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Alpha 2A adrenergic receptor agonist, guanfacine, attenuates cocaine-related impairments of inhibitory response control and working memory in animal models

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Abstract

There is considerable evidence that centrally acting α 2A adrenergic receptor agonists can attenuate impairments in executive function that result from dysfunction of the prefrontal cortex. Such positive effects resulted in the recent approval by the United States Food and Drug Administration (FDA) of the α 2A agonists clonidine and guanfacine for the treatment of Attention-Deficit/Hyperactivity Disorder (ADHD), but also suggest that they could have beneficial effects in substance abuse disorders and other neuropsychiatric conditions. The purpose of this study was to evaluate guanfacine for its ability to attenuate behavioral alterations associated with acute cocaine exposure in rats trained to perform a task of sustained attention, the five choice serial reaction time task (5C-SRTT) and monkeys trained to perform a task of working/short term memory, the delayed match to sample task (DMTS). In the rodent 5C-SRTT acute intraperitoneal (i.p.) administration of cocaine (3.5–15.0 mg/kg) did not affect accuracy, but was associated with dose-dependent increases in premature responses and timeout responses. Guanfacine (0.1–1.0 mg/kg i.p.) dose-dependently decreased premature responses and timeout responses associated with cocaine and it attenuated similar deficits in inhibitory response control observed in a variable ITI version of the 5C-SRTT. In the DMTS task in monkeys, acute intramuscular (i.m.) administration of cocaine (4.0 mg/kg) was associated with impairments in accuracy at long delay intervals, an effect that was attenuated by guanfacine (0.4 mg/kg). These animal studies suggest that guanfacine may have therapeutic potential for treating impairments of executive function that are associated with the abuse of cocaine.

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Keywords

drug abuse; addiction; cocaine; cognition; impulsivity; compulsivity; executive function

1. Introduction

The generic term “executive function” is commonly used to designate the variety of cognitive processes that are important to successful human behaviors and adaptive abilities including attention, working memory, problem solving, task planning, cognitive flexibility (task or rule switching) and the inhibition of inappropriate responses (Luria, 1973; Baddeley and Hitch, 1974; Lezak 1983; for recent reviews see Jurado and Rosselli, 2007; Ardila, 2008). Deficits in executive function are now thought to contribute to all components of the substance abuse cascade including the vulnerability, initiation and maintenance of addiction-related behaviors, as well as recidivism in patients who have attempted to end their dependence (Everitt et al., 2007; Porrino et al., 2007; Li and Sinha, 2008; Verdejo-García et al., 2008; Goldstein and Volkow, 2011). Accordingly, it has been argued that targeting the impairments of executive function in substance abuse disorders is an important strategy for improving overall treatment efficacy (Sofuoglu, 2010).

There is considerable evidence from both animal models and humans that centrally acting α 2A adrenergic receptor agonists (e.g., clonidine, guanfacine) can attenuate alterations in executive function that result from dysfunction of the prefrontal cortex (PFC) including inattention and distractibility, poor impulse control, and impairments of working memory, planning and decision-making, and the regulation of emotion (e.g., impulsive aggression) (see review, Arnsten 2010). The positive effects of clonidine and guanfacine on inattention, distractibility and impulse control resulted in their recent approval by the United States Food and Drug Administration (FDA) for the treatment of Attention-Deficit/Hyperactivity Disorder (ADHD). The evidence described above might also suggest that α 2A adrenergic receptor agonists such as clonidine and guanfacine could have beneficial effects in substance abuse disorders and addiction, although this line of research has not been rigorously pursued to date.

One advantage of α 2A adrenergic agonists as a potential therapy for substance-abuse-related disorders is their previous clinical track record. In addition to their recently approved clinical indication for ADHD, clonidine and guanfacine have been prescribed for hypertension for decades and they have also been commonly used (off label) to treat the symptoms of a number of other conditions including menopause, sleep disorders, pain disorders, drug and alcohol withdrawal, and some neuropsychiatric conditions (e.g., tic disorders, anxiety disorders, post-traumatic stress disorder, schizophrenia, see review, Crassous et al., 2007). The antihypertensive effects of α 2A adrenergic agonists and their ability to decrease heart rate are thought to result from the activation of brainstem α 2A adrenergic receptors to suppress sympathetic nerve activity from the vasomotor center to the heart and blood vessels (reviewed, Sorkin and Heel, 1986), while their beneficial effects on behavior are thought to result from the stimulation of post-synaptic α 2A receptors on dendritic spines of pyramidal cells in the PFC, effectively increasing the functional connectivity of PFC networks (Wang et al., 2007; Arnsten, 2010).

There is evidence to suggest that guanfacine might offer some advantages over clonidine for the treatment of neuropsychiatric disorders since it has weaker antihypertensive and sedative actions than clonidine, but is more potent in enhancing PFC function (Arnsten et al., 1988), advantages that may be related to its more selective affinity for the α_2A subtype (Uhlen et al., 1994) and lower affinity for the imidazoline receptor (Coupry et al., 1989). This argument, especially the superior effects on PFC function, was supported in a clinical study in young healthy volunteers where guanfacine, but not clonidine improved spatial working memory and planning ability (Jäkälä et al., 1999).

The purpose of the study described here was to evaluate guanfacine for its ability to attenuate behavioral alterations associated with acute cocaine exposure in rats trained to perform a task of sustained attention, the five choice serial reaction time task (5C-SRTT) and monkeys trained to perform a task of working/short term memory, the delayed match to sample task (DMTS).

2. Material and Methods

All procedures employed during this study were reviewed and approved by the Georgia Regents Sciences University Institutional Animal Care and Use Committee and are consistent with AAALAC guidelines. Measures were taken to minimize pain and discomfort in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Significant efforts were also made to minimize the total number of animals used while maintaining statistically valid group numbers.

2.1 Drugs

Cocaine hydrochloride was generously provided by the National Institute on Drug Abuse. Guanfacine hydrochloride was obtained from Sigma-Aldrich Corporation., St. Louis, MO, USA)

2.2 Rodent Studies

2.2.1 Test Subjects—Male albino Wistar rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN, USA) approximately 2 months old were housed individually in a temperature controlled room (25°C), maintained on a 12:12h normal light-dark cycle (lights on at 7AM) with free access to water and food during the first week (see subsequent food restriction procedures below).

