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Alpha-2 Adrenoceptor Activation Inhibits Phencyclidine-Induced Deficits of Spatial Working Memory in Rats

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N-methyl-D-aspartate (NMDA)/glutamate receptor antagonists, such as phencyclidine (PCP), induce behavioral abnormalities (locomotor hyperactivity, sensorimotor gating deficits, impairments of cognition) in animals that are thought to model aspects of schizophrenia. The administration of PCP increases noradrenaline transmission in the rat prefrontal cortex, a brain structure required for normal cognitive processes. Noradrenaline, in turn, works through a set of receptors that have themselves been implicated directly in NMDA antagonist-induced deficits; we recently reported that the alpha-2 agonist, clonidine, is effective at preventing PCP-induced deficits of working memory and visual attention in rats. Here, we further investigated the role for alpha-2 adrenoreceptors in the effects of PCP on spatial working memory performance. The alpha-2 agonist clonidine (0.001–0.01 mg/kg, subcutaneously (s.c.)) produced a significant amelioration of PCP-induced working memory deficits; the effects of PCP (1.0 mg/kg, s.c.), but not clonidine, were reduced in noradrenaline-depleted rats. In addition, the alpha-2A-preferring agonist guanfacine (0.05–1.0 mg/kg, s.c.) dose-dependently prevented the deficits of spatial working memory performance produced by PCP. Although the highly selective alpha-2 receptor antagonist, atipamezole (ATI), failed to affect spatial working memory on its own, at the doses studied (0.1–0.5 mg/kg, s.c.), it dramatically enhanced the working memory deficit produced by PCP. These data indicate that alpha-2 adrenoreceptors tonically inhibit PCP-induced deficits of spatial working memory, suggesting an important role for these receptors in cognitive deficits associated with NMDA receptor hypofunction.

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INTRODUCTION

The neural mechanisms mediating cognitive deficits in schizophrenia are far from clear. Although the hypotheses separately implicate dopaminergic, cholinergic, noradrenergic, serotonergic, glutamatergic, and/or GABAergic deficits in schizophrenia sympatomatology, it is very likely that the actual circuit dysfunction in psychotic disorders involves inter-related changes in each of these systems. Indeed, studies of the neurochemical and behavioral effects of NMDA antagonists support just such a notion.

NMDA/glutamate receptor antagonists, such as PCP and ketamine, trigger cognitive deficits with face validity for certain impairments of attention and memory in schizophrenia (Grottick and Higgins, 2000; Javitt and Zukin, 1991; Jentsch and Roth, 1999; Malhotra *et al*, 1996; Newcomer *et al*, 1999; Newcomer and Krystal, 2001; Verma and Moghaddam, 1996). Increasing evidence supports the notion that neurochemical changes secondary to NMDA receptor hypofunction directly mediate these cognitive deficits, inasmuch as pharmacologic strategies working on nonglutamatergic systems can be effective at alleviating cognitive impairments in NMDA antagonist-treated animals (Jentsch and Anzivino, 2004; Verma and Moghaddam, 1996). For example, activation of dopaminergic and cholinergic systems triggered by NMDA blockade mediate both behavioral and neurotoxic effects of PCP and related drugs (Kim *et al*, 1999; Verma and Moghaddam, 1996), and an overall increase in excitatory glutamatergic transmission is thought to link NMDA receptor hypofunction to altered function of these reticular systems (Moghaddam *et al*, 1997).

Recent work has implicated the noradrenergic system in behavioral alterations produced by NMDA antagonists. Both PCP and ketamine dramatically increase noradrenaline release and turnover in brain (Bowers and Morton, 1994;

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Deutch *et al*, 1987; Kubota *et al*, 1999a; Lorrain *et al*, 2003; Rasmussen *et al*, 1991). In a recent study, Harkin *et al* (2001) demonstrated that the potent alpha-2 agonist, clonidine, strongly attenuated NMDA antagonist-induced hyperlocomotion in mice, while the potent alpha-2 receptor antagonist, yohimbine, produced opposite effects; Swanson and Schoepp (2003)reported similar effects of clonidine in rats. Alpha-2 agonists appear to exert broad actions against NMDA antagonist-induced effects as we recently reported that the alpha-2 agonist, clonidine, was also effective at ameliorating deficits of spatial working memory and visuospatial attention elicited by administration of the NMDA antagonist PCP (Jentsch and Anzivino, 2004).

The reported cognitive-enhancing effects of clonidine in PCP-treated animals (Jentsch and Anzivino, 2004) may be predictive of effects on cognitive function in schizophrenia patients produced by alpha-2 agonists (Fields *et al*, 1988; Friedman *et al*, 2001). Although clonidine and related alpha-2 agonists such as guanfacine are not as active, clinically, as they are in preclinical models, there is sufficient evidence of procognitive effects of these drugs to warrant further study. We therefore designed the current set of experiments to explore (1) the dose-dependency of the effects of clonidine in PCP-treated animals, (2) the degree to which the actions of clonidine vary with respect to the integrity of the presynaptic noradrenergic system, (3) whether guanfacine, another alpha-2 agonist, also produces an attenuation of PCP-induced cognitive deficits similar to clonidine, and (4) if a highly selective alpha-2 antagonist ATI produces effects opposite to those of clonidine.

MATERIALS AND METHODS

Subjects

Male and female Long–Evans rats (Harlan, Indianapolis IN) were used in these experiments. Subjects were housed into same-sex groups of two to three on a 14.5/9.5 h light/dark cycle. Training and testing were conducted only during the light phase.

The rats were 55–75 days old at the initiation of testing. Food restriction was initiated 3 days before training began, during which time they were exposed to small quantities of the reinforcer (45 mg Bio-Serv dustless precision pellets; Bio-Serv, Frenchtown, NJ). After training began, they were fed $\sim$10–15 g/rat after the daily test sessions. Water was always available in the home cage. The experimental protocols used were consistent with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and were approved by the Chancellor's Animal Research Committee at the University of California, Los Angeles.

Behavioral Testing Apparatus

The design of the behavioral testing chambers is based upon that described by Carli *et al* (1983). The apparatus (Model MED-NPW-9L; MED Associates, St Albans, VT) is an extra-tall aluminum and Plexiglas operant conditioning chamber. On one wall of the chamber is a pellet delivery magazine equipped with a cup light and an infrared beam sensor. The wall opposite the magazine is curved and is fitted with five nose-poke apertures, each of which is equipped with an internal LED lamp and an infrared beam sensor. The chambers are housed in sound-attenuating cubicles with background white noise broadcast during the session and are illuminated by a house light (a light diffuser placed outside the chamber, but inside the cubicle).

