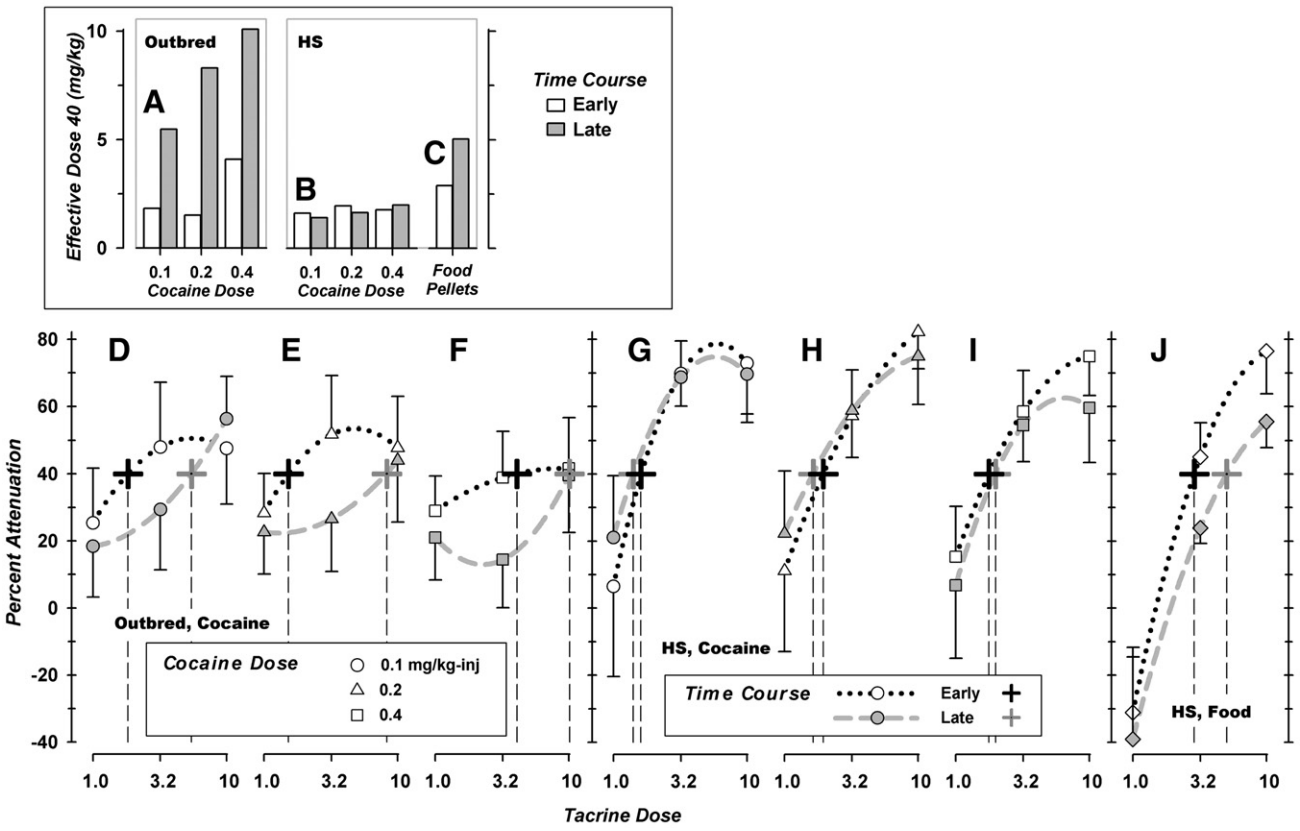

Fig. 4. Early and late effects of tacrine treatment on self-administration under different conditions. Panels D through J show results of second-order regression for early and late effects (dotted lines with open symbols and dashed lines with gray symbols, respectively). Results for self-administration of cocaine by outbred and HS rats are shown on the left (panels D,E, and F) and center (panels G,H, and I), respectively, with data for food pellet self-administration by HS rats shown on the right (panel J). Tacrine doses which attenuated self-administration by 40% were calculated for the self-administration experiments presented in Figs. 1 through 3 (40% effective dose or ED40 values). Cross-hair symbols with drop lines show points on second-order regression curves which correspond to ED40 values, which are plotted across different conditions in Panels A to C.

occurs as re-exposure to drugs of abuse increases DA levels and relieves symptoms associated with DA depletion (Khantzian, 1997). In vitro experiments have shown that micromolar concentrations of tacrine can inhibit DA reuptake (Jossan et al., 1992) and metabolism by monoamine oxidase (Adem et al., 1989), with both of these effects expected to augment DA transmission after clinically relevant does of tacrine. Treatment with tacrine increases cerebrospinal fluid metabolites of DA and serotonin in Alzheimer's disease patients, with levels of the serotonin metabolite correlated with clinical improvement (Alhainen et al., 1993). Based on these findings, tacrine may have attenuated the reinforcing effects of cocaine by augmenting DA levels prior to and following self-administration sessions in the present study. Tacrine is a non-selective inhibitor of both AChE and BuChE