2.2.2 Behavioral Studies—Rats were tested using an automated 5-Choice Serial Reaction Time Task (5C-SRTT) as we have described previously (Terry et al., 2012). Training and testing in the 5C-SRTT was conducted using eight ventilated, sound attenuated operant chambers (Med Associates, St. Albans, VT, USA). Each operant chamber consisted of nine nose pokes/apertures (2.5 cm wide, 4 cm deep), four of which were closed off with metal inserts; thus, every other nose poke was available. The apertures, arranged on a curved panel 2 cm above the floor of the chamber, were equipped with a photocell beam to detect nose pokes. Each aperture was equipped with a lamp (2.8 W) on the rear wall that could be illuminated randomly and for varying durations. Food pellets (45 mg chow pellet, BioServ,

Frenchtown, NJ, USA) were delivered automatically to a magazine, located on the opposite wall to the nose pokes, that was also equipped with a light that turned on to indicate that a pellet had been dispensed. The food magazine was equidistant from all nose poke apertures. The house light remained on for the entire session unless an error or omission occurred. The apparatus was controlled using MedPC software (Med Associates, St. Albans, VT, USA).

5C-SRTT Training: After arrival test subjects were separated into single housed conditions in preparation for food restriction and handled daily for one week to reduce stress and anxiety in preparation for training. From week 2 until the end of the study animals were food restricted to approximately 85% of their age-dependent, free-feeding weights based upon Harlan Laboratories growth rate curves. At week 3, animals began habituation to the 5C-SRTT apparatus in preparation for training consisting of a non-spatial habituation program. Study subjects were transferred (in their home cages) to the behavioral testing rooms each morning approximately 30 min before testing. 5C-SRTT training began with a stimulus duration (SD) of 10 seconds, each session being 100 trials or 30 minutes in duration with inter-trial intervals (ITI) of 5 seconds. Animals were trained 5 days per week until they reached stable performance levels (defined as 2 consecutive days at >80% accuracy, <20% omissions and completion of all 100 trials) at the 10 seconds stimulus duration. Once criterion was achieved at a given stimulus duration, the animals were moved to the next more challenging stimulus duration (5, 2.5, 2.0, 1.5, 1.25 and 1.0 second). After these training criterion were achieved, rats were tested in a protocol that used a fixed stimulus duration (1.0 sec) and inter-trial interval (5.0 sec) or a protocol that used the pseudorandom presentation of variable inter-trial intervals (VITI, 1.0, 5.0, and 10.0 sec). To assess performance the following parameters were measured: % correct ($(\# \text{ correct} / (\# \text{ correct} + \# \text{ incorrect})) \times 100$), % of omissions, premature responses (total # of responses performed after the trial began but before onset of the light stimulus), timeout responses (total # of nose pokes made in any aperture during a timeout period, perseverative responses (total # of nose pokes performed after the correct response had been made but before collecting the reward), trials completed, latency to correct response (time taken from onset of nose poke light stimulus to making the correct nose poke response), latency to incorrect response (time taken from onset of nose poke light stimulus to making the incorrect nose poke response), and latency to reward (i.e., the magazine latency, time taken from making a correct nose poke response to retrieving the reward from the magazine).

2.2.3 Drug administration—Following baseline (vehicle) assessments in the Standard and VITI versions of the 5C-SRTT, test subjects were treated acutely with cocaine and later with a combination of cocaine and the alpha 2 adrenergic agonist guanfacine. Drug testing occurred twice per week (e.g., Tuesday and Thursday or Wednesday and Friday) with 5C-SRTT maintenance training intervening between the drug test sessions; testing occurred only when animals maintained their performance accuracy (>80% accuracy, <20% omissions and completion of all 100 trials). Cocaine was dissolved in vehicle (0.9% saline) and administered by intraperitoneal injection (i.p.) 15 min before 5C-SRTT testing. In the studies in which the combination of guanfacine and cocaine were evaluated, guanfacine (dissolved in vehicle) was administered by i.p. injection 30 min before 5C-SRTT testing followed by cocaine administered by i.p. injection 15 min before 5C-SRTT testing. Drugs

and vehicle were administered in a pseudorandom manner to obviate any effects associated with the order of drug administration.

The rationale for the doses selected for guanfacine (dose range 0.1–1.0 mg/kg) and cocaine (dose range 3.5–15.0 mg/kg) in the 5C-SRTT was based on previous published experiments in rats (Fernando et al., 2012 and Fletcher et al., 2012, respectively).

2.3 Monkey Studies

2.3.1 Test Subjects—Non-human primate subjects included 4 male and 3 female pigtail macaques (*Macaca nemestrina*, see Table 3 for additional details). Each animal was well trained (>100 individual sessions) in the delayed matching-to-sample (DMTS) task. The animals were maintained on tap water (unlimited) and standard laboratory monkey chow (Harlan Teklad Laboratory monkey diet, Madison, WI) supplemented with fruits and vegetables. Food was removed from cages at about 0630 hours, and replaced after the completion of testing of all subjects for the day (at about 1630 hours). Additional nourishment was derived from 300 mg reinforcement food pellets (commercial composition of standard monkey chow and banana flakes, Noyes Precision food pellets, P.J. Noyes Co., Lancaster, NH) obtained during experimental sessions. On weekends animals were fed without time restrictions. Room temperature and humidity were maintained at $22 \pm 0.6^{\circ}\text{C}$ and $52 \pm 2\%$, respectively.

Each test subject had previously participated in one or more short-term studies assessing the effects of reversible drugs on DMTS performance. Prior drug experience produced no observable untoward effects in the animals, and each subject received at least a 4-week washout period (with continued weekday DMTS testing) prior to the start of this study.

2.3.2 Behavioral Studies

Delayed Match to Sample (DMTS) Testing: DMTS testing was conducted as we have described previously (Callahan et al., 2013). Test panels attached to each animal's home cage presented the task by using a computer-automated system. A touch-sensitive screen (15 in. AccuTouch LCD Panelmount TouchMonitor)/pellet dispenser units (Med Associates) mounted in light-weight aluminum chasses was attached to the home cage. The stimuli included rectangles of various colors (e.g., red, blue, yellow). A trial was initiated by presentation of a sample square composed of one of three colors. The sample rectangle remained in view until the monkey touched within its borders to initiate a pre-programmed delay (retention) interval. Following the delay interval, the two choice rectangles located below where the sample had been were presented. One of the two choice colors was presented with the color matching the stimulus, whereas the other (incorrect) color was presented as one of the two remaining colors. A correct (matching) choice was reinforced. Non-matching choices were neither reinforced nor punished. The inter-trial interval was 5 sec and each session consisted of 96 trials. The presentation of stimulus color, choice colors, and choice position were fully counterbalanced so as to relegate non-matching strategies to chance levels of accuracy. Three different presentation sequences were rotated through each daily session to prevent the subjects from memorizing the first several trials. Delay intervals were established during numerous non-drug or vehicle sessions prior to initiating the study.