Initial Training

Training started with a single 45 min session of magazine training, during which the house light was on constantly and pellets were delivered into the magazine on a fixed-time 20-s schedule. On the second day of training, rats were reinforced with a single food pellet for making a nose-poke response into an illuminated aperture. With the house light on, an aperture was selected at random and illuminated for up to 30 s. If the rat made a response (nose poke) into the illuminated aperture before the 30 s expired, the aperture light was extinguished, the magazine was illuminated, a pellet was delivered, and a correct response was recorded. An entry into the magazine would extinguish the magazine light and initiate the start of a new trial 5 s later. If, instead, the rat made a response into an unlit aperture, an incorrect choice would be scored, a 3-s time out would ensue, and a new trial would begin 5 s later. If the rat failed to respond during stimulus presentation, an omission would be recorded, a time out would be initiated, and a new trial would begin 5 s later. Each session terminated after 100 trials were completed or after 45 min elapsed, whichever came first. Each rat was trained on this procedure until it reached a session accuracy of at least 80% (accuracy was defined as the number of correct responses divided by the total number of correct and incorrect responses) with no more than 20% omissions (total number of omissions as a proportion of all trials).

After meeting the aforementioned criterion, the duration of illumination of the nose-poke aperture on individual trials was reduced to 5 s. As above, a response into the illuminated aperture during signal presentation resulted in a food pellet being delivered, while an incorrect response (into a darkened aperture) or no response within the 5-s period led to a time out. Each rat was again trained until achieving an accuracy of at least 80% with no more than 20% omission, after which it was advanced to the delayed nonmatch-to-sample task.

Delayed Nonmatch-to-Sample Training

Each trial in the delayed nonmatch-to-sample task consisted of both a sample phase and a choice phase. During the sample phase, one aperture is illuminated temporarily, followed by a delay period in which all aperture lights are extinguished. After the delay, two apertures are illuminated (including the sample hole) and the rats' job is to nonmatch, or respond into the aperture not illuminated during the sample phase. The inter-trial interval was 5 s, and the task ended after 125 trials were completed or 60 min passed, whichever came first. The task utilized here is operationally similar to a recent task described by Chudasama and Robbins (Chudasama *et al*, 2004; Chudasama and Robbins, 2004a), with the exception that

their task uses shorter stimulus durations (to tax attentional processes) and involves a match contingency.

In the sample phase, one of the five apertures was chosen at random and illuminated for up to 5 s. A response into the illuminated aperture (correct sample-phase choice) caused the aperture light to extinguish and the magazine to be illuminated. The magazine remained lit until an entry into the magazine was detected, after which the choice phase was initiated (see below). Alternatively, a response in an unlit nose-poke aperture (incorrect sample-phase choice) or a failure to respond within 5 s (sample-phase omission) resulted in the trial being aborted, and a 5 s time-out period ensued.

During the choice phase of the trial, two apertures were illuminated: the sample aperture (the match location) and a new aperture selected at random (the nonmatch location). Both of these apertures were illuminated for up to 15 s. A response in any aperture other than that of the nonmatch location would result in a 5-s time out, and an incorrect response would be recorded. Failure to make a response within 15 s would result in a time out. Responses to the nonmatch location triggered magazine illumination, and in some cases, pellet delivery. All correctly completed trials that followed a correctly completed trial were reinforced, while none of the correctly completed trials that followed an incorrectly completed trial were reinforced. At 5 s after entry into the magazine, a new trial would commence.

For all completed trials, the actual interval between the response in the sample phase and the response in the choice phase was considered to be the trial-specific 'delay'. This means that the range of encountered delays could vary from animal to animal during training. During training, trials were binned according to delay to evaluate the relationship between delay and performance.

In order to standardize encountered delays on treatment days, we implemented sessions in which 'minimum' delay periods were enforced. The minimum delays were either 0, 5, or 10 s, and the delay period administered was selected at random from trial to trial. On nontreatment days, animals were tested on the free delay period described above.

Experiment 1

In this experiment, we sought to determine whether the cognitive-enhancing effects of the alpha-2 agonist, clonidine, in PCP-treated animals (Jentsch and Anzivino, 2004) was altered by prior exposure to a noradrenergic neurotoxin. To address this issue, rats were trained to perform the spatial working memory task before being distributed into two groups (with similar numbers of male and female subjects in each group), based upon their baseline performance measures; they were subsequently treated with either saline (2 ml/kg, i.p.) or the noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4; 40 mg/kg in 2 ml/kg saline, i.p.; Sigma, St. Louis MO). This dosage of DSP-4 was chosen because of its ability to produce a neurochemically selective but substantial reduction in forebrain tissue noradrenaline levels (Tellez *et al*, 1999).

At 7 days after DSP-4 injection, testing was resumed, and several baseline sessions were delivered in order to determine whether any performance differences could be measured that were related directly to DSP-4 treatment. After this baseline period, the effects of clonidine (0, 0.001, or 0.01 mg/kg) and PCP (0 or 1.0 mg/kg) were determined using a 3×2 *within-subject* design. Clonidine (as the hydrochloride; Sigma) and PCP (as the hydrochloride; Sigma) were dissolved in 0.9% sterile saline and injected s.c. at 30 min and 10 min prior to testing, respectively. The order of doses was determined by a cyclic Latin square design. The ordering of doses was balanced across the two groups (control *vs* DSP-4), and 3–4 days of drug-free training separated each drug challenge.

After completion of the behavioral tests conducted above, conscious rats were killed, during the light phase, by rapid decapitation. Brains were extracted, and the prefrontal cortex was dissected on a cold platform and immediately stored at -80°C. The tissue samples were later analyzed by high-pressure liquid chromatography with electrochemical detection (HPLC-EC) precisely as described previously (Jentsch *et al*, 1997b). Tissue protein levels were determined spectrophotometrically according to Lowry's method (Lowry *et al*, 1954). Total tissue levels of noradrenaline, dopamine, and serotonin (in ng) were calculated and expressed as a proportion of the total protein content of the sample.

