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Effects on spontaneous and cocaine-induced behavior of pharmacological inhibition of noradrenergic and serotonergic systems

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Abstract

Cocaine-induced increases in dopamine (DA) contribute importantly to cocaine effects on behavior but, the role of concomitant increases in norepinephrine (NE) and serotonin (5-HT) is less well understood. In order to selectively block the increases in NE and 5-HT evoked by cocaine, autoreceptor preferring low doses (0.01, 0.025 and 0.05 mg/kg) of the a2 agonist, Clonidine or the 5-HT_{1A} agonist, 8-OHDPAT were given as pretreatments 20 min prior to saline or cocaine (10.0 mg/kg) in separate groups of rats ($N=10$). With pharmacological stimulation of NE and 5-HT autoreceptors, release of these neurotransmitters would be suppressed and, therefore, less available for re-uptake blockade by cocaine. With increasing dose levels, Clonidine had marked inhibitory effects on spontaneous and cocaine-induced locomotion, grooming and rearing. 8-OHDPAT pretreatment also suppressed spontaneous locomotion, grooming and rearing; but, in contrast, did not reduce the cocaine locomotor stimulant effects. 8-OHDPAT, however, did suppress central zone entry and rearing in cocaine treated rats. Using *ex vivo* methods, we found that 8-OHDPAT selectively reduced 5-HT metabolism in the medial frontal cortex (MFC) and subcortical limbic brain. Clonidine selectively reduced NE metabolism in the MFC, but decreased both DA and 5-HT metabolism in the subcortical limbic brain without affecting NE metabolism. This diverse and broad spectrum of Clonidine effects upon neurotransmitters and behavior is striking and points-up the important, complex and integrative role of NE in brain function. While both Clonidine and 8-OHDPAT can substantially attenuate a number of cocaine behavioral effects, these inhibitory effects appear to be secondary to reductions in the behavioral baseline rather than reversals of cocaine effects.
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It has been established for sometime (Koe, 1976; Ritz et al., 1990) that cocaine binds with similar efficacy to the dopamine (DAT), norepinephrine (NET), and serotonin transporters (SERT). This interference with the neurotransmitter re-uptake process can lead to a rapid increase in extracellular dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) [e.g., Reith et al., 1997; Müller et al., 2002a). The increases in extracellular neurotransmiter levels induced by cocaine depend upon the degree of transporter binding (i.e., cocaine dose) and also on the amount of neurotransmitter being released. Consequently, cocaine regardless of dose is ineffective in extreme neuropathological conditions wherein neurons that produce the neurotransmitters are lost (e.g., the loss of dopamine neurons in Parkinson's disease). In several recent studies, we have employed pharmacological treatments at dose levels designed to stimulate autoreceptors of specific neurotransmitter neuron populations (Carey et al., 2001, 2004) to suppress neurotransmitter release. Primarily, we have targeted 5-HT_{1A} autoreceptors and have shown that low doses of the 5-HT_{1A} agonist 8-OHDPAT (Arvidsson et al., 1981; Hjorth et al., 1982), when administered prior to cocaine treatment,

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prevent the typical extracellular 5-HT increase generated by cocaine (Müller et al., 2003); whereas, the extracellular increase in DA induced by cocaine remains unaffected (Müller et al., 2002b). Employing this pharmacological approach, we have undertaken a series of studies using selective 5-HT_{1A} agonists/antagonists to target 5-HT autoreceptors in order to diminish/enhance the cocaine-induced increases in extracellular 5-HT (for a review see Müller et al., 2007).

In the present study, we used two different drug pretreatments designed to stimulate either 5-HT or NE autoreceptors, respectively, in order to selectively inhibit either 5-HT or NE neuronal activity prior to the cocaine treatment. In this way, we undertook to selectively blunt the cocaine-induced increases in either 5-HT or NE extacellular transmitter levels. We report the effects of the 5-HT_{1A} agonist, 8-OHDPAT and of the α_2 agonist Clonidine (Drew, 1976) upon spontaneous behavior and upon cocaine-induced behavioral activation. We also assessed the neurochemical effects of the 8-OHDPAT and Clonidine upon 5-HT and NE by measuring the effects of each drug upon 5-HT and NE metabolism in the medial frontal cortex and limbic subcortical brain structures as expressed in *ex-vivo* metabolite/transmitter ratios.

1. Methods

1.1. Subjects

Thirty naive male Sprague–Dawley rats from Taconic Farms (Germantown, NY), 4 months old and weighing approximately 400 g at the start of the experiments were used. Upon arrival, the animals were housed in individual 48×27×20 cm clear polycarbonate cages in a climate-controlled room at 22–24 °C with a 12-h dark and 12 h light cycle. During the first week after arrival, all animals were handled and weighed daily for 7 days. During the second week, the animals received three injections I.P. (1.0 ml/kg) and three injections S.C. (0.5 ml/kg) of 0.9% saline in order to acclimate the animals to the injection procedure. All experiments occurred during the 12-h light cycle (6 AM–6 PM). The protocol was approved by the Veterans Administration Medical Center's Subcommittee for Animal Studies (IACUC 4-E).

1.2. Drugs

Cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile distilled H_2O to a concentration of 10.0 mg/ml. Cocaine injections were administered I.P. in a volume of 1 ml/kg. 8-OHDPAT (±8-hydroxy-2-(di-*n*-propylaminotetralin)) (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile distilled H_2O to concentrations of 0.025, 0.05 and 0.1 mg/ml. Clonidine (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile distilled H_2O to concentrations of 0.025, 0.05 and 0.1 mg/ml. 8-OHDPAT and Clonidine injections were administered S.C. in a volume of 0.5 ml/kg. Saline injections (0.9% sodium chloride) were administered in a volume of 1.0 ml/kg (I.P.) as a treatment or 0.5 ml/kg (S.C.) as a pretreatment.