Training sessions: The duration for each delay interval was adjusted for each subject until three levels of group performance accuracy were approximated: zero delay (85–100% of trials answered correctly); short delay interval (70–84% correct); and long delay interval (50–65% correct). The assignment of retention intervals based upon an individual's baseline task accuracy is necessary to avoid ceiling effects in the most proficient animals during drug studies, while also serving to insure that each animal begins testing at relatively the same level of task difficulty. In addition to session accuracy, two response latencies also were measured: the “sample latency”, which is the time between presentation of the sample color and the animal pressing in sample rectangle; and the “choice latency”, which is the time between presentation of the choice colors and the animal pressing one of the choice rectangles.

2.3.3 Drug Administration—Once the duration for each delay interval was adjusted and stabilized so that performances of the monkeys were within the ranges of accuracy described above, pharmacological studies were initiated. The standard weekly regimen for each drug dose included, Monday: the administration of vehicle followed by DMTS testing; Tuesday: drug administration followed by DMTS test session; Wednesday: DMTS testing (in the absence of drug or vehicle) initiated 24 hours after Tuesday's dosing; Thursday and Friday: same as Tuesday and Wednesday. This timing of administration (i.e., the particular day of drug and/or placebo administration) could be modified in response to some husbandry or housing-related constraints and adapted to each monkey. Guanfacine, cocaine, and the combinations of guanfacine and cocaine were administered one hour before DMTS testing according to guidelines specified above. Given that neither guanfacine nor cocaine had previously been evaluated in pigtail monkeys at our facility, a non-randomized (ascending order) approach to dosing was conducted due to the potential for adverse reactions especially at the higher doses. The rationale for the doses selected for guanfacine (dose range 0.2–0.6 mg/kg) and cocaine (dose range 0.5–4.0 mg/kg) was based on previous published behavioral experiments in monkeys (Franowicz and Arnsten, 1998 and Jentsch et al., 2002, respectively).

In addition to the DMTS analysis, each subject was monitored by a behavioral technician (locally in the testing room) just prior to and immediately after testing (and from outside the testing room through a peep hole during DMTS testing) to ascertain and record any visible adverse reactions (e.g., salivation, diarrhea, stereotypy, failure to eat the normal daily rations, etc.).

2.4 Statistical Analyses

Statistical analysis was performed using SigmaPlot 11.2 and JMP 5.0. Statistical significance was assessed using an alpha level of 0.05.

Rodent Studies: In the standard version of the 5C-SRTT (fixed ITI), for analyses of accuracy (% correct), premature responses, perseverative responses, omissions, timeout responses, and response latencies, a one factor analysis of variance (with repeated measures) was used followed by the Dunnett's method (for comparisons to vehicle controls only) for post hoc analysis. In the variable ITI version of the task, a two-factor repeated measures

ANOVA was performed. The fixed effects in the model included dose and ITI, as well the two-factor interaction between dose and ITI. A Bonferroni adjustment to the overall alpha level was used to control for the number of comparisons within ITI.

Monkey Studies: For accuracy (% correct) in the DMTS experiments, a two-factor repeated measures ANOVA was performed. The fixed effects in the model included treatment (or dose) and delay as well the two-factor interaction between treatment and delay. Animal nested within treatment group was considered a random effect. Of statistical interest was the F-test for the two-factor interaction between treatment and delay and if statistically significant would indicate that the effect of delay on matching accuracy was different in the different treatment groups. Regardless of the statistical significance of the interaction term there were specific post hoc pairwise comparisons of interest within the treatment by delay interaction. As the only post hoc comparisons of interest were those within delay between treatments, a Bonferroni adjustment to overall alpha level of 0.05 was used to examine post hoc pairwise comparisons on the adjusted least square means from the treatment by delay interaction term. For the number of trials completed and the session duration, a one-factor repeated measures ANOVA was performed with Dunnett's method (for comparisons to vehicle controls only) for post hoc analysis. Median response latencies (in sec) for both sample and choice were analyzed by the nonparametric method of Kruskal-Wallis (analysis of variance on ranks).

With the exception of median latencies associated with DMTS testing in monkeys provided in Table 4, in all figures and tables, error values denoted by $\pm$ indicates the standard error of the mean.

3. Results

3.1 Rodent behavioral studies

3.1.1 Rat-Standard 5C-SRTT—In the standard version of the 5C-SRTT (single 1.0 sec SD and 5 sec ITI), three doses of cocaine were evaluated for effects on performance. The results of these experiments are provided in Fig 1 and Table 1. As indicated in Fig 1A and 1C, cocaine (at the doses evaluated) did not alter accuracy (% correct) of the task or the number of perseverative responses compared to vehicle ($p > 0.05$ for the main effect of dose for both comparisons). Conversely, cocaine significantly increased the number of premature responses, $F_{(3,39)} = 22.8$, $p < 0.001$ (Fig 1B), and timeout responses, $F_{(3,39)} = 19.6$, $p < 0.001$ (Fig 1D) in a dose-dependent manner. Cocaine was also associated with dose-related decreases in response latencies associated with correct, $F_{(3,39)} = 17.1$, $p < 0.001$, and incorrect, $F_{(3,39)} = 4.8$, $p < 0.01$ responses. An increase in omissions was observed, $F_{(3,39)} = 12.1$, $p < 0.001$, but only at the 15 mg/kg dose of cocaine. None of the test doses of cocaine significantly ($p > 0.05$) affected magazine latencies or the number of trials completed (see Table 1). In the second phase of the study, four doses of guanfacine were evaluated for their ability to attenuate the effects of cocaine 15.0 mg/kg in the standard version of the 5C-SRTT. The results of these experiments are presented in Fig 2 and Table 1. Neither cocaine nor the combinations of cocaine and guanfacine affected accuracy of the task (Fig 2A) or the number of perseverative responses (Fig 2C) compared to vehicle ($p > 0.05$ for the main effect of treatment for both comparisons). Guanfacine significantly attenuated the cocaine-related

increase in premature responses, $F_{(5,65)} = 17.4$, $p < 0.001$ (Fig 2B) and timeout responses, $F_{(5,65)} = 12.2$, $p < 0.001$ (2D) in a dose-dependent manner. Guanfacine also attenuated the cocaine-related decreases in response latencies associated with correct, $F_{(5,65)} = 25.7$, $p < 0.001$ and incorrect, $F_{(5,65)} = 8.0$, $p < 0.001$ responses (Table 1). Guanfacine (particularly at the higher doses) when combined with cocaine was also associated with a slight increase in magazine latencies $F_{(5,65)} = 7.3$, $p < 0.001$, an increase in omissions, $F_{(5,65)} = 19.9$, $p < 0.001$, and a decrease in trials completed $F_{(5,65)} = 9.8$, $p < 0.001$ when compared to cocaine-vehicle combination (Table 1).