Experiment 2

In Experiment 2, the degree to which an alpha-2A-preferring agonist could mimic the ability of clonidine to attenuate PCP-induced deficits of spatial working memory was assessed. A total of 12 rats were used to evaluate whether guanfacine hydrochloride (Sigma) blocked deficits of spatial working memory performance induced by PCP. Rats were treated with 0.9% saline or guanfacine (0.5 or 1.0 mg/kg, s.c.) 30 min prior to testing, followed by 0.9% saline or PCP (1.0 mg/kg, s.c.) 10 min prior to testing. The order of treatment conditions were given in a cyclic Latin square design (within-subject design), and at least 3–4 days of drug-free training separated each drug challenge. Based upon the substantial sedating effects of these higher doses of the drug, a follow-up study was executed in which saline or lower doses of guanfacine (0.05 or 0.1 mg/kg, s.c.) were used as a pretreatment to saline or PCP (1.0 mg/kg, s.c.). Again, the orders of treatments were given in a cyclic Latin squares design, and 3–4 days of drug-free training separated each drug challenge.

Experiment 3

Experiment 3 was designed to evaluate whether an alpha-2 receptor antagonist could alter PCP-induced deficits of spatial working memory in a manner symmetrical to that produced by the alpha-2 agonist clonidine. The effects of the alpha-2-selective antagonist ATI (Orion-Pharma, Turku, Finland) given alone, or in combination with PCP, on spatial working memory performance were determined in a set of nine rats. For these subjects, saline (1 ml/kg) or ATI (0.1 or 0.5 mg/kg) was administered s.c. 30 min prior to testing (doses based upon Millan *et al*, 2000), followed by an injection of saline or PCP (1.0 mg/kg) 10 min prior to test. All rats received all treatments, the order of which was

balanced using a cyclic Latin square design, and 3–4 days of drug-free training separated each drug challenge.

Data Analysis

Data from the behavioral experiments were analyzed with repeated measures analysis of variance (ANOVA). For each session, multiple delays were imposed, and therefore, delay was factored in as the repeated measure for the analyses of response accuracy. In addition, all pharmacological studies were *within-subject* designs, so treatment conditions (eg saline *vs* guanfacine 0.05 *vs* guanfacine 0.1) were likewise treated as repeated measures. *Between-subject* factors included sex and group (control *vs* DSP-4), where appropriate. *Post hoc* comparisons included Tukey's tests when permitted by the presence of a significant main effect or interaction.

Data from the *post-mortem* neurochemical experiments were analyzed by unpaired *t*-tests to confirm group differences in the three parent amine levels.

RESULTS

Experiment 1: Clonidine + PCP Effects in DSP-4-Treated Animals

Baseline performance. After training on the delayed nonmatch-to-sample task, rats were treated systemically with saline (2 ml/kg, i.p.) or DSP-4 (40 mg/kg, i.p.). They were first tested 10 days after toxin treatment to determine whether a reduction in forebrain noradrenaline affected baseline working memory performance. As expected, ANOVA revealed a highly significant main effect of delay ($F_{(2,28)} = 10.3$, $p < 0.001$); performance ranged from ~85–90% at delays less than 5 s to between ~70 and 75% at delays more than 10 s, supporting the validity of the test as a measure of working memory. At 10 days after toxin treatment, there were no measured differences between DSP-4 and control rats for choice accuracy (Figure 1), as revealed by no main effect of group or no group × delay interaction. Additionally, there were no main effects of sex or no higher level interactions involving sex for response accuracy (Figure 1). The lack of effect of DSP-4 treatment on working memory performance, which is generally consistent with earlier observations by Wenk *et al* (1987), persisted for the duration of testing (more than 60 days; see Figure 2).

At 10 days after DSP-4 exposure, omission rates (either for the sample or choice phases) were also not affected by sex (F's < 1) but were affected by DSP-4 treatment (sample omissions: $F_{(1,14)} = 14.6$, $p = 0.002$; choice omissions: $F_{(1,14)} = 5.1$, $p < 0.05$). Both main effects of DSP-4 treatment were mediated by significant reductions in omissions in the depleted animals, relative to normal control rats. The effects of DSP-4 treatment on omissions did not vary with respect to sex.

Clonidine/PCP effects on choice accuracy. Subsequently, the rats were challenged with clonidine (0, 0.001 mg/kg or 0.01 mg/kg, i.p.) followed by PCP (0 or 1 mg/kg, s.c.) in a 3 × 2 *within-subject* design (Figure 2). Overall repeated measures ANOVA revealed main effects of delay

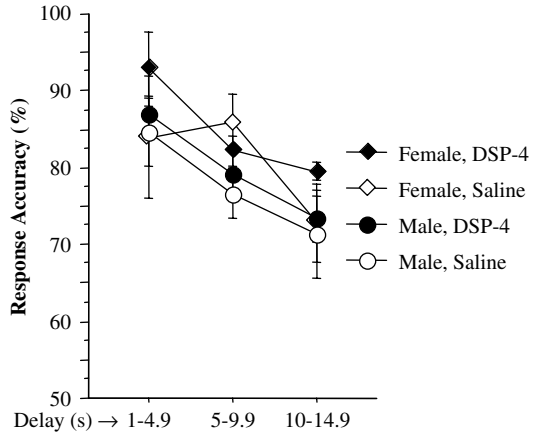

Figure 1 Curves describing the response accuracy–delay function for male and female rats exposed to either saline (as a control) or DSP-4 (40 mg/kg, i.p.; to reduce forebrain noradrenaline levels). Testing was conducted 10 days after the dosing. No main effects of sex or treatment group were apparent, indicating that the amount of noradrenaline depletion produced in this experiment did not affect working memory performance, on its own. Data represent means ± SEM. Significant differences between saline and PCP-treated rats are indicated as follows: ***$p < 0.0001$; *$p < 0.05$. $N = 9$ control rats; $N = 9$ DSP-4 rats.