1.3. Apparatus

All of the behavioral tests were conducted in two square 60×60×40 cm and in two round (68 cm: diameter×40 cm: height) open-field compartments of approximately equal enclosed area. Testing was conducted in two similar subsections of the testing room with a circular and square chamber in each subsection. While we had previously found that there were no differences in activity levels related to chamber shape or room subsection, we always equated these factors across groups and treatments to eliminate any possibility that chamber shape or room subsection could be potential uncontrolled variables. In the present experiments, neither test room subsection nor test chamber shape were statistically significant variables on any behavioral measure ($P>0.05$). Closed-circuit video cameras (Sanyo VCB-5123B) were mounted 50 cm above the open-field enclosures. All signals were analyzed by a video tracking system using a distance criterion of 2 cm for a movement to be scored (Ethovision, Noldus Information Technology, Inc, Leesburg, VA). The accuracy of the system for measurement of distance was validated by moving objects a fixed distance and confirming that the tracking system generated the same distances. A central zone (CZ) comprising one-ninth of the floor area was monitored independently from the rest of the area by the computer software and was distinguished only by the computer software. Both time spent in the central zone (CZ duration) and the number of penetrations into the central zone (CZ entries) were recorded automatically. Measurement of central zone activity is pertinent to the study of cocaine effects upon open-field behavior (Carey and Gui, 1998; Franklin and Druhan, 2000). The behavioral data were imported into a PC compatible computer for statistical analysis and graphing. The walls of the chamber were white and the floor of the open-field was covered by plain white paper that was changed after each animal. Masking sound (75 dB) was provided by a white noise generator (San Diego Instruments, San Diego, CA) and was turned-on immediately prior to placement of the animal into the test chamber and turned-off upon removal from the test chamber. Each chamber was illuminated by two 12 V projection lamps placed overhead 50 cm above the chamber adjacent to the video camera. Each lamp contained a red filter. Testing was conducted under conditions of red light illumination to avoid the possible aversive quality of white light and to enhance the contrast between the subject and background as well as to reduce the animal's shadow. Testing under red light conditions is less stressful and it also facilitates locomotor activation as the rats are transferred from the ambient light of the vivarium to the red light of the testing room (Nasello et al., 1998). The animal's head was blackened with a non-toxic marker and the camera tracked only this feature of the rat's body. Tracking the location of the animals' head is of critical importance for the measurement of central zone penetrations. During each session, data were calculated every 2.5 min by the software. The computer screen tracings of the animal's patterns of locomotion were constantly present on monitors outside of the test room and saved by the software. In previous reports (Carey and Gui, 1998; Dai and Carey, 1994), we have presented the tracing of

the locomotion patterns generated by animals in this test environment. In the present study, the locomotion patterns were similar to those previously presented (Carey and Gui, 1998; Dai and Carey, 1994). The tracings recorded by the tracking system could readily be used to identify small repetitive movements. Such tracings occurred infrequently and idiosyncratically. The complete test procedure was conducted automatically without the presence of the experimenter in the testing room. In addition, a VHS VCR was connected to each camera to videotape session. The videotapes were reviewed after each session in order to validate that the recording of the tracings represented the animal's actual locomotor patterns. In addition, the videotapes were evaluated by trained observers to score behaviors not amenable to image analysis. Two experimenters uninformed of the treatment protocols scored behaviors observed in the videotapes that include facial and flank grooming and rearing behavior (both forelimbs raised off the floor or against the wall). Prior to viewing the videotapes from the experiments, the experimenters were trained on other similar videotapes until they achieved inter and intra-experimenter reliability correlations of $r > 0.9$.

1.4. Biochemical analyses

The experimental animals were sacrificed immediately following the last day of behavioral testing. The animals were placed into a plastic restraining cone (Braintree Products, Braintree, MA) and sacrificed by guillotine decapitation. In the *ex vivo* preparation of tissue samples, the brains were rapidly removed and immediately placed in a chilled mold of the rat brain which has a series of spaced slots for obtaining comparable sections of brain tissue. The first coronal section is a 3 mm thick section as measured from the frontal poles. From this section, medial prefrontal cortex tissue samples, approximately 10 mg in weight were obtained. The second coronal section is the next 3 mm block of tissue. From this section, we obtained samples of limbic structures, including the nucleus accumbens and the paraolfactory nucleus. Tissue samples from these structures are obtained by dissection under magnification.