3.1.2 Rat-5C-SRTT Variable ITI—In order to increase the demands of the task, particularly on inhibitory control, we evaluated the performance effects of guanfacine alone using the variable inter-trial interval (VITI) version of the 5C-SRTT. The results of these experiments are presented in Fig 3 and Table 2. As indicated in Fig 3A and 3C, the pseudorandom presentation of three different ITIs did not significantly alter task accuracy (% correct) or the number of perseverative responses following vehicle or guanfacine alone (i.e. the main effects of dose, ITI, and the dose x ITI interactions were not significant, $p > 0.05$). However, for premature responses (Fig 3B) the following statistical results were obtained, main effect of dose, $F_{(4,52)} = 16.5$, $p < 0.001$; ITI, $F_{(2,104)} = 59.6$, $p < 0.001$; dose x ITI interaction, $F_{(8,104)} = 16.1$, $p < 0.001$. Post hoc analysis indicated that there was a significant increase in premature responding at the longest ITI and that the two higher doses of guanfacine (0.6 and 1.0 mg/kg) significantly attenuated this increase. A similar effect was observed with the number of timeout responses, main effect of dose, $F_{(4,52)} = 10.7$, $p < 0.001$; ITI, $F_{(2,104)} = 15.4$, $p < 0.001$; dose x ITI interaction, $F_{(8,104)} = 4.0$, $p < 0.001$. Again, the two higher doses of guanfacine (0.6 and 1.0 mg/kg) significantly attenuated the ITI-related increase in timeout responses. For the other dependent measures in the VITI task, the following observations were made: there was a slight increase in response latencies associated with incorrect responses, $F_{(4,52)} = 4.6$, $p < 0.01$, magazine latencies, $F_{(4,52)} = 8.3$, $p < 0.001$, and omissions, $F_{(4,52)} = 13.7$, $p < 0.001$, most notably associated with the higher doses of guanfacine. Interestingly, there was a slight decrease in latencies associated with correct responses, $F_{(4,52)} = 2.7$, $p = 0.04$, significant for the 0.1 dose of guanfacine versus vehicle upon post hoc analysis ($p < 0.05$) and there was a (guanfacine-related) trend toward an increase the number of trials completed, ($F_{(4,52)} = 2.3$, $p < 0.07$).

3.2 Monkey Studies

3.2.1 Monkey DMTS

Guanfacine: To ensure stable and consistent baseline responding in the subjects, vehicle (saline) was administered on 3 occasions during the guanfacine dose-effect study. Performance following the vehicle administration tests was not significantly different from one another (data not shown); therefore, the data obtained from these sessions were averaged and used for comparison with guanfacine treatment sessions. As a group ($N=7$) the mean performance accuracies in the DMTS task after vehicle administration met the performance criteria set forth above (see Methods): Zero delay, 89.0%, Short delay 72.6%, and Long delay, 55.9% trials correct and always followed a significant ($p < 0.001$) delay-dependent decline in accuracy (Fig. 4A). Guanfacine was not associated with dose-dependent effects on DMTS accuracy; main effect of dose, $F_{(3,6)} = 0.34$, $p = 0.77$, delay, $F_{(2,66)}$

=99.0, $p<0.001$, dose x delay interaction, $F_{(6,66)}=0.50$, $p=0.81$. While there appeared to be a trend toward enhanced performance associated with guanfacine 0.4 mg/kg at the long delays, none of the post hoc comparisons met the required level of statistical significance (i.e., all p values for drug effects compared to vehicle were > 0.05).

Cocaine: In the second phase of the monkey studies, several doses of cocaine were evaluated for effects on DMTS performance. Monkey V6t began exhibiting formication-related behaviors and excessive grooming upon exposure to the higher doses of cocaine (2.0 and 4.0 mg/kg) and was, therefore, removed from the remainder of the study. These behaviors quickly resolved in this monkey and were not observed in the other test subjects. In the remaining six subjects the mean accuracies in the DMTS task after vehicle administration again met the predetermined performance criteria, Zero delay, 87.1%, Short delay 71.0%, and Long delay, 57.7% trials correct and always followed a significant ($p<0.001$) delay-dependent decline in accuracy. Again, to ensure stable behavioral performance over the course of testing, vehicle was administered on 6 occasions during the cocaine dose-effect study and the data obtained from these sessions were averaged and used for comparison with cocaine treatment sessions. The following statistical results were obtained for the cocaine dose-effect analysis, main effect of dose, $F_{(4,5)}=0.74$, $p=0.57$, delay, $F_{(2,69)}=141.6$, $p<0.001$, dose x delay interaction, $F_{(8,69)}=0.72$, $p=0.67$. Post hoc analysis indicated that the 4.0 mg/kg dose significantly ($p<0.05$) impaired DMTS accuracy (compared to vehicle) at the long delay intervals.

Guanfacine + Cocaine: In the third phase of the study, 3 doses of guanfacine were evaluated for their ability to attenuate the negative effects of cocaine 4.0 mg/kg on DMTS performance ($N=6$). Under vehicle conditions the mean accuracies in the DMTS task after vehicle administration again met the predetermined performance criteria, Zero delay, 88.1%, Short delay 74.4%, and Long delay, 59.0% trials correct and followed a significant ($p<0.001$) delay-dependent decline in accuracy (see Fig. 4C). The following statistical results were obtained for the guanfacine + cocaine analysis, main effect of treatment, $F_{(4,5)}=0.85$, $p=0.50$, delay, $F_{(2,66)}=48.5$, $p<0.001$, treatment x delay interaction, $F_{(8,66)}=0.73$, $p=0.66$. Post hoc analysis indicated that the 4.0 mg/kg dose of cocaine impaired DMTS accuracy (compared to vehicle) at the long delay intervals and that the 0.4 mg/kg dose of guanfacine completely blocked this effect of cocaine. In addition, there was a trend toward an attenuation of the effect of cocaine associated with the other two doses of guanfacine ($p<0.08$ in each case).