($F_{(2,30)} = 52.1$, $p < 0.0001$), as well as effects of both clonidine dose ($F_{(2,30)} = 6.3$, $p < 0.01$) and PCP treatment ($F_{(1,15)} = 51.2$, $p < 0.0001$). There was a statistically significant interaction between clonidine dose and PCP dose ($F_{(2,30)} = 4.0$, $p < 0.05$). Based upon the clonidine dose × PCP treatment interaction, follow-up ANOVA were performed to determine whether PCP evoked a deficit, relative to saline challenge, after saline, clonidine 0.001 mg/kg, or clonidine 0.01 mg/kg. These analyses revealed that PCP treatment significantly impaired performance in rats pretreated with saline (Figure 2a; $F_{(1,17)} = 30.9$, $p < 0.0001$) and 0.001 mg/kg clonidine (Figure 2b; $F_{(1,16)} = 14.7$, $p < 0.01$) but not 0.01 mg/kg clonidine (Figure 2c). These data support our earlier report that PCP impairs working memory performance and that clonidine dose-dependently attenuates this effect (Jentsch and Anzivino, 2004).

Overall ANOVA also revealed a significant PCP treatment × group interaction ($F_{(1,15)} = 8.5$, $p < 0.01$), in the absence of a clonidine dose × group interaction. This interaction indicates that DSP-4 treatment altered the behavioral effects of PCP but not clonidine. In rats not receiving clonidine, PCP evoked a performance deficit in both control ($p < 0.001$) and DSP-4-pretreated ($p < 0.05$) rats; however, the drug-induced decline in performance was less substantial in DSP-4-pretreated rats than in control subjects.

In this experiment, there were no main effects of sex or no higher level interactions involving sex, for the choice accuracy measure (all F's < 1). Therefore, sex did not affect response accuracy, which is related to spatial working memory performance, nor did it affect the response to clonidine or PCP.

Clonidine/PCP effects on omissions. Clonidine, within this dose range, sedated the subjects, while PCP stimulated them (Figure 3). Considering first sample omissions (Figure 3a), repeated measures ANOVA revealed separate main effects

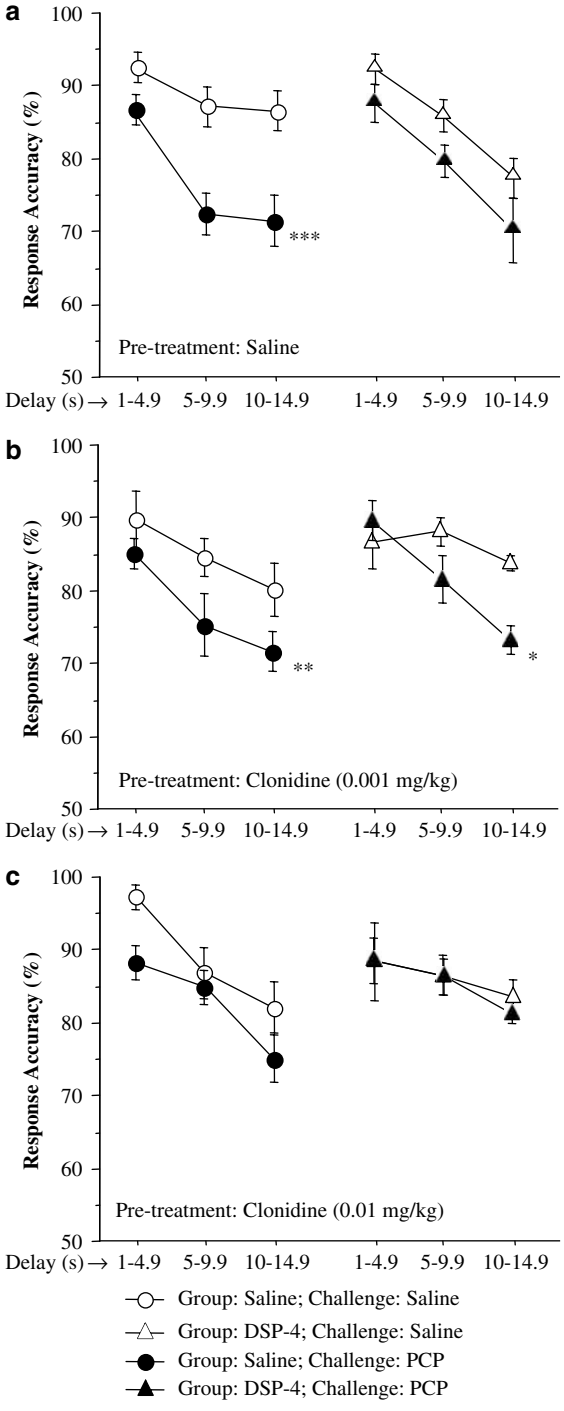

Figure 2 Clonidine (0, 0.001, or 0.01 mg/kg, s.c.) and PCP (0 or 1.0 mg/kg, s.c.) were administered alone, and in combination, to control rats or to subjects previously treated with DSP-4 (40 mg/kg, i.p.) to decrease forebrain noradrenaline concentrations (Table 1). PCP impaired response accuracy in the spatial working memory test, and clonidine dose-dependently reversed that deficit. PCP appeared to elicit a less substantial deficit of working memory in DSP-4 relative to control rats. Data are not split by sex due to no significant effects of sex or interactions involving sex. Data represent means ± SEM. Significant differences between saline and PCP-treated rats are indicated as follows: ***$p<0.0001$; **$p<0.01$; *$p<0.05$. $N=9$ control rats; $N=9$ DSP-4 rats.