Immediately after dissection the samples of brain tissue are weighed, placed in tubes containing 0.5 ml of 0.1 M perchloric acid and 4.5 μl of 10 μg/ml dihydroxybenzylamine (DHBA) as an internal standard, and then homogenized and centrifuged. The resulting supernatant is filtered through 0.2 μm pore filters and the extracts are stored at −70 °C until the HPLC-BC analysis, which is completed within 24 h. The tissue samples are analyzed for dopamine: DA (3-hyroxytryptamine), the dopamine metabolite, HVA (homovanillic acid): 5-HT (5-hydroxytryptamine), the 5-HT metabolite, 5HIAA (5-hydroxyindole-3-acetic acid, and also for NE (arterenol bitartrate) as well as the NE metabolite VMA (vanillymandelic acid). For the DA and 5-HT analyses, a BAS biophase column [C18 reverse phase (4.6×250 mm 5 μm)] was used. The buffer used is 0.15 M monochloroacetic acid, pH 3.1, with 2 mM EDTA and 0.86 mM SOS (sodium octyl sulfate). This is added to 35 ml acetonitrile (3.5%) to make 1 L. This solution was then filtered and degassed and 18 ml (1.8%) tetrahydrofuran (THF) was added. The mobile phase flow rate is 1.2 ml/min and a BAS 4B EC detector set at 0.8 V. NE and VMA are extracted using an Alltech Adsorbosphere catecholamine column (C 18 reverse phase, 100×4.6 mm, 3 μm). A 0.2 M sodium phosphate monobasic buffer is used at pH 2.5 with 0.25 mM SOS and 0.07 mM EDTA and the pH is adjusted with 0.2 M phosphoric acid. The mobile phase flow rate is 0.5 ml/min and a BAS/LC 4C dual electrode EC detector is used with the electrodes arranged in parallel and the potential set 0.8 V.

1.5. Procedure

1.5.1. Phase 1

Three days following completion of handling and acclimation to saline injections, all animals ($N=30$) were given five daily 20 min tests in the open-field apparatus. On the basis of individual locomotion distance scores on these tests, which were also designed to habituate the animals to the test environment, the animals were assigned to one of three groups ($N=10$) equated on total distance scores. Three days later, the groups were administered four daily 20 min tests in the open-field test environment. On day 1, all groups received two saline injections before testing: first an S.C. injection followed 20 min later by an I.P. injection. The next three tests were drug test sessions. One group (the saline group) received saline S.C. and I.P. injections prior to each test as it received on the first day. The other two groups received either 8-OHDPAT (S.C.) (8-OHDPAT group) or Clonidine treatment (S.C.) (Clonidine group) 20 min prior to testing and then saline treatment (I.P.) immediately before testing. An ascending order of drug dose administration was used for each drug on these three tests: 0.01, 0.025 and 0.05 mg/kg.

1.5.2. Phase 2

Three days after completion of the tests in phase 1, the three groups received five daily 20 min tests in the open-field. On the first test session, all groups received saline (S.C.) 20 min before testing and saline (I.P.) immediately before testing. On the next four sessions, all groups received cocaine (I.P.), 10 mg/kg, immediately before testing. The pretreatment in the first cocaine session was the same for all groups, saline S.C. 20 min prior to the cocaine injection. On the next three sessions, the groups received either saline (S.C) (saline group), 8-0HDPAT (S.C) (8-OHDPAT group) or Clonidine (S.C.) (Clonidine group) 20 min prior to testing. Cocaine (10.0 mg/kg was administered (I.P.) immediately before testing. Dose levels of 8-OHDPAT and Clonidine were the same as in phase 1: 0.01, 0.025 and 0.05 mg/kg.

1.5.3. Phase 3

Three days after the animals completed the Phase 2 tests, the three groups were again tested in the open-field test environment for 20 min on two successive days of testing. On the first day, all groups received saline (S.C.) 20 min before and saline (I.P.) immediately before testing. On the second test session, the saline group again received the same saline pretreatment/saline treatment. The other two groups received either 8-OHDPAT (0.05 mg/kg, S.C.) (8-OHDPAT group) or

Clonidine (0.025 mg/kg, S.C.) (Clonidine group) 20 min before testing and then saline I.P. immediately before testing. These dose levels were selected on the basis of the results in Phase 1 which indicated approximately equivalent inhibitory behavioral effects of 0.025 Clonidine and 0.05 8-OHDPAT. At completion of this test, each animal was immediately sacrificed so that the effects of the drug treatments upon 5-HT, NE and DA metabolism could be measured in selected brain tissue areas.

1.6. Statistical analysis

Two-Way Analyses of Variance (ANOVA) were used to assess possible drug treatment effects upon the behavioral responses. Whenever statistically significant interaction effects were obtained, One-Way ANOVAs followed by Duncan's multiple range tests were performed. Paired *t*-tests were used to make intra-group comparisons between Phase 1 and Phase 3 behavioral testing for each group. One-way ANOVA was used to make inter-group comparisons on the biochemical measurements. The $P<.05$ level of statistical significance was used for null hypothesis rejection.

2. Results

Figs. 1 and 2 summarize the findings obtained in Phases 1 and 2 of the experiment. As can be seen in Figs. 1 and 2, Clonidine and 8-OHDPAT had marked effect upon spontaneous behavior. All pretreatment effects on spontaneous behavior were statistically significant: distance ($F2,27=11.8$, $P<.001$), rearing ($F2,27=8.5$, $P<.001$), grooming ($F2,27=24.7$, $P<.001$), and CZ/meter ($F2,27=5.7$, $P<.01$). The treatment × dose interactions were also significant: distance ($F6,81=23.9$, $P<.001$), rearing ($F6,81=8.8$, $P<.001$), grooming ($F6,81=10.5$, $P<.001$), and CZ/meter ($F6,81=2.2$, $P<.05$). It is apparent in Figs. 1 and 2 that the cocaine treatment enhanced locomotion distance and increased CZ/meter but sharply suppressed grooming and had little effect on rearing. In fact, there were no statistically