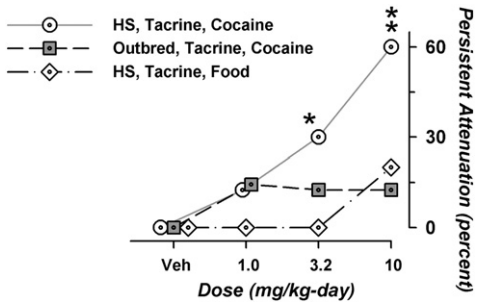

Fig. 5. Rates of persistent attenuation of cocaine and food pellet reinforced responding under different conditions. Percentages of rats exhibiting persistent attenuation, for different strains and reinforcers. * and ** correspond to $p<0.017$, and $p<0.001$ respectively, for comparisons with vehicle-treated animals by the likelihood ratio chi-square test.

(Wagstaff and McTavish, 1994). In addition to contributing to the degradation of ACh, BuChE can metabolize cocaine and other exogenous compounds (Carmona et al., 2005). Because cocaine is a substrate for BuChE, effects of tacrine may also have occurred through increased brain levels of cocaine.

Behaviors motivated by psychostimulant reinforcement are known for their persistence over time (Weiss et al., 2001; Grimm et al., 2002). Incentive sensitization is the process by which drug-associated cues confer more than routine sensory information, instead signaling a greater importance that can motivate compulsive and pathological behaviors (Berridge, 2007). When allowed daily access to intravenous cocaine self-administration, animals typically either maintain a stable pattern of drug intake, or increase levels of self-administration after exposure to either increased cocaine dose (Mantsch et al., 2001) or extended access to self-administration sessions (Ahmed and Koob, 1998). In addition, most patients who are motivated to achieve abstinence will relapse to stimulant abuse within the first three months of initiating treatment for cocaine dependence (Schmitz et al., 2008). Accordingly, our results show that control animals maintained a consistent pattern of cocaine self-administration over periods of up to one-week after pretreatment with infusions of vehicle.

In rat brain, tacrine is eliminated with a half-life of approximately 100 min (Telting-Diaz and Lunte, 1993). Because group means for cocaine self-administration were attenuated in HS rats three days after pretreatment was discontinued, actions of tacrine in the present study are unlikely to have been mediated by direct pharmacological effects. Instead, these effects must be explained by neurochemical or learned mechanisms that persist beyond the presence of active drug levels. It is possible that the combination of tacrine and cocaine has an