Drug effects on Latencies, Trials Completed and Time Elapsed: The effects of guanfacine, cocaine, and the combination of guanfacine and cocaine (4.0 mg/kg) on median response latencies during performance of the DMTS task are provided in Table 4. As indicated, when administered alone, the higher doses of guanfacine were associated with slightly higher sample and choice latencies (both in association with correct and incorrect responses). There were no other significant, drug-related effects on response latencies. The effects of guanfacine, cocaine, and the combination of guanfacine and cocaine (4.0 mg/kg) on the number of trials completed and on session durations are provided in Table 5. Guanfacine (when administered alone) was not associated with significant effects on the

number of trials completed or session duration (main effects of dose, $p > 0.05$). Conversely, cocaine was associated with dose dependent decreases in the number of trials completed, $F_{(4,20)} = 50.2$, $p < 0.001$, as well as increases in the duration of the test sessions, $F_{(4,20)} = 40.7$, $p < 0.001$. Post hoc analysis indicated that all 3 doses of cocaine significantly ($p < 0.05$) affected these dependent measures when compared to the vehicle-related response. The combination of guanfacine with cocaine (4.0 mg/kg) was also associated with decreases in the number of trials completed, $F_{(4,19)} = 23.7$, $p < 0.001$, as well as increases in the duration of the test sessions, $F_{(4,19)} = 25.0$, $p < 0.001$. Post hoc analysis indicated that all drug combinations significantly ($p < 0.05$) affected these dependent measures when compared to the vehicle-related response, thus indicating that guanfacine (at the doses that were evaluated) failed to attenuate these negative effects of cocaine.

Additional Behavioral Observations: Throughout the study the test subjects were visually monitored for possible drug-related side effects. The following observations were recorded: There were no apparent side effects associated with guanfacine administration. The animals' behaviors appeared normal during the pretreatment phase (before the DMTS task was initiated), they were alert, they moved and climbed around their cages normally, and after the DMTS task began, they focused on (and completed) the task in a similar fashion to when vehicle was administered. Conversely, when cocaine (most notably at the 2.0 and 4.0 mg/kg doses) and the guanfacine-cocaine 4.0 mg/kg combinations were administered, some visible drug effects were observed (i.e., within a few minutes after drug administration and for an extended period afterwards). These effects included drooling, stereotypic grooming, staring, visual tracking, and visual checking. The effects appeared to be subject specific (i.e., one or more, but not all, of the effects described above were observed in individual animals), and they eventually subsided within 1–2 hours after drug administration. Typically, the subjects would not perform the DMTS task until these signs resolved, but afterwards finished the remaining trials normally. All test subjects (irrespective of drug treatment) ate all of their regular food later in the afternoon after DMTS testing and would take (and consume) food treats throughout the study period. In addition, there were no residual effects noted after the study ended.

4. Discussion

The results of this study can be summarized as follows: 1) in rats using the standard version of the 5C-SRTT, acute cocaine administration did not affect accuracy, but was associated with dose-dependent increases in premature responses and timeout responses, and a slight decrease in response latencies, but not reward latencies, 2) guanfacine dose-dependently attenuated each of the cocaine-related alterations in the 5C-SRTT in rats, 3) guanfacine (administered alone) also attenuated deficits in inhibitory response control induced by the presentation of VITIs in rats. 4) in monkeys, guanfacine (at the doses that were evaluated) did not significantly affect DMTS accuracy, 5) acute cocaine administration (4.0 mg/kg) was associated with a modest decrease in DMTS accuracy primarily at the long delay intervals in monkeys, but it also resulted in non-cognitive behavioral effects such as drooling, stereotypical grooming, staring, a decrease in the number of trials completed, and longer session durations, 6) guanfacine (0.4 mg/kg) significantly attenuated the negative

effects of cocaine on DMTS accuracy in monkeys, but did not significantly improve the non-cognitive behavioral effects, decrease in trials completed, or increased session times.

The observation of cocaine-related elevations in premature responding (i.e., inappropriate nose pokes during the intertrial interval before the target stimulus) in the rat 5C-SRTT observed here agrees with previously published studies (van Gaalen et al., 2006; Fletcher et al., 2011) and suggests that cocaine increases impulsivity-like behaviors in animals models. It should be noted that in the Fletcher et al., 2011 study, a dose-dependent decrease in accuracy (i.e., at 10 and 20 mg/kg) was also observed in the 5C-SRTT which contrasts with our findings where no effects on accuracy were observed at doses up to 15.0 mg/kg. The basis for this discrepancy is unclear. We used the same strain of rats (Wistar) with similar weights and ages, however there were a few differences in methodology that could contribute to the differences. In our studies, the rats were singly housed, testing during the light cycle, with cocaine pretreatment time of 15 min. In the Fletcher et al., 2011 study, the rats were double housed, testing during the dark cycle, with cocaine pretreatment time of 20 min.

The increase in timeout responses (i.e., nose pokes made during the timeout interval which occurred after an incorrect response or premature response) observed in our study are also indicative of (cocaine-related) impulsive-like behaviors and/or cognitive inflexibility (i.e., the inability to alter behavior in reaction to changing situational demands, in this case, disorganized responses that are not tied to the stimulus presentation, see review, Robbins 2002; Bari et al., 2008; Amitai and Markou, 2011). Interestingly, deficits of inhibitory response control have commonly been associated with acute exposure to cocaine and other stimulants (e.g., amphetamine) in humans and are thought to facilitate the development of uncontrolled patterns of drug intake (i.e., “bingeing”, see Gawin 1991; Fillmore et al. 2002, 2003; van Gaalen et al., 2006).

An additional objective of the rodent experiments described in this report was to determine if guanfacine might improve impulsivity-like behaviors when the demands of the task were increased in normal (i.e., not cocaine-treated) rats. The VITI version of the 5C-SRTT is often used to increase the demand on the inhibition of inappropriate responding by making the appearance of the stimuli unpredictable. As expected, when the test subjects were exposed to a pseudorandom presentation of different ITIs, there was a significant increase in premature responses (compared to when fixed ITIs were presented), particularly at the longer (10 sec) and, moreover, this effect was attenuated by guanfacine. These studies in the 5C-SRTT appear to complement other studies that were focused on alterations of attention and impulsivity in rodent models. For example, in a lever-based, visual discrimination task, guanfacine improved sustained attention, reduced overactivity, and decreased impulsivity, in the spontaneous hypertensive rat, an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD) (Sagvolden 2006). Likewise, guanfacine reduced premature responding in the 5C-SRTT (without affecting accuracy, similar to our findings with cocaine) in rats selected for high impulsivity (Fernando et al., 2012).