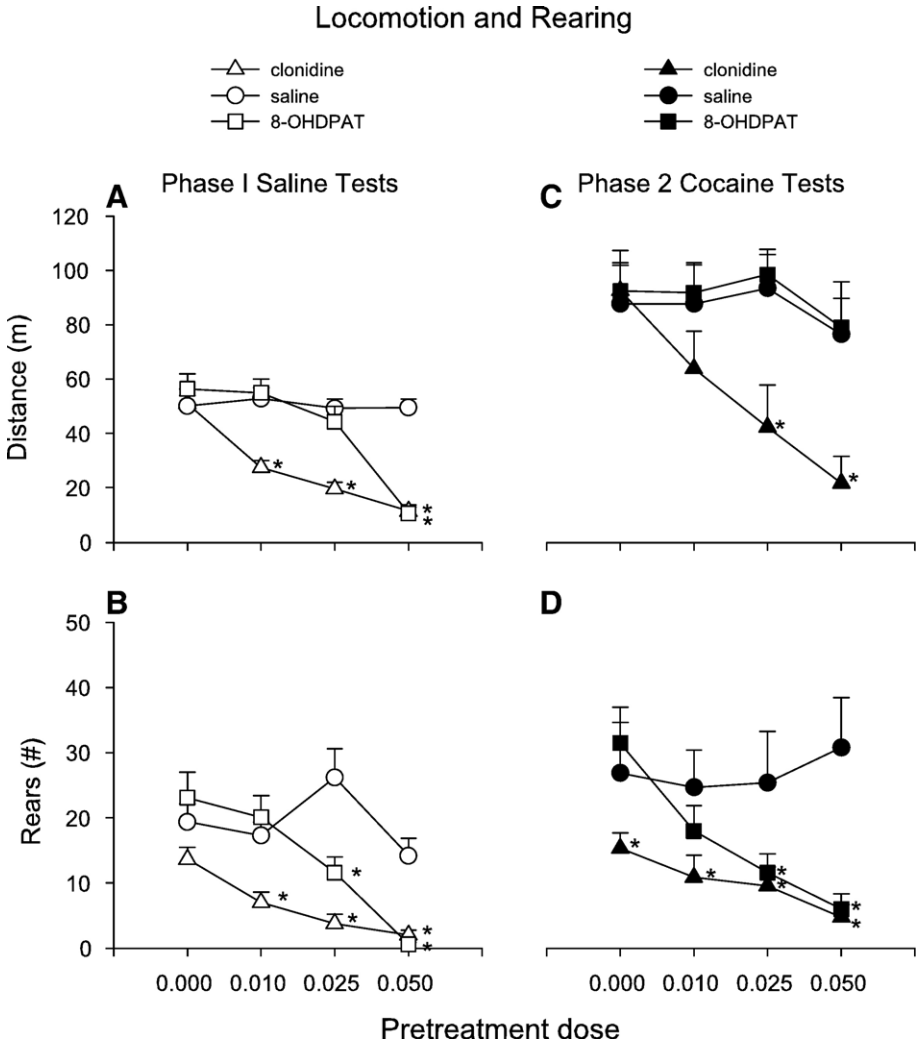

Fig. 1. Means and SEMs for: A. locomotor distance (m); and, B. rears. Panels A and B show the effects of the Clonidine and 8-OHDPAT pretreatments on (Phase 1); whereas, Panels C and D show the effects of these same pretreatments given prior to 10 mg/kg cocaine (Phase 2). For all groups, 0.0 is a saline injection. In the pretreatment sessions the saline group received saline, the 8-OHDPAT groups received, 0.01, 0.025 or 0.05 mg/kg 8-OHDPAT; and, the Clonidine groups received, 0.01, 0.025 or 0.05 mg/kg Clonidine. *$P<.05$ for comparisons *vs*. the saline group.