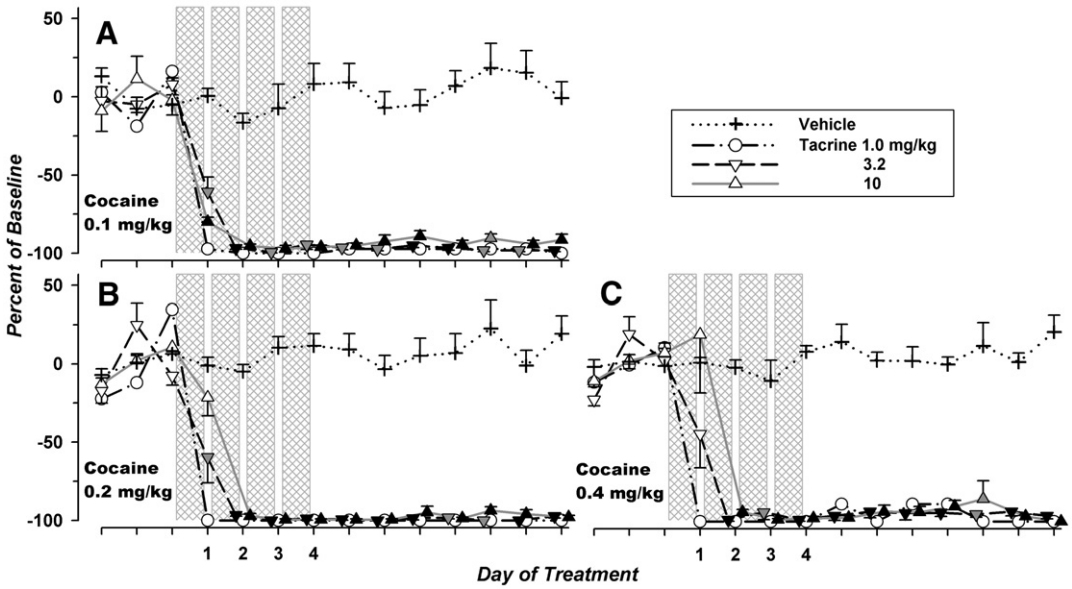

Fig. 6. Cocaine self-administration by HS rats exhibiting persistent attenuation. Data is shown for a subset of the animals presented in Fig. 1 that both met criterion for persistent attenuation of cocaine-reinforced responding and maintained patent catheters over a one-week period after discontinuation of tacrine treatment. The axes and legend are similar to that used for Fig. 1. For comparison, results are shown for an additional group of five vehicle-treated rats, none of which met criterion for persistent attenuation. Data is shown for 1, 3, and 5 rats that met criteria for persistent attenuation after receiving 1.0, 3.2, and 10 mg/kg-day of tacrine, respectively. Filled symbols indicate significant differences for post-hoc comparisons to vehicle-treated rats, with gray and black corresponding to $p<0.017$ and $p<0.001$ respectively.

aversive property that decreases the incentive salience of cocaine in genetically susceptible animals. From a therapeutic perspective, this possibility is intriguing. Patients with substance abuse disorders are known for their poor compliance with ongoing treatment requirements, and a potential therapy that did not require continuous administration would provide an important advantage in this patient population.

If used as a potential drug treatment in substance abuse disorders, AChE inhibitors such as tacrine would be administered in divided doses over a period of one or more days. Based on this, the present

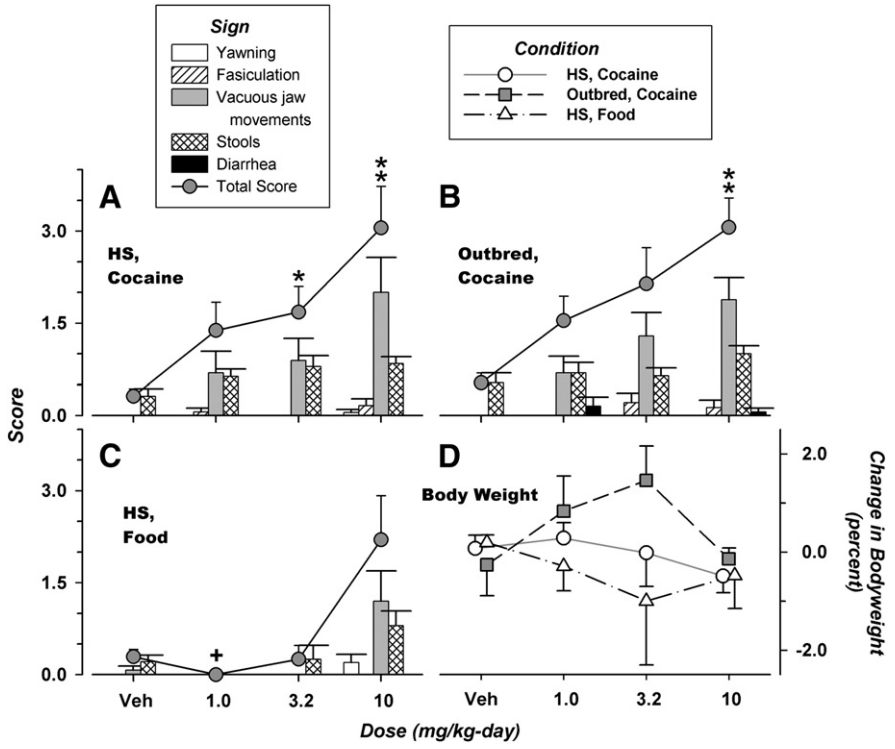

Fig. 7. Signs of cholinergic stimulation and change in body weight under different conditions. Panels A,B, and C show signs of cholinergic stimulation prior cocaine and food pellet reinforced responding. Change in body weight, recorded across a four-day period, as animals received daily infusions of vehicle or tacrine is shown in panel D. * and + indicate significant differences for comparison of total score with vehicle-treated rats or with animals that received the same dose of tacrine under different conditions, respectively. One and two symbols correspond to $p<0.017$ and $p<0.001$, respectively.

Measure	R	R^2	F	P
Early – Total Score	0.162	0.026	0.697	NS
Late – Total Score	0.139	0.019	0.513	NS