Another goal of the current study was to determine, if guanfacine could attenuate working/short-term memory deficits induced by cocaine in non-human primates. The impetus for

these experiments was previously published literature where cocaine and guanfacine have been associated with deleterious and positive effects on working memory (and other domains of cognition), respectively, in monkeys and humans. For example, in rhesus monkeys, both acute cocaine injections and chronically self-administered cocaine have been associated with deficits in DMTS performance (Porter et al., 2011; Porrino et al., 2013). In humans, impairments in working memory (primarily associated with chronic exposure) have been reported in multiple studies (O'Malley et al., 1992; Hoff et al., 1996; Bechara and Martin, 2004; Kübler et al., 2005; Verdejo-Garcia and Perez-Garcia, 2007), although there are exceptions (see Bolla et al., 1999; Pace-Schott et al., 2008). Guanfacine, in contrast, has been shown to improve working memory in young and aged monkeys (Franowicz and Arnsten 1998) and to attenuate impulsive behavioral choices in young monkeys (Kim et al., 2012). Likewise, guanfacine improved working memory performance in healthy human volunteers (Jakala et al., 1999), patients with frontal and temporal lobe epilepsy as well as healthy volunteers (Swartz et al., 2008) and it enhanced sustained attention in patients with schizophrenia (Friedman et al., 2001) or ADHD (Scahill et al., 2001).

In the current study, we did not observe statistically significant improvements in DMTS task performance in monkeys administered guanfacine as were observed in the aforementioned Franowicz and Arnsten 1998 study. The basis for our disparate results is unclear, although there were some differences in the experimental approaches. In the Franowicz and Arnsten study, a somewhat wider range of doses was evaluated (0.1–0.7 mg/kg versus 0.2–0.6 mg/kg in our study), the monkey species was different (rhesus versus pigtail macaque), and the DMTS procedure was different. Specifically, in the Franowicz and Arnsten study, a manual Wisconsin General Testing Apparatus (WGTA) procedure with two food wells (in a sound attenuating room) was used, whereas our procedure employed a computer-automated color discrimination task using a touch screen attached to the subject's home cage. In the cocaine dose-effect study, we did observe impairments in DMTS performance as have been observed in the aforementioned publications, while (to our knowledge) our study is the first to report an attenuation of such negative effects of cocaine by an $\alpha 2A$ adrenergic receptor agonist.

The neurochemical basis for the positive effects of guanfacine (administered alone) in rats and on cocaine-related behavioral deficits in rats and monkeys are unclear, but are likely related to effects at $\alpha 2A$ adrenergic receptors. As noted in the Introduction, the beneficial effects of $\alpha 2A$ receptor agonists on executive function observed previously (in ADHD animal models and humans with ADHD) have been hypothesized to result from the stimulation of post-synaptic $\alpha 2A$ receptors on dendritic spines of pyramidal cells in the PFC, effectively increasing the functional connectivity of PFC networks (Wang et al., 2007; Arnsten, 2010). However, recent work in rodents also suggests that presynaptic $\alpha 2A$ adrenergic receptors in the hippocampus may be important. Specifically, local infusions of guanfacine into the ventral hippocampus (where $\alpha 2A$ receptors are primarily found on presynaptic terminals) led to a reduction in impulsive decision-making in a delay discounting task (Abela and Chudasama, 2014). In this manuscript the authors hypothesized that the positive effects of guanfacine on impulsive decision-making (specifically in the delay discounting task) might be related to an attenuation of the stress response during the

wait for the large reward (mediated by a reduction of noradrenergic tone through presynaptic mechanisms in the ventral hippocampus).

Finally, there are some potential limitations to this study that should be discussed. As noted in the Results, in the rodent studies, guanfacine (when administered alone in the VITI task) was associated with a modest increase in some of the response latencies and in the number of omissions. In this context, it could possibly be argued that a motor slowing effect, sedating effect, or decreases in motivation induced by guanfacine could contribute to the apparent attenuation of cocaine-related increases of inhibitory response control. It should be noted, however, that the increases in latencies were quite modest, they did not occur in the “latency to correct response” measure, and further, guanfacine (alone) was not associated with impairments in accuracy in the 5C-SRTT. In the monkey studies, guanfacine (administered alone) was also associated with a slight increase in sample and choice latencies, but (again), no alterations in accuracy (% correct) were observed (as might be expected if significant sedation was present).

The non-cognitive behavioral effects of cocaine in monkeys described above typically manifested during the period before the test subjects began performing the DMTS procedure. The DMTS software initially presents the sample stimulus and if no response occurs within 3 minutes a new sample is presented (i.e., the next trial is initiated automatically). As can be observed in Table 4, test subjects typically finished about one-half of the total trials that were presented and there was up to a 2 hour delay in the completion time (for all presented sessions). Given the relatively short half-life of cocaine, it might be argued that a confounding effect of diminishing cocaine levels *in vivo* during DMTS testing might limit conclusions about cocaine and cocaine + guanfacine-related effects on working memory. However, in the one published study we were able to find where cocaine was administered acutely by i.m. administration in macaques (rhesus monkeys), the reported half-life of cocaine in plasma was 1.4 hours (Duhart et al., 1993). Accordingly, significant levels of cocaine would be expected to have remained in our test subjects over the full DMTS session (i.e., the mean session duration in monkeys administered the 4.0 mg/kg dose of cocaine was approximately 3 hours).

In conclusion, the results of these studies indicate that guanfacine can attenuate the deficits of inhibitory response control and working/short term memory that are induced by acute cocaine exposure. These animal studies suggest that guanfacine may have therapeutic potential for treating the impairments of specific components of executive function that are associated with the abuse of cocaine. Our studies combined with the results of other recently published studies where guanfacine and clonidine decreased cue-induced reinstatement in cocaine seeking in rats (Smith and Aston-Jones, 2011), and where guanfacine reduced cocaine-, nicotine- and alcohol-induced cravings, as well as anxiety and arousal in individuals dependent on these substances (Fox et al., 2012, 2014), further support the premise that α_2A selective adrenergic agonists have therapeutic potential for substance abuse-related conditions.

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Research Highlights

Acute cocaine exposure impairs inhibitory response control in rats.

Acute cocaine exposure impairs working memory in monkeys.

Guanfacine attenuates cocaine impairments of inhibitory response control in rats.

Guanfacine attenuates cocaine impairments of working memory in monkeys.