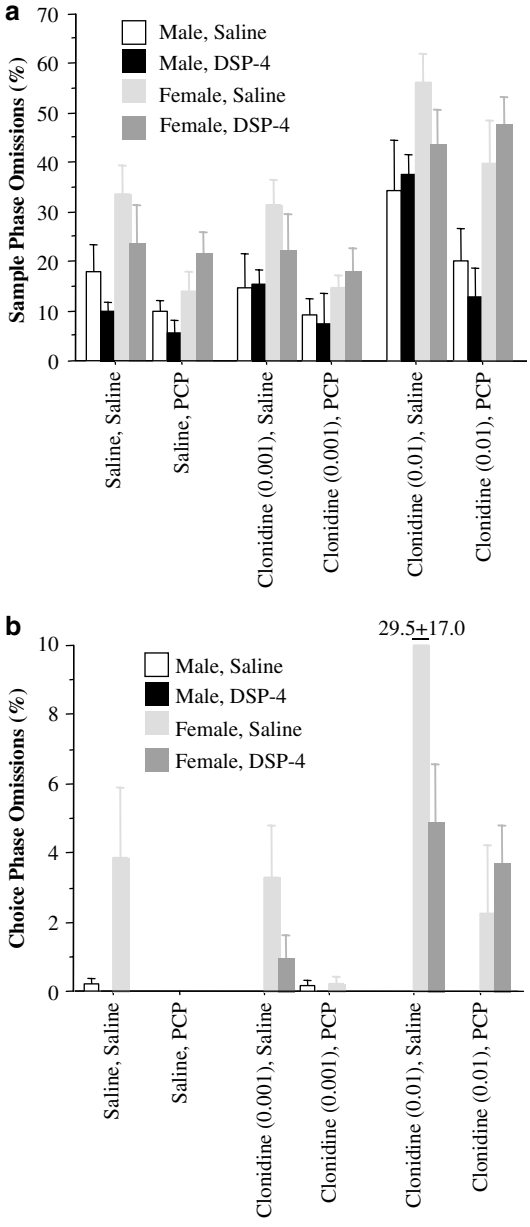

Figure 3 Female rats made more sample-phase omissions than male rats, and clonidine increased, while PCP decreased, these omissions. Sedating effects of clonidine were limited to the highest dose (0.01 mg/kg, s.c.). Although more rare in frequency, choice-phase omissions were also affected by sex (females made more omissions than males), clonidine (dose-dependent increase), and PCP (reduction). Data represent means ± SEM. $N=9$ control rats; $N=9$ DSP-4 rats.

of sex ($F_{(1,14)}=13.5$, $p=0.003$), clonidine dose ($F_{(2,28)}=43.0$, $p<0.0001$), and PCP dose ($F_{(1,14)}=22.9$, $p<0.001$); these main effects did not interact, nor did group (control *vs* DSP-4) enter into any significant interactions. Overall, females made more sample-phase omissions than males, clonidine dose-dependently increased omission rates (only the 0.01 mg/kg dose differed from saline, $p<0.0001$), and PCP decreased omission rates.

Repeated measures ANOVA were next performed on omission rates in the choice phase (Figure 3b). Choice-phase omissions were much rarer than sample omissions, occurring with a frequency of <4% at baseline. Main effects of sex ($F_{(1,14)}=6.1$, $p<0.05$), clonidine dose ($F_{(2,28)}=5.8$, $p<0.01$), and PCP dose ($F_{(1,14)}=4.7$, $p<0.05$) were

revealed, along with a statistically significant sex × clonidine dose interaction ($F_{(2,28)} = 5.9$, $p < 0.01$). Essentially, females made more omissions than males, clonidine dose-dependently increased omissions in both males and females (only the 0.01 mg/kg dose different from saline; $p < 0.01$), and clonidine more strongly increased omissions in females. An additional clonidine dose × PCP dose interaction ($F_{(2,28)} = 3.9$, $p < 0.05$) indicated that PCP reduced the increase in omissions produced by clonidine.

Group (control *vs* DSP-4) did not affect omission rates or enter into any interactions with other factors for omissions, unlike what was found at 10 days post-DSP-4. This suggests that the suppression of omissions found at the earlier time point was a transient effect of noradrenaline depletion.

Confirmation of DSP-4-induced noradrenergic depletion. After completion of the behavioral testing, the rats were killed and monoamine levels were measured in the prefrontal cortex using HPLC-EC. As predicted, ~60 days after DSP-4 treatment (40 mg/kg), prefrontal cortical noradrenaline levels were substantially (~50%) but selectively decreased (Table 1; $p < 0.0001$. by unpaired *t*-test); dopamine and serotonin levels were not significantly affected (Table 1; NS).

Experiment 2: Guanfacine + PCP Effects on Spatial Working Memory

Guanfacine (0.5 and 1.0 mg/kg). In order to further evaluate whether alpha-2 agonists, as a class, attenuate the deleterious effects of PCP on working memory, we also assessed the effects of the alpha-2 agonist guanfacine in a separate, experimentally naive cohort of rats ($n = 6$ males and $n = 6$ females). Guanfacine has slightly higher affinity for the alpha-2A *vs* alpha-2B and alpha-2C receptors (Uhlen *et al*, 1995, 1997a).

Initially, the effects of guanfacine (0.05–1.0 mg/kg, s.c.) pretreatment on PCP-induced deficits of spatial working memory were investigated. Both doses of guanfacine exerted sedating effects, complicating interpretation of their effects on spatial working memory *per se*. For these two higher doses, there was a main effect of guanfacine ($F_{(2,11)} = 105.0$, $p < 0.0001$) and a main effect of PCP ($F_{(1,11)} = 5.3$, $p < 0.05$) for total trials completed (Figure 4). *Post hoc* analyses confirmed that the main effect of guanfacine was driven by a dose-dependent *decrease* in the number of trials completed (several of the rats treated with the highest dose of guanfacine fell asleep during the 30 min drug-to-testing interval); the main effect of PCP reflected an overall *increase* in the number of trials completed by animals treated with the NMDA antagonist. These two main effects appeared to be purely additive with one another.

Table 1 Tissue Monoamine Levels from Rats Treated with Vehicle or DSP-4 (40 mg/kg, i.p.)

Group	Brain Region	Noradrenaline	Dopamine	Serotonin
DSP-4	Prefrontal cortex	2.3 ± 0.1***	1.2 ± 0.2	6.3 ± 0.4
Control	Prefrontal cortex	4.6 ± 0.1	1.2 ± 0.1	6.3 ± 0.2

All values are ng analyte/mg protein.
***Significantly different than control levels, $p < 0.0001$.