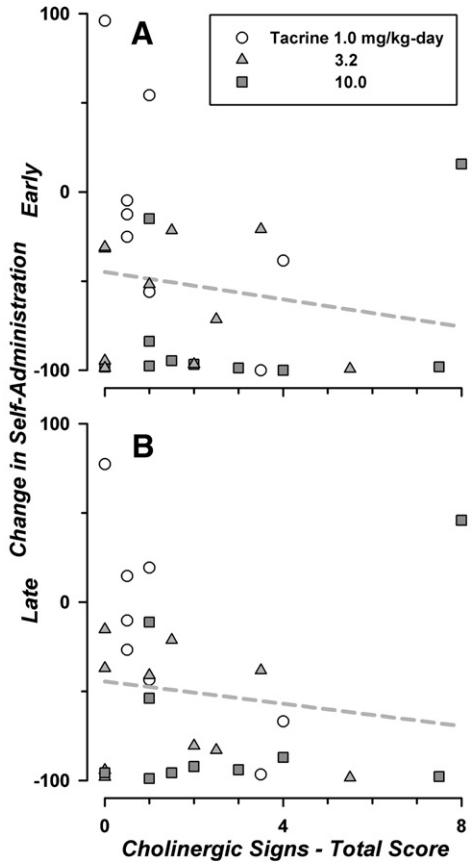

Fig. 8. Correlation of cocaine self-administration and signs of cholinergic stimulation for HS rats. The vertical axes show change in cocaine self-administration during the final three days of tacrine treatment, and the three days immediately after treatment with tacrine was discontinued (early and late periods, panels A and B respectively). Total score for cholinergic stimulation after the initial 24 h of treatment and after the final day of treatment were averaged to obtain one score for each individual animal. Dashed-lines show results of simple linear regression with *R* values shown above.

study administered tacrine as a series of infusions delivered over an extended period of hours, rather than through bolus dosing. Even so, the results obtained are in agreement with our previous finding that pretreatment with tacrine administered to outbred rats as bolus injections is much less potent in attenuating self-administration of high-dose cocaine (Grasing et al., 2008). Interestingly, doses of tacrine found to be effective in producing early effects on low- and intermediate-dose cocaine in outbred rats, and early and late effects on all doses of cocaine in HS rats in the present study are within the dose range that modify cognitive function in rodents and humans (Wagstaff and McTavish, 1994).

In patients with Alzheimer's disease, treatment with tacrine can cause typical symptoms of cholinergic stimulation, which include anorexia, nausea, and vomiting (Imbimbo, 2001). Dose-related signs of cholinergic stimulation are also observed in animals that receive tacrine and other AChE inhibitors. Attenuation of cocaine self-administration during treatment with tacrine may have occurred because of symptoms of cholinergic stimulation caused nonspecific declines in performance. Given that we observed a greater number of signs in cocaine-reinforced rats relative to animals self-administering food pellets, cocaine dependence may potentiate both signs and symptoms of cholinergic stimulation. If so, this property could explain the ability of tacrine to selectively attenuate cocaine self-administration. Nonetheless, when administered by infusion over an extended period, signs of cholinergic stimulation occurred at much lower rates than in previous reports when tacrine was administered as bolus injections (Dronfield et al., 2000; Ogura et al., 2001), suggesting against a role for cholinergic toxicity for its behavioral effects in the present study. Doses of tacrine that attenuated cocaine self-administration in HS and outbred rats did not produce significant changes in body weight, also indicating that effects on behavior are unlikely to have been caused by drug-induced toxicity. Furthermore, early and late effects of tacrine on cocaine and food pellet reinforced responding were not correlated with the degree to which change in body weight or signs of cholinergic stimulation were exhibited by individual animals.

In addition to its effects cocaine self-administration, pretreatment with tacrine also produced a persistent attenuation of food pellet reinforced responding. This effect both resolved more quickly and required higher doses of tacrine relative to late effects on cocaine self-administration in HS rats. In food-reinforced rats, pretreatment with tacrine resulted in relatively few animals that met criterion for persistent attenuation of food pellet reinforced responding. In contrast, approximately 30 and 60% of cocaine-reinforced rats met criterion for persistent attenuation of cocaine self-administration after receiving intermediate- and high-doses of tacrine, respectively; indicating that late effects of tacrine occur more selectively in cocaine- relative to food-reinforced HS rats.

For the present study, cocaine injections were self-administered under FR-5, with food pellet reinforcement made available under FR-15. Under these conditions, self-administration of food pellets and low-dose injections of cocaine produced similar hourly rates of reinforcement. Nonetheless, the greater response requirement supported by food pellets may have diminished the ability of tacrine to attenuate their self-administration. An additional limitation of the present study is that rats were utilized across a range of breeding generations as the HS line was being selected. Heterogeneity in the HS rats evaluated may have influenced the results obtained. Even so, early and late effects of tacrine on cocaine and food pellet reinforced responding were not correlated with breeding generation, suggesting against a large effect of this variable.

In summary, pretreatment with tacrine can produce a long-lasting attenuation of cocaine-reinforced responding. Although early effects on responding reinforced by low- and intermediate-dose cocaine occurred with similar potency in outbred and HS rats, higher doses of tacrine were required to produce late effects in outbred animals and outbred rats did not meet criteria for persistent attenuation of cocaine self-administration in significant numbers. Late effects of tacrine exhibited good selectivity for cocaine- relative to food-reinforced responding in HS rats. Further study is needed to determine the specific genetic elements that increase the sensitivity of the HS strain to late effects of tacrine.

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