Guanfacine may have therapeutic potential in abusers of cocaine.

Fig. 1.

Dose-related effects of cocaine on performance of the 5C-SRTT in rats. Rats were trained to meet specific performance criterion (described in the Methods section) at a stimulus duration (SD) of 1.0 sec in the 5C-SRTT. Subsequently, vehicle and several doses of cocaine (administered i.p., 15 min before testing) were evaluated for effects on 5C-SRTT performance. **A.** Percent Correct (accuracy); **B.** Premature Responses (impulsive behavior); **C.** Perseverative Responses (compulsive behavior); **D.** Timeout Responses (compulsive behavior/cognitive inflexibility). Each symbol represents the mean $\pm$ SEM for each test group. *(p<0.05); ***(p<0.001) =significantly different compared to vehicle-associated performance level. N=14

Fig. 2.

Dose-related effects of guanfacine on cocaine-related alterations in performance of the 5C-SRTT in rats. Rats were trained to meet specific performance criterion (described in the Methods section) at a stimulus durations (SD) of 1.0 sec in the 5C-SRTT. Subsequently, vehicle and several doses of guanfacine (administered i.p. 30 min before testing) were evaluated for their ability to attenuate the negative effects of cocaine (15.0 mg/kg administered i.p., 15 min before testing). **A.** Percent Correct (accuracy); **B.** Premature Responses (impulsive behavior); **C.** Perseverative Responses (compulsive behavior); **D.** Timeout Responses (compulsive behavior/cognitive inflexibility). Each symbol represents the mean $\pm$ SEM for each test group. * ($p < 0.05$); *** ($p < 0.001$) = significantly different compared to vehicle-associated performance level. + = significantly different ($p < 0.05$) compared to cocaine-associated performance level. $N = 14$

Fig. 3.

Dose-related effects of guanfacine on performance of a variable intertrial interval (ITI) version of the 5C-SRTT in rats. Rats were trained to meet specific performance criterion (described in the Methods section) at a stimulus durations (SD) of 1.0 sec in the 5C-SRTT. On specific days variable intertrial intervals (1.0, 5.0, and 10.0 sec) were presented in a pseudorandom order. Subsequently, vehicle and several doses of guanfacine (administered i.p. 30 min before testing) were evaluated for their effects on performance. **A.** Percent Correct (accuracy); **B.** Premature Responses (impulsive behavior); **C.** Perseverative Responses (compulsive behavior); **D.** Timeout Responses (compulsive behavior/cognitive inflexibility). Each bar represents the mean $\pm$ SEM for each test group. *($p < 0.05$); =significantly different compared to the vehicle-associated performance level. $N = 14$.

Fig. 4.

(A) Dose-effect relationship for each delay in the Delayed Match to Sample (DMTS) task by 7 pigtail monkeys, 2 hr after the intramuscular (i.m.) administration of guanfacine. The baseline was determined from the average of 3 vehicle sessions conducted during the study.

(B) Dose-effect relationship for each delay in the DMTS task by 6 pigtail monkeys, 20 min, after the i.m. administration of cocaine. The baseline was determined from the average of 6 vehicle sessions conducted during the study.

(C) Effect of vehicle + vehicle, vehicle + cocaine 4.0 mg/kg or guanfacine (across 3 doses) + cocaine 4.0 mg/kg in the DMTS task by 6 pigtail monkeys. In each case the first vehicle or guanfacine injection was administered 2 hr before DMTS testing and the second vehicle or cocaine injection was administered 20 min before DMTS testing. The baseline was determined from the average of 7 vehicle sessions conducted during the study. Each bar represents the mean (% correct) $\pm$ S.E.M. over 96 trials per session. * $p < 0.05$, = significant difference from vehicle response. ⁺ $p < 0.05$, = significant difference from cocaine + vehicle related response.

Table 1

Effects of cocaine and the combination of cocaine and guanfacine on Latencies, Omissions, and Trials Completed in the Standard Five Choice Serial Reaction Time Task in Rats

Treatment	Latency Correct (s)	Latency Incorrect (s)	Magazine Latency (s)	Omissions	Trials Completed
Vehicle + Vehicle	0.95±0.03	2.13±0.17	1.66±0.09	2.29±0.41	99.64±0.36
Vehicle + Cocaine 3.5 mg/kg	0.88±0.02	1.64±0.13	1.72±0.10	2.93±0.63	97.29±1.52
Vehicle + Cocaine 7.5 mg/kg	0.83±0.02*	1.70±0.16*	1.86±0.13	2.57±0.62	97.64±1.52
Vehicle + Cocaine 15.0 mg/kg	0.71±0.03*	1.47±0.12*	1.62±0.11	8.64±2.12*	93.79±2.84
Guanfacine 0.1 mg/kg + Cocaine 15.0 mg/kg	0.77±0.03*	1.36±0.10	2.05±0.24	3.71±1.04	78.86±5.35**
Guanfacine 0.3 mg/kg + Cocaine 15.0 mg/kg	0.89±0.03#	1.57±0.12	2.05±0.19	9.14±2.39*	90.93±3.74
Guanfacine 0.6 mg/kg + Cocaine 15.0 mg/kg	1.16±0.04#	2.18±0.20#	2.62±0.26**	19.29±2.69**	86.71±3.74*
Guanfacine 1.0 mg/kg + Cocaine 15.0 mg/kg	0.89±0.04#	2.54±0.27#	2.77±0.41**	21.43±2.85**	72.43±3.44**

Data are presented as the mean ± SEM.

* p<0.05; statistically significant difference from Vehicle + Vehicle treated group;

statistically significant difference (p<0.05) from the Vehicle + Cocaine 15.0 mg/kg-treated group. N=14

Effects of guanfacine on Latencies, Omissions, and Trials Completed in the Variable Intertrial Interval (VTI) Version of the Five Choice Serial Reaction Time Task in Rats

Table 2

Treatment	Latency Correct (s)	Latency Incorrect (s)	Magazine Latency (s)	Omissions	Trials Completed
Vehicle	1.14±0.08	2.09±0.10	1.57±0.08	9.10±1.37	62.31±3.95
Guanfacine 0.1 mg/kg	0.98±0.06	1.89±0.16	1.67±0.07	13.93±2.20	71.43±5.54
Guanfacine 0.3 mg/kg	1.00±0.05	2.11±0.22	1.69±0.12	16.07±2.42*	73.79±4.86
Guanfacine 0.6 mg/kg	1.04±0.03	2.25±0.15	1.92±0.15*	22.14±2.96*	72.29±4.48
Guanfacine 1.0 mg/kg	1.12±0.04	2.73±0.13*	2.16±0.15*	22.57±1.59*	66.71±3.61

Data are presented as the mean ± SEM.