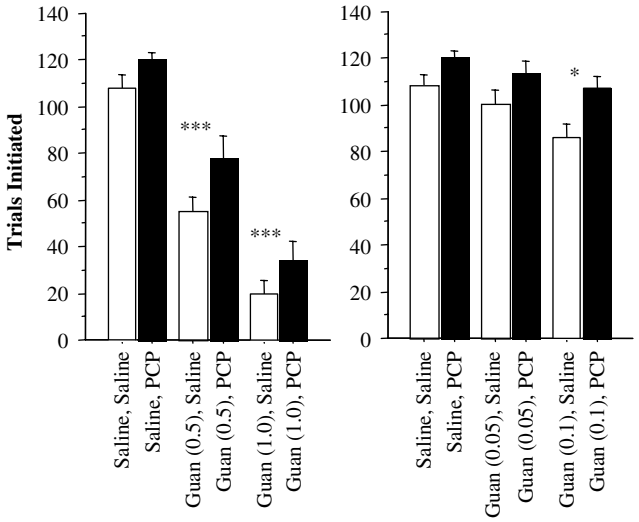

Figure 4 In two subsequent experiments, guanfacine was shown to dose-dependently suppress the total number of trials initiated by rats, evidence for substantially sedative effects of the drug. Doses of 0.1 mg/kg (s.c.) and higher reduced the total number of trials initiated. The lowest dose of guanfacine studied (0.05 mg/kg, s.c.) did not significantly affect this measure. Data represent means ± SEM. Significant differences between saline and guanfacine-treated rats are indicated as follows: ***$p < 0.0001$; *$p < 0.05$. $N = 12$ rats (six males, six females) received all treatments in a *within-subject* design.

The decrease in trials completed produced by guanfacine was associated with a main effect of agonist treatment for omissions during both the sample phase ($F_{(2,22)} = 23.6$, $p < 0.001$) and choice phase ($F_{(2,10)} = 4.9$, $p < 0.01$; one animal was excluded from this analysis because it initiated no trials after 1 mg/kg of guanfacine). In other words, the high doses of the alpha-2 agonist decreased the numbers of trials initiated and increased the total number of omissions. Owing to the substantial sedation produced by guanfacine, no clear conclusions about the effects of the high doses on cognitive performance could be drawn.

Guanfacine (0.05 and 0.1 mg/kg)/PCP effects on choice accuracy. The experiment was subsequently repeated in the same rats, using 0.05 and 0.1 mg/kg of guanfacine *vs* PCP (1.0 mg/kg). Main effects of guanfacine dose ($F_{(2,22)} = 11.2$, $p < 0.001$) and PCP treatment ($F_{(1,11)} = 8.8$, $p < 0.01$) were again detected for trials completed (Figure 4). The main effect of guanfacine dose was due to a dose-dependent reduction in trials initiated; *post hoc* comparisons demonstrated that only the 0.1 mg/kg dose differed from saline. The reduction in trial completion was not confounding for either dose because all subjects still performed a large enough number of trials (>80) to permit adequate analyses to be conducted.

Figure 5a displays the effects of 0.05–0.1 mg/kg of guanfacine on PCP-induced deficits of choice accuracy in the spatial working memory task. Repeated measures ANOVA revealed a main effect of PCP treatment ($F_{(1,10)} = 5.4$, $p < 0.05$), which statistically interacted with

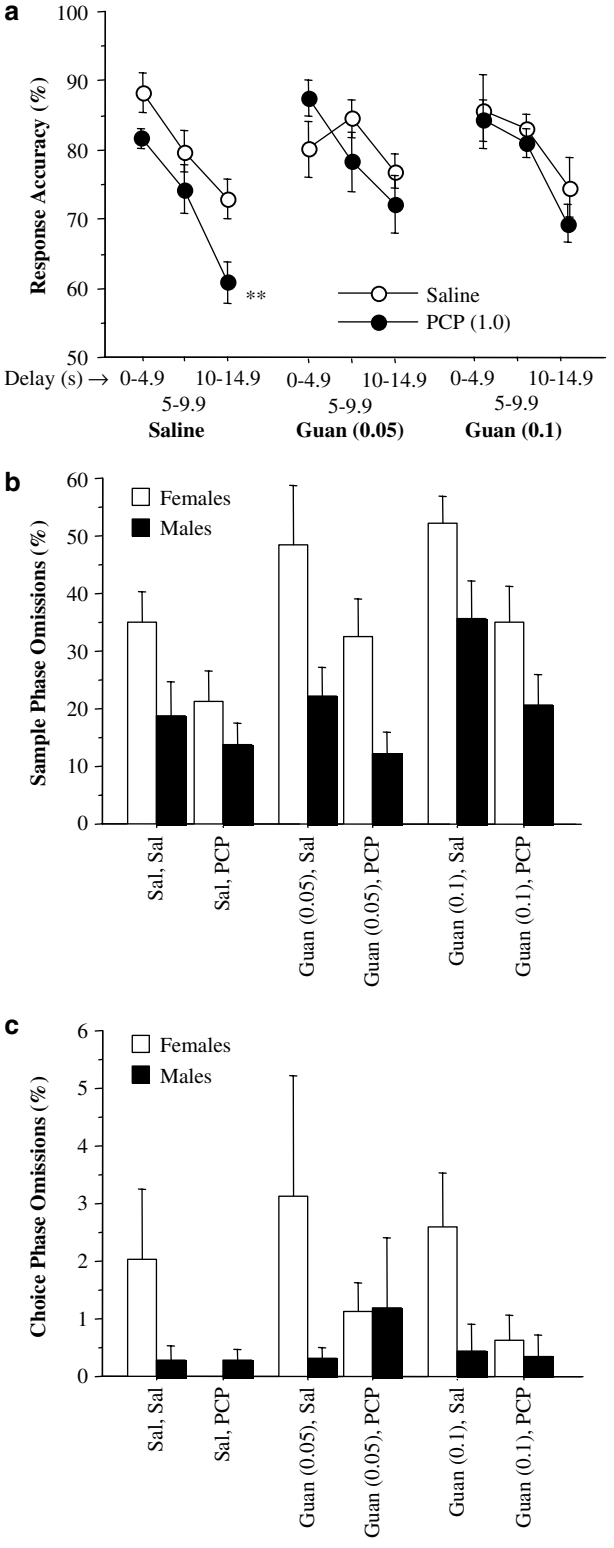

Figure 5 Choice accuracy and omissions were affected by guanfacine and PCP treatments. PCP impaired choice accuracy and reduced omissions, while guanfacine blocked the deficit in working memory performance while increasing omission rates. Data represent means ± SEM. Significant differences between saline and PCP-treated rats are indicated as follows: $^{**}p<0.01$. $N=12$ rats (six males, six females) received all treatments in a *within-subject* design.

delay ($F_{(2,20)}=4.4$, $p<0.05$). Although there was only a trend for a main effect of guanfacine dose ($F_{(2,20)}=3.1$, $p=0.07$), ANOVA did uncover a significant guanfacine dose × PCP treatment interaction ($F_{(2,20)}=3.6$, $p<0.05$). There was no main effect of sex or no significant interactions involving sex (all $p>0.05$). *Post hoc* analyses confirmed that these main effects and interactions were driven by a delay-dependent deficit of performance produced by PCP that was alleviated by pretreatment with both of the doses of guanfacine.