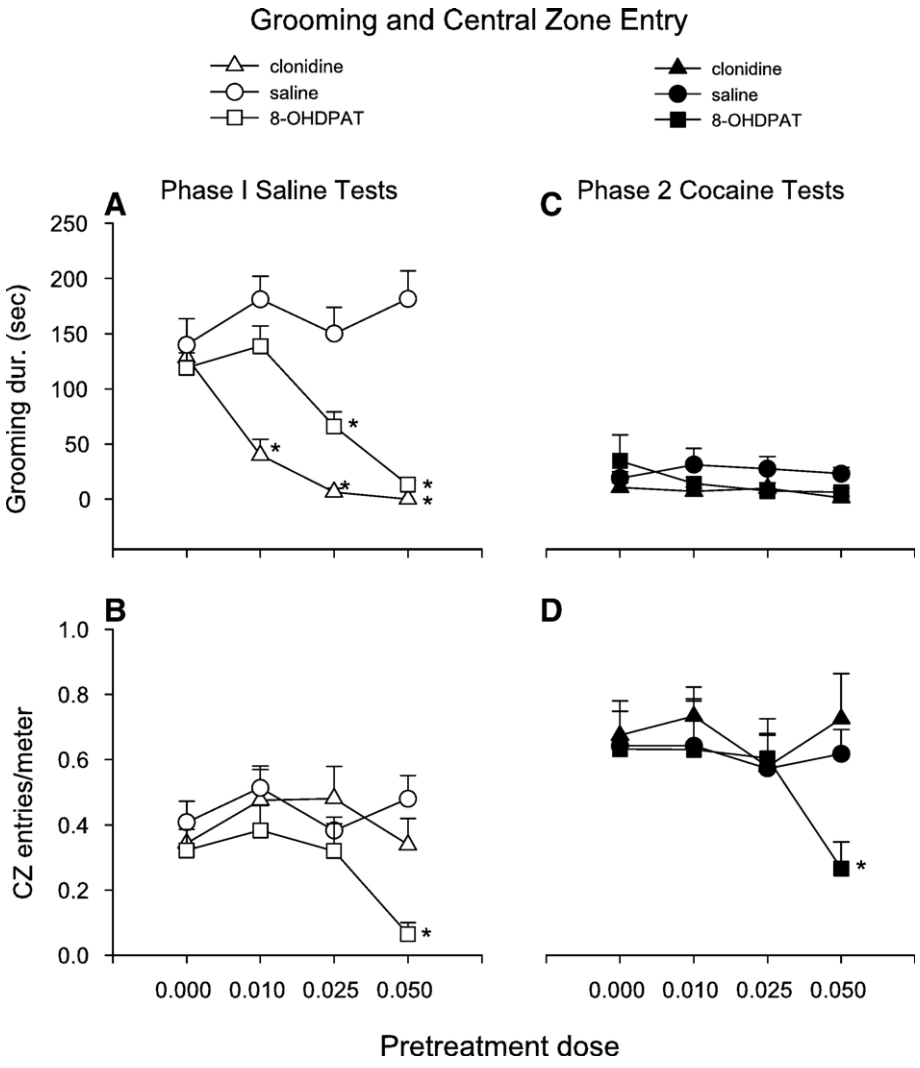

Fig. 2. Means and SEMs for: A. grooming duration; and B. central zone entry per meter of locomotion distance (CZ/m). Panels A and B show the effects of the pretreatments on spontaneous behavior (Phase 1); and, Panels C and D, the pretreatment effects on 10 mg/kg cocaine (phase 2) induced locomotor behavior. *$P<0.05$ for comparisons *vs*. the saline group.

significant drug pretreatment main effects on behavior in the cocaine test ($P>.05$). There were, however, treatment × dose interaction effects: distance ($F6,81=3.5$, $P<.01$); rearing ($F6,81=6.2$, $P<.001$); and CZ/meter ($F6,81=2.9$, $P<.01$). For grooming the treatment × dose interaction was not significant ($P>.05$). In consideration of the marked effect of Clonidine and 8-OHDPAT on spontaneous behavior, Fig. 3 presents the results such that the effects for each treatment group are compared on the saline vs. cocaine test sessions. Presented in this way, the effects of cocaine are apparent; and, it can also be seen that cocaine had similar parallel effects to changes in baseline behavior for each of the treatment groups: saline, 8-OHDPAT and Clonidine. The only exception occurred for the 8-OHDPAT group at the 0.05 dose level as 8-OHDPAT markedly suppressed locomotion in the saline test but did not reduce locomotion in the cocaine test.

Fig. 4, presents the effect of the 0.025 Clonidine and 0.05 mg/kg 8-OHDPAT pretreatments upon NE, DA, and 5-HT metabolism as reflected in metabolite/transmitter ratios for medial frontal cortex (A); and limbic subcortical tissue samples (B). These dose levels were used since the results in Phase 1 indicated that the drugs at these dose levels had similar behavioral effects. In both brain tissue samples, none of the groups differed ($P>.05$) in mean concentrations of NE, DA and 5-HT. In the frontal cortex, the dopamine metabolite HVA was not always reliably detected; so that, the HVA/DA ratios were not used in the data analysis. As can be seen in Fig. 4A, the Clonidine and 8-OHDPAT groups were differentiated on the VMA/NE and 5-H1AA/5-HT ratios ($F2,27=11.1$, 10.2, $P<.01$, respectively). Clonidine pretreatment selectively suppressed NE metabolism while 8-OHDPAT selectively suppressed 5-HT metabolism. In the limbic subcortical samples, the results for 8-OHDPAT (Fig. 4B) indicated selective suppression of 5-HT metabolism, whereas, Clonidine had a radically different effect, compared to its effect in the frontal cortex. Clonidine had no effect on the VMA/NE ratios but, rather, markedly suppressed 5-HTAA/5-HT ($F2,27=25.6$, $P<.01$) and HVA/DA ratios ($F2,27=12.3$, $P<.01$). These results indicate that Clonidine had