* p<0.05; statistically significant difference from Vehicle + Vehicle treated group. N=14

Table 3

Monkey Subject Information

Subject ID	Gender	Age	Wt (kg)	Delay intervals (sec)	
				Short	Long
c8r	M	14	11.6	5	20
p18	F	17	8.4	5	60
v6t	M	15	17.2	5	60
pa1	M	20	16.2	5	30
p2j	M	17	13.2	5	100
770	F	25	7.6	5	40
119	F	24	8.6	10	80
Mean				5.7	55.7
SEM				0.7	10.7

Effects of guanfacine, cocaine, and the combination of guanfacine and cocaine and on response latencies in the Delayed Match to Sample (DMTS) Task in Monkeys.

Table 4

Treatment	Response Latencies (sec)					
	Sample (Correct)			Sample (Incorrect)		
	Percentile			Percentile		
	Median	25	75	Median	25	75
Vehicle	1.23	0.82	2.12	1.09	0.84	2.21
Guanfacine 0.2 mg/kg	1.20	0.91	2.43	1.43	0.96	2.31
Guanfacine 0.4 mg/kg	1.60*	1.40	2.84	1.74*	1.27	3.48
Guanfacine 0.6 mg/kg	1.92*	1.56	4.32	1.53*	1.37	4.70
Vehicle	1.85	0.84	2.34	1.81	0.83	2.18
Cocaine 0.5 mg/kg	1.27	0.87	2.24	1.35	0.85	2.76
Cocaine 1.0 mg/kg	1.30	0.67	2.40	1.31	0.69	1.88
Cocaine 2.0 mg/kg	1.84	0.80	2.86	1.71	0.84	2.65
Cocaine 4.0 mg/kg	1.65	1.27	4.20	1.84	1.30	4.43
Vehicle + Vehicle	1.54	0.83	2.00	1.44	0.85	2.14
Vehicle + Cocaine 4.0 mg/kg	1.65	1.27	4.20	1.84	1.30	4.43
Guanfacine 0.2 mg/kg + Cocaine 4.0 mg/kg	1.63	1.11	2.11	1.48	0.98	1.87
Guanfacine 0.4 mg/kg + Cocaine 4.0 mg/kg	1.71	1.38	2.28	1.45	1.28	2.75
Guanfacine 0.6 mg/kg + Cocaine 4.0 mg/kg	0.90	0.68	6.42	0.89	0.70	7.73

	Choice (Correct)			Choice (Incorrect)		
	Percentile			Percentile		
	Median	25	75	Median	25	75
Vehicle	1.58	1.50	1.75	2.04	2.00	2.51
Guanfacine 0.2 mg/kg	1.76	1.43	1.82	2.12	1.87	2.43
Guanfacine 0.4 mg/kg	1.78*	1.37	2.25	2.30	2.21	2.64
Guanfacine 0.6 mg/kg	1.87*	1.64	2.28	2.55*	2.48	3.12
Vehicle	1.70	1.59	2.32	2.20	2.04	2.64
Cocaine 0.5 mg/kg	1.42	1.22	1.58	2.09	1.64	2.67

	Choice (Correct)			Choice (Incorrect)		
	Percentile			Percentile		
	Median	25	75	Median	25	75
Cocaine 1.0 mg/kg	1.80	1.40	2.05	2.37	1.97	2.85
Cocaine 2.0 mg/kg	1.81	1.48	2.50	3.32	2.24	3.79
Cocaine 4.0 mg/kg	1.54	1.25	1.83	2.19	1.59	2.51
Vehicle + Vehicle	1.52	1.42	1.82	2.07	1.86	2.38
Vehicle + Cocaine 4.0 mg/kg	1.54	1.25	1.83	2.19	1.59	2.51
Guanfacine 0.2 mg/kg + Cocaine 4.0 mg/kg	1.60	1.28	1.83	1.86	1.35	2.91
Guanfacine 0.4 mg/kg + Cocaine 4.0 mg/kg	1.68	1.18	2.55	2.06	1.68	9.11
Guanfacine 0.6 mg/kg + Cocaine 4.0 mg/kg	1.68	1.24	2.90	2.28	1.97	9.81

* significantly different from vehicle control

Table 5

Effects of guanfacine, cocaine, and the combination of guanfacine and cocaine and on the number of trials completed and the session duration in the Delayed Match to Sample (DMTS) Task in Monkeys.

Treatment	Trials Completed		Session Duration (min)	
	(Mean $\pm$ S.E.M.)		(Mean $\pm$ S.E.M.)	
Vehicle	94.67 $\pm$ 1.07		52.14 $\pm$ 7.27	
Guanfacine 0.2 mg/kg	96.00 $\pm$ 0.00		45.71 $\pm$ 5.53	
Guanfacine 0.4 mg/kg	96.00 $\pm$ 0.00		46.57 $\pm$ 5.72	
Guanfacine 0.6 mg/kg	96.00 $\pm$ 0.00		49.50 $\pm$ 5.67	
Vehicle	92.89 $\pm$ 2.82		55.97 $\pm$ 9.34	
Cocaine 0.5 mg/kg	96.00 $\pm$ 0.00		90.25 $\pm$ 1.87*	
Cocaine 1.0 mg/kg	79.50 $\pm$ 3.36*		98.83 $\pm$ 8.04*	
Cocaine 2.0 mg/kg	59.00 $\pm$ 6.04*		137.67 $\pm$ 13.26*	
Cocaine 4.0 mg/kg	46.25 $\pm$ 4.14*		183.58 $\pm$ 10.04*	
Vehicle + Vehicle	94.66 $\pm$ 1.25		49.90 $\pm$ 6.82	
Vehicle + Cocaine 4.0 mg/kg	46.25 $\pm$ 4.14*		183.58 $\pm$ 10.04*	
Guanfacine 0.2 mg/kg + Cocaine 4.0 mg/kg	55.67 $\pm$ 6.57*		158.83 $\pm$ 16.23*	
Guanfacine 0.4 mg/kg + Cocaine 4.0 mg/kg	49.67 $\pm$ 4.27*		168.33 $\pm$ 8.96*	
Guanfacine 0.6 mg/kg + Cocaine 4.0 mg/kg	46.20 $\pm$ 4.57*		181.00 $\pm$ 10.17*	

* significantly different from vehicle control