GUAN/PCP effects on omissions. Consistent with the dose-dependent decrease in trials initiated (see above), guanfacine dose-dependently increased sample-phase omissions (Figure 5b; $F_{(2,20)}=12.9$, $p<0.001$), while PCP reduced them (Figure 5c; $F_{(1,10)}=62.7$, $p<0.0001$). Neither of the manipulations significantly affected omissions in the choice phase.

Experiment 3: ATI + PCP Effects on Spatial Working Memory

ATI/PCP effects on choice accuracy. Response accuracy during the choice phase was significantly affected by both ATI dose (Figure 6a; $F_{(2,14)}=10.8$, $p<0.001$) and PCP dose (Figure 6; $F_{(1,7)}=74.5$, $p<0.0001$). The main effect of PCP dose statistically interacted with delay ($F_{(2,14)}=6.2$, $p=0.01$), providing evidence for a delay-dependent deficit in PCP-treated rats. In addition, ANOVA revealed a significant interaction between the main effects of ATI dose, PCP dose, and delay ($F_{(4,28)}=2.6$, $p<0.05$). *Post hoc* follow-up tests were performed to reveal the basis of this higher level interaction; pretreatment with ATI (either 0.1 or 0.5 mg/kg) failed to affect saline-treated animals, but worsened performance in PCP-treated rats under long-delay conditions ($p<0.01$ for ATI (0.1) + PCP *vs* saline + PCP and ATI (0.5) + PCP *vs* saline + PCP).

In this experiment, ANOVA revealed a main effect of sex ($F_{(1,7)}=9.4$, $p=0.01$) for the response accuracy measure. This main effect was driven by overall better performance in female than male rats. However, this main effect did not enter into any interactions with other factors including ATI dose, PCP dose, or delay.

ATI/PCP effects on omissions. Omissions during the sample phase were not significantly affected by sex or PCP dose (Figure 6b); these effects only reached the trend level ($p=0.07$ for sex and $p=0.05$ for PCP dose). Choice-phase omissions (Figure 6c), on the other hand, were significantly affected by both sex ($F_{(1,7)}=13.1$, $p<0.01$) and PCP dose ($F_{(1,7)}=8.1$, $p<0.05$) but not ATI dose ($p=0.15$). The main effects of sex and PCP dose were driven by overall higher rates of omissions in female compared to male rats and overall lower rates of omissions in PCP- compared to saline-treated rats. ATI did not influence any of these effects.

DISCUSSION

Alpha-2 agonists (clonidine and guanfacine) ameliorate the deficit of spatial working memory induced by PCP. Conversely, the deficits of working memory produced by PCP are exacerbated by pretreatment with the highly

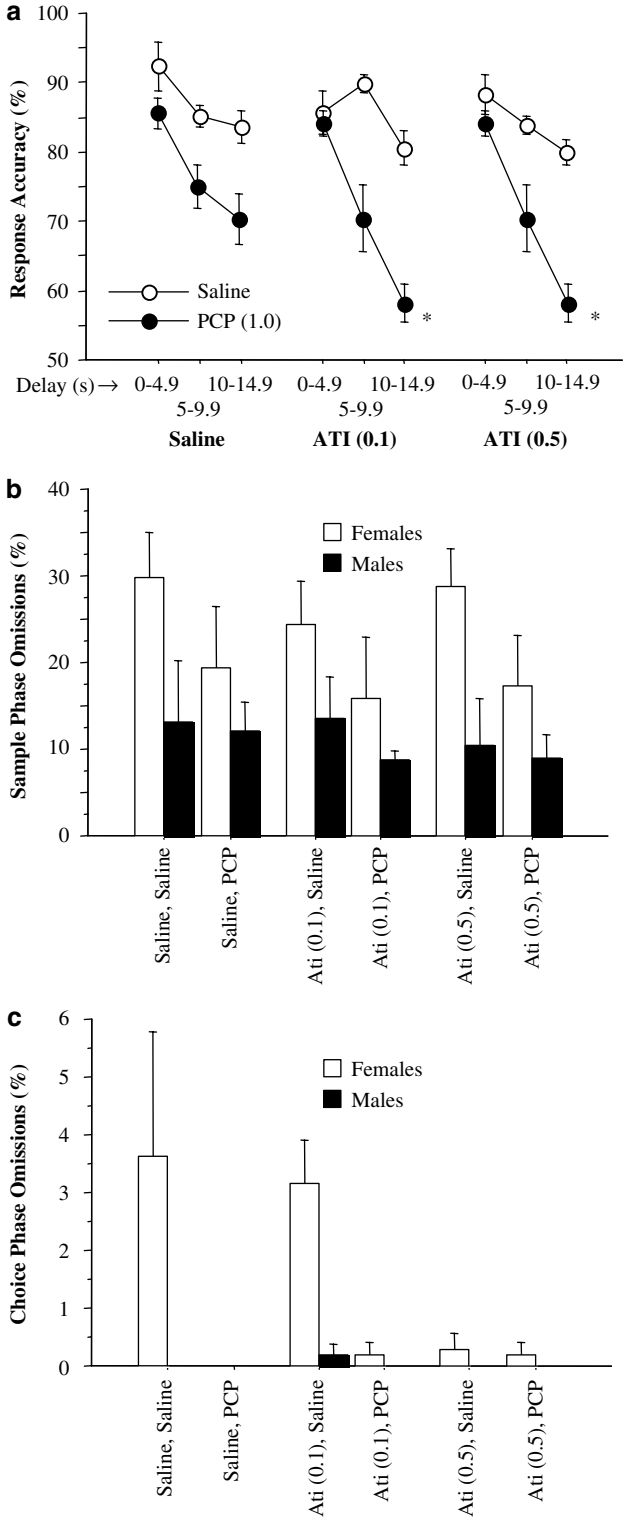

Figure 6 ATI augmented the deficits of choice accuracy produced by PCP. There were no drug interactions measured for omissions. Data represent means ± SEM. Significant differences between saline and ATI-treated rats are indicated as follows: $*p < 0.05$. $N = 12$ rats (six males, six females) received all treatments in a *within-subject* design.

selective alpha-2 receptor antagonist ATI. These data indicate that alpha-2 receptors tonically inhibit NMDA antagonist-induced deficits of cognition. Our hypothesis is that the increased release of noradrenaline that occurs after PCP administration (Bowers and Morton, 1994; Deutch *et al*, 1987; Kubota *et al*, 1999a) contributes directly to the cognitive deficits produced by the NMDA antagonist; this view is supported by the interaction between group (control *vs* DSP-4) and PCP dose, which was mediated by diminished PCP effects in animals (DSP-4 treated) with a compromised noradrenergic system.