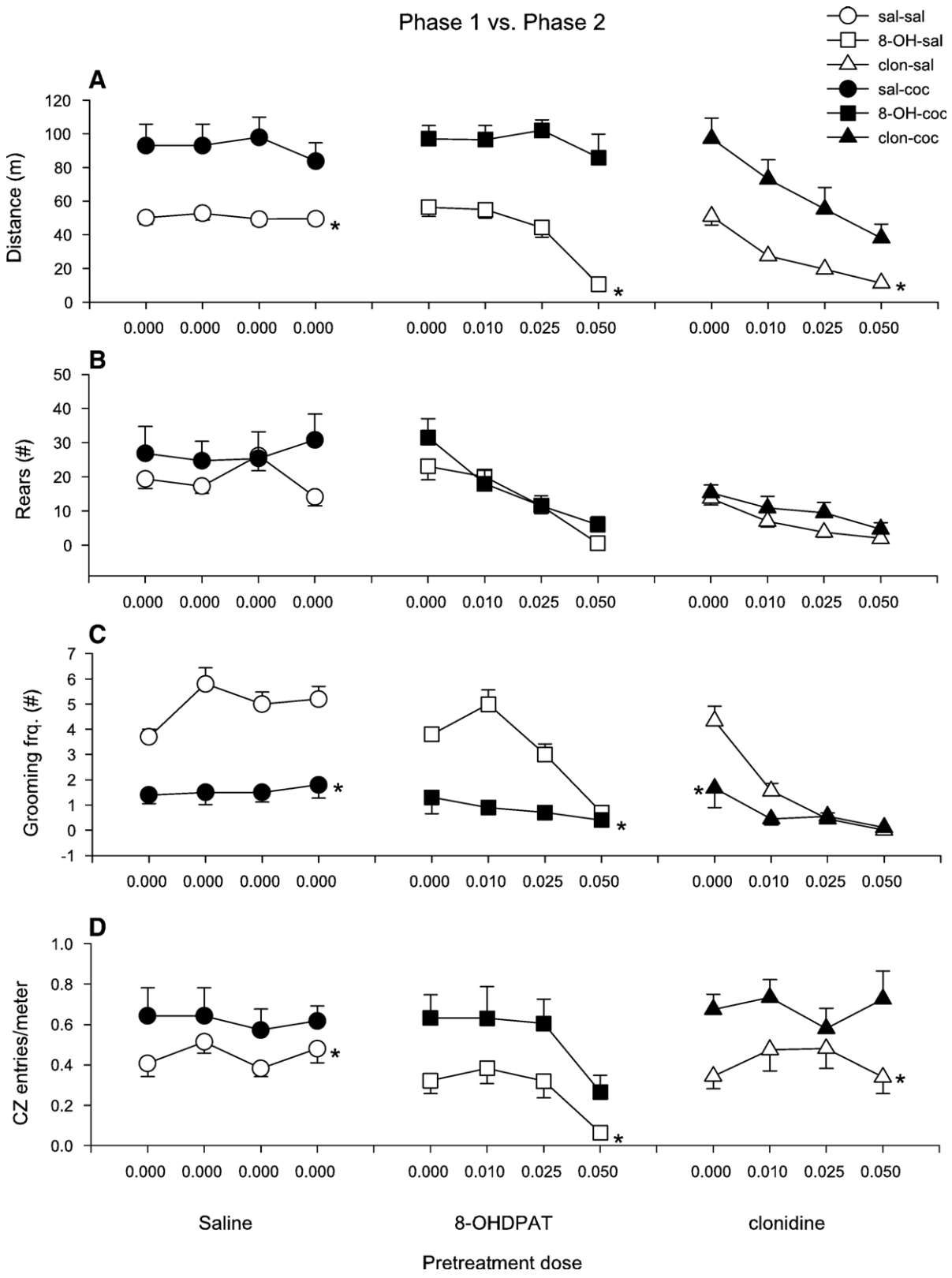

Fig. 3. Means and SEMs for: A. locomotion distance (m); B. rears; C. grooming (duration); and D. CZ entries per meter of distance traveled (CZ/m). The unfilled symbols on the left are the scores for these groups on the saline baseline test and the filled symbols are the cocaine test score. *$P<0.05$ for comparisons *vs*. the cocaine treatment.

no effect upon NE metabolism but an inhibitory effect upon DA and 5-HT metabolism. It also needs to be noted that in the behavioral test (data not shown) which preceded the collection of brain tissue samples, the effects of the pretreatments were statistically equivalent to those presented in Figs. 1 and 2 for the 0.025 mg/kg Clonidine pretreatment and for the 0.05 mg/kg

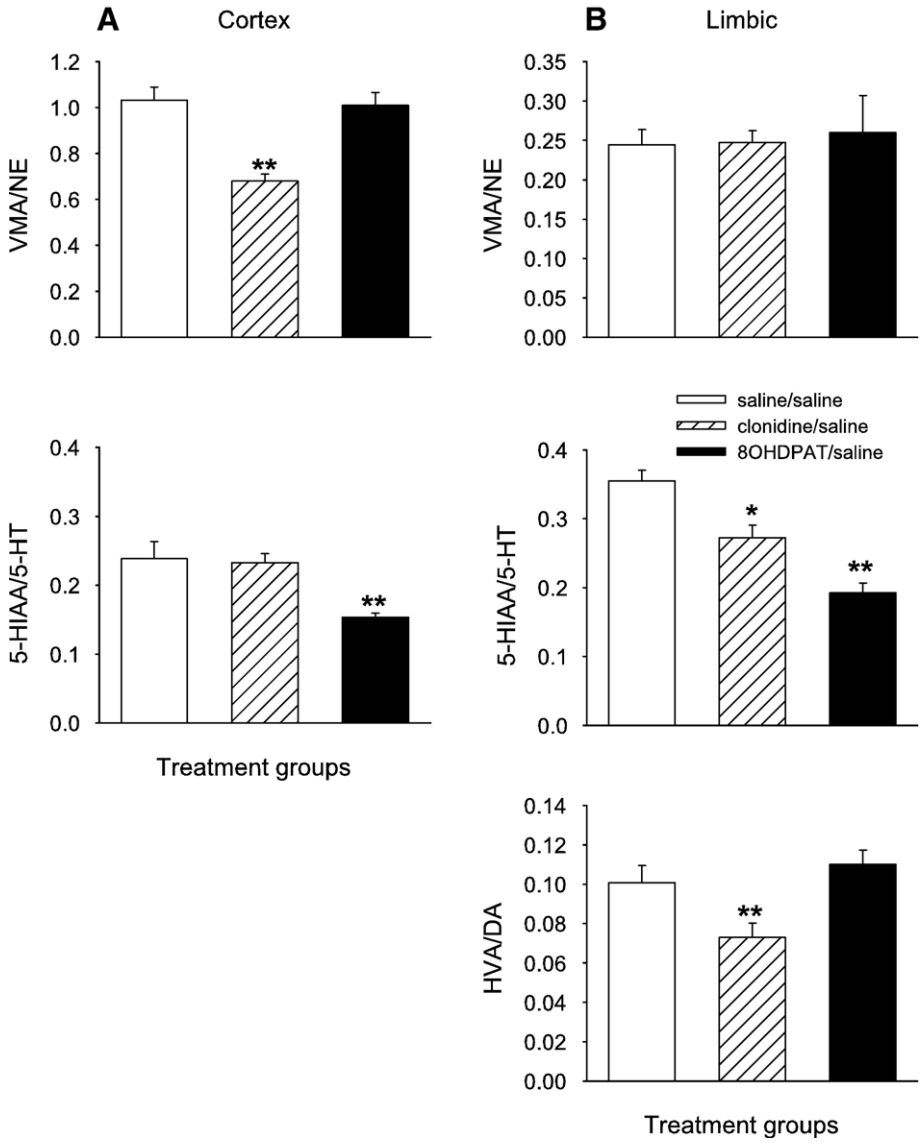

Fig. 4. Means and SEMs for *ex-vivo* VMA/NE, 5-H1AA/5-HT and HVA/DA ratios obtained from medial frontal cortex samples (left panel) and subcortical limbic samples (right panel). *$P<0.05$ *vs*. saline group. **$P<0.05$ *vs*. all other groups.

8-OHDPAT pretreatment (i.e., none of the paired *t*-tests for each group, phase 1 vs. phase 3, were statistically significant ($P>.05$). In addition, the behavioral differences between the Clonidine (0.0025 mg/kg) and 8-OHDPAT (0.05 mg/kg) were not statistically significant ($P>.05$ independent sample *t*-tests) but were different ($P<.01$) from the saline group in the test immediately preceding sacrifice.

3. Discussion

Overall, the present results show that Clonidine and 8-OHDPAT each had profound dose-related suppressive effects upon spontaneous behavior. These findings are consistent with previous reports. For all the behaviors measured, both drugs had a suppressive effect with greater sensitivity exhibited to Clonidine. In fact, at the highest dose levels, both drugs induced a virtual complete suppression of spontaneous behavior (Antelman et al., 1989; Capasso and Loizzo, 2001; Drouin et al., 2002; Carey et al., 2004). The major behavioral difference observed between the two drugs was on CZ entry. When the CZ entry scores were adjusted for locomotion distance, 8-OHDPAT but not Clonidine suppressed CZ entry. In that a decrease in central zone penetration has been frequently related to an increase in fear/anxiety processes (Ramos et al., 2003; Stefanski et al., 1992), the CZ results suggest that 8-OHDPAT which reduced 5-HT activity enhanced fear and/or anxiety. Consistent with this suggestion, we have previously reported that a different 5-HT_{1A} selective drug, WAY 10065 which is a 5-HT_{1A} antagonist, attenuated fear in an animal predator model designed to elicit fear/anxiety (Barros et al., 2003).

Cocaine elicited behavioral facilitation (increased locomotion distance and increased CZ entry) as well as behavioral suppression (decreased grooming). This overall pattern of results agrees with previous findings (Cooper and Van der Hoek,

1993; Franklin and Druhan, 2000; Carey and Gui, 1997). Our intent in conducting the present experiment was to use the receptor selective drug pretreatments in order to suppress central release of NE (Clonidine) and 5-HT (8-OHDPAT). As a consequence of such effects, cocaine would, then, have a decreased impact on either NE (Clonidine pretreatment) or 5-HT (8-OHDPAT pretreatment). The changes in behavior induced by the inhibition of NE by Clonidine and of 5-HT by 8-OHDPAT were expected to also become manifested in cocaine treated animals. In general, our findings are consistent with this assumption. The only major exception was that 8-OHDPAT at the 0.05 mg/kg dose severely inhibited locomotion in saline treated animals but had no effect in cocaine treated animals. However, as we have previously reported (Carey et al., 2004), this dose of 8-OHDPAT, when given within an ascending dose regimen, does not suppress locomotion in cocaine treated animals. If given acutely, the 0.05 mg/kg dose of 8-OHDPAT does suppress cocaine-induced locomotion (Carey et al., 2005b). On the other hand, additional pairings of 8-OHDPAT (0.05 mg/kg) with cocaine (10.0 mg/kg) can reverse this inhibitory effect of 8-OHDPAT. In the ascending dose treatment regimen of the present study, the animals had received two combined 8-OHDPAT/cocaine treatments 0.01 and 0.025 mg/kg 8-OHDPAT and 10 mg/kg cocaine prior to the 0.5 mg/kg 8-OHDPAT/cocaine treatment. Seemingly, these pairings with cocaine at the lower 8-OHDPAT dose levels may have been sufficient to reverse the inhibitory effect of the 0.05 mg/kg 8-OHDPAT given as a pretreatment to cocaine. Nonetheless, the ascending dose regimen of the combined Clonidine-cocaine treatment did not reverse the Clonidine inhibitory effect on cocaine-induced hyperlocomotion. There are, however, other reports (Vandenschuren et al., 2003) indicating that Clonidine, (0.05 mg/kg) does not suppress cocaine-induced behavioral stimulation. In this particular report (Vandenschuren et al., 2003), Clonidine was given in combination with cocaine after it had been given paired with amphetamine. This suggests again, as in previous observations with 8-OHDPAT/cocaine pairings, that the suppression effects of Clonidine may be reversed when the combined Clonidine/stimulant drug treatment is repeatedly administered. We have also reported similar reversal effects when autoreceptor dose levels of the D2 agonist Apomorphine are combined with cocaine (Carey et al., 2005a). Since such effects are observed in paired but not in unpaired Apomorphine/cocaine or 8-OHDPAT/cocaine treatments, we have suggested that such drug–drug interactions are not fixed but are subject to neuroplasticity effects (Carey et al., 2005c). It can be noted alongside these reversal effects with repeated paired treatments of cocaine with autoreceptor agonists, that the suppression of cocaine stimulant effects by D2 postsynaptic receptor antagonists such as haloperidol (Carey et al., 2002) is not reversed with repeated combined haloperidol/cocaine treatments.