Using a match variant of the task employed here, Chudasama and Robbins (2004a) also provided evidence for an important neuromodulatory influence of dopamine D1 agonists in working memory performance. PCP also evokes the release of dopamine into the prefrontal cortex (Bowers and Morton, 1994; Deutch *et al*, 1987; Hertel *et al*, 1995), and clonidine is effective at suppressing dopaminergic hyperactivity evoked by PCP (Jentsch *et al*, 1998). Taken together, these neurochemical and behavioral data indicate that a role for dopamine in the current results must also be considered.

Opposite Effects of Alpha-2 Agonists and Antagonists

As we reported previously, clonidine, at a dose of 0.01 mg/kg, attenuates PCP-induced deficits of working memory (Jentsch and Anzivino, 2004); this effect is found here to be dose-dependent, in that a 10-fold lower dose is without significant effect. The higher dose of clonidine studied here did produce sedation, while a lower dose (0.001 mg/kg) was neither effective against PCP nor sedating. Guanfacine, on the other hand, prevented PCP-induced deficits of working memory at a dose (0.05 mg/kg) that produced only minimally sedating effects. Of course, the dose–response curve for guanfacine employed smaller increments, so it may be that a dose between 0.001 and 0.01 mg/kg of clonidine is effective against PCP effects while also being nonsedating. This issue deserves further attention.

A concern related to these findings emerges from a report by Millan and colleagues (Newman-Tancredi *et al*, 1998); both clonidine and guanfacine exhibit weak to moderate 5-HT1A agonist properties (measured by actions on recombinant human receptors expressed in CHO cells) (Newman-Tancredi *et al*, 1998). Taken with the fact that many conventionally used alpha-2 antagonists (such as yohimbine and idazoxan) also exhibit actions at this receptor, the potential role for 5-HT1A in the cognitive effects of clonidine and guanfacine is worth considering. To address this, we show that the alpha-2 antagonist, ATI, which is highly selective for alpha-2 receptors (Newman-Tancredi *et al*, 1998), enhanced the effects of PCP on working memory performance. Therefore, a drug that acts as an alpha-2 antagonist, but which lacks any actions on the 5-HT1A receptor, produces behavioral changes opposite to those produced by clonidine and guanfacine, thereby bolstering the claim that these are alpha-2 receptor-mediated effects.

Involvement of Presynaptic Noradrenergic Mechanisms?

Alpha-2 receptors are found on both presynaptic axonal terminals and on postsynaptic targets (Aoki *et al*, 1994, 1998). Presynaptically, they can act as both autoreceptors and heteroreceptors (Cooper *et al*, 2003). One prominent

idea is that they may work presynaptically to suppress hypernoradrenergic function induced by NMDA antagonists (Bowers and Morton, 1994; Deutch *et al*, 1987; Jentsch *et al*, 1997a; Kubota *et al*, 1999b; Murase *et al*, 1992), in turn preventing a neurochemical substrate that contributes to the deficits of spatial working memory. If this hypothesis were correct, then depletion of presynaptic noradrenaline levels (produced by DSP-4 administration) should have altered responsiveness to clonidine. This hypothesis was not supported by the current experiment, which instead indicated that the effects of PCP were diminished by noradrenaline depletion. Although these results do not resolve whether a presynaptic action of clonidine on noradrenergic systems is critical or not to its ameliorative effects against PCP-induced behavioral impairments, they do suggest that a releasable pool of noradrenaline participates in cognitive deficits elicited by NMDA antagonists. Studies using more substantial noradrenaline depletions will probably be required to firmly resolve this situation.

Role for alpha-2 receptor subtypes. The current results do not resolve the question of which alpha-2 receptor subtype(s) may be involved in the beneficial effects of clonidine and guanfacine in PCP-treated rats. In the brain, the alpha-2A receptor subtype is both pre- and postsynaptically localized, while the alpha-2B and alpha-2C receptor subtypes are largely postjunctional. Both alpha-2A and alpha-2C receptors are expressed in significant amounts in the neocortical and basal ganglia structures (Renouard *et al*, 1994; Uhlen *et al*, 1997b; Winzer-Serhan *et al*, 1997a, b) needed for efficient working memory performance (Bailey and Mair, 2004; Chudasama and Muir, 1997; Floresco *et al*, 1999; Mair *et al*, 2002; Seamans *et al*, 1998). In addition, alpha-2B receptors are expressed in thalamic nuclei (Tavares *et al*, 1996; Winzer-Serhan and Leslie, 1997), and dorsomedial and anterior nuclei of the thalamus also contribute substantially to performance (Bailey and Mair, 2004; Floresco *et al*, 1999). So far, both the alpha-2A and alpha-2C subtypes have been implicated in cognition, as reflected by deficiencies in working memory performance in 2A or 2C knockout mice (Franowicz *et al*, 2002; Tanila *et al*, 1999). Therefore, all three subtypes are positioned to modulate potentially working memory function. Further studies utilizing selective antagonists and/or knockout mice will be necessary to resolve which receptor subtype regulates PCP-induced deficits of cognition.

Summary. Alpha-2 agonists are effective at reducing PCP-induced deficits of working memory in rats. Inasmuch as NMDA antagonist-induced cognitive deficits may 'model' aspects of neuropsychological impairments in schizophrenia (Chudasama and Robbins, 2004b; Jentsch and Roth, 1999; Krystal *et al*, 2002; Newcomer *et al*, 1999), these data may support further attempts to characterize the potential cognitive-enhancing effects of alpha-2 agonists in schizophrenia (Fields *et al*, 1988; Friedman *et al*, 2001). Indeed, accurate dissection of the subtype mechanisms involved may maximize the cognitive benefits of alpha-2 agonists while obviating the sedating and hypotensive side effects.

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