For the 8-OHDPAT pretreatment, the biochemical findings were in agreement with expectations. That is, 8-OHDPAT decreased 5-HTAA/5-HT ratios in both brain areas sampled without any corresponding effect on VMA/NE and HVA/DA ratios obtained from the same brain tissue samples. In contrast, the biochemical findings obtained for Clonidine were only consistent with a selective NE effect for the medial frontal cortex tissue samples but not for the limbic subcortical tissue samples. In the cortex, Clonidine decreased VMA/NE ratios but did not alter 5-HTAA/5-HT ratios. Unexpectedly, in the limbic subcortical brain tissue samples, Clonidine did not alter VMA/NE ratios but substantially decreased 5-HTAA/5-HT ratios and HVA/DA ratios. Thus, Clonidine had a broad impact upon neurotransmitters and it decreased metabolic activity in all three neurotransmitter systems directly affected by cocaine, namely, NE, 5-HT and DA. The unanswered and intriguing aspect of this observation is that these effects were varied and dependent upon brain region. Evidently, the loss of NE activity can diminish DA and 5-HT activity in some brain regions. The basis for a neuroanatomically variegated neurochemical impact of Clonidine remains to be determined. The widespread neurotransmitter inhibition by Clonidine however, appears to provide a basis for understanding the profound behavioral inhibitory effect produced by Clonidine. The present neurochemical findings were obtained with *ex-vivo* methods; thus, there remains the need to employ an *in-vivo* methodology. Importantly, Florin et al. (1994) have shown that Clonidine can block the increase in NE induced by cocaine in the medial frontal cortex and hippocampus. Previously, we have shown that 8-OHDPAT neurochemical effects obtained with *in-vivo* microdialysis (Müller et al., 2003) are consistent with the *ex-vivo* findings of the present study. Given this consistency between *ex-vivo* and *in-vivo* 8-OHDPAT effects upon 5-HT, it will be important to use *in-vivo* microdialysis to determine if Clonidine blocks cocaine-induced increases in neurotransmitters differentially in different target sites (e.g., medial frontal cortex *vs*. nucleus accumbens *vs*. hippocampus). As shown by Florin et al. (1994), Clonidine (0.05 mg/kg) blocks cocaine-induced increase in NE in the medial prefrontal cortex and hippocampus but the present finding suggests that Clonidine may also block cocaine-induced DA increases in brain areas important for reward and motoric properties of cocaine such as the nucleus accumbens (Koob, 1992). If Clonidine does block cocaine-induced neurotransmitter increases differentially in different brain locations, this would point to the complexity of the neuropharmacological properties of systematically administered Clonidine and also suggest a broad and important role for NE in brain function.

The findings obtained with 8-OHDPAT suggest that when the 5-HT contribution to cocaine neurochemical activation is suppressed by 8-OHDPAT (Müller et al., 2003) only some facets of cocaine behavioral activation are diminished. While cocaine-induced locomotor activation can be enhanced with combined 8-OHDPAT/cocaine treatments (Carey et al., 2005b; DeLaGarza & Cunningham, 2000), at the same time, the proclivity to enter the central zone of the open-field as well as rearing behavior remain sharply reduced. Indeed, the locomotor activation in a cocaine treated animal which had received 8-OHDPAT pretreatment resembles the locomotor activation generated by high doses of dopamine selective drugs such as apomorphine which activate locomotor behavior but shift locomotion to the periphery of the open-field (Damianopoulos & Carey, 1992; Mattingly et al., 1997) and suppresses rearing behavior and grooming (Bloise et al., 2007). Seemingly, the blockade of 5-HT activation by 8-OHDPAT would tilt the balance of the neurochemical impact of

cocaine more toward dopamine and alter the cocaine behavioral effect to become more like that of a selective DA agonist (Müller et al., 2007; Szumlinski et al., 2004).

The neurochemical mechanisms that mediate cocaine effects have been long known (Koe, 1976; Ritz et al., 1990). As an indirect acting agonist, the neurotransmitter impact of cocaine is dependent upon the state of ongoing neural activity in the catecholaminergic and indoleaminergic neurons and this activity can be modulated by organismic as well by situational variables. Indeed, the organismic and situational variables can seemingly have different effects upon different neurotransmitter systems. It is such differences in baseline neural activity as well as the interplay among neurotransmitters systems that contribute to the variety and complexity of cocaine effects upon behavior. As the present study shows, efforts to pharmacologically manipulate the state of activity in selected neurotransmitter systems affected by cocaine does not provide an uncomplicated strategy to assess the differential contribution of different neurotransmitter systems to cocaine effects. Not only can such manipulations alter behavioral baselines but, in addition, the neurotransmitter profile impacted upon by the neurotransmitter selective drug pretreatments may be more complex than intended. In fact, if this study had not incorporated an *ex-vivo* measurement of neurotransmitters and neurotransmitter metabolites, then a prominent role for a selective decrease in NE in spontaneous behavioral activation effects as well as for cocaine behavioral activation would have been suggested. Given that Clonidine had a substantial impact upon the availability of DA and 5-HT as well as on NE, the present report highlights the limitation of experiments designed to examine neurochemical mechanisms which rely solely upon assumptions of the impact of drug pretreatment manipulations upon neurotransmitters.

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