Repeated 7-OH-DPAT Treatments: Behavioral Sensitization, Dopamine Synthesis, and Subsequent Sensitivity to Apomorphine and Cocaine

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Abstract. Male Wistar rats (250 - 350 g) were injected (SC) daily with the putative selective dopamine D_3 receptor agonist, 7-OH-DPAT (0.01, 0.10, or 1.0 mg/kg) or vehicle for 10 days. Fifteen min after each injection, the rats were tested for locomotor activity in photocell arenas for 20 min or 2 hr. In two experiments, following this subchronic treatment, all rats received a challenge injection of apomorphine (1.0 mg/kg, SC), or cocaine (10 mg/kg, IP) on day 11, and were tested for locomotor activity. In a third experiment, dopamine synthesis in striatal and mesolimbic (nucleus accumbens-olfactory turbercle) tissue was assessed following acute or chronic 7-OH-DPAT treatments by measuring the accumulation of dihydroxyphenylalanine (DOPA) after treatment with a DOPA decarboxylase inhibitor. Major findings were as follows: a) acute 7-OH-DPAT treatment produced a dose-dependent decrease in locomotor activity; b) when tested for two hours, the 1.0 mg/kg dose of 7-OH-DPAT produced a progressively greater increase in activity across the 10 test days (i.e., behavioral sensitization); c) subchronic treatment with 7-OH-DPAT did not result in cross-sensitization to either apomorphine or cocaine; d) acute treatment with the 1.0 mg/kg dose of 7-OH-DPAT significantly decreased dopamine synthesis in both striatal and mesolimbic regions; and e) chronic 7-OH-DPAT treatments did not affect basal dopamine synthesis in either brain region. Although the behavioral effects of 7-OH-DPAT were similar to the reported effects of the D_2/D_3 dopamine agonist quinpirole, the effects of repeated 7-OH-DPAT treatments differed from those of quinpirole in terms of cross-sensitization and basal dopamine synthesis. These results suggest that locomotor inhibition produced by low doses 7-OH-DPAT is not related to dopamine autoreceptor stimulation, and the development of behavioral sensitization to high doses of 7-OH-DPAT is not due to the development of dopamine autoreceptor subsensitivity.

Key Words: behavioral sensitization - cross-sensitization - 7-OH-DPAT - cocaine - locomotor activity - dopamine D_2-type receptors - dopamine D_3 receptors

The repeated administration of both direct (e.g., apomorphine) and indirect (e.g., amphetamine) dopamine receptor agonists in rodents results in the development of behavioral sensitization, characterized by a progressive enhancement of various drug-induced motor behaviors (see Kalivas & Stewart 1991; Stewart & Badiani 1993; Robinson & Becker 1986, for reviews). Although many dopamine agonists which induce behavioral sensitization stimulate both D_1- and D_2-type dopamine receptors, available evidence suggests that the development of behavioral sensitization is mediated by the repeated stimulation of D_1-type receptors. For example, the co-administration of selective D_1-type, but not D_2-type, dopamine antagonists prevents the development of behavioral sensitization to the direct dopamine agonist, apomorphine (Mattingly et al. 1991), and the indirect agonist, amphetamine (Drew & Glick 1990; Stewart & Vezina 1989; Vezina & Stewart 1989). Moreover, rats repeatedly treated with the selective D_1-type dopamine agonist, SKF 38393, subsequently display a sensitized locomotor response to apomorphine (Mattingly et al. 1993).

Although the co-administration of D_2-type antagonists with mixed D_1/D_2 dopamine agonists fails to prevent the development of behavioral sensitization, recent evidence suggests that behavioral sensitization may develop as a result of stimulation of dopamine receptors within the D_2 family (i.e., D_2, D_3, & D_4). For example, repeated daily treatment with the dopamine D_2 receptor agonist, bromocryptine, or the D_2/D_3 receptor agonist, quinpirole, results in a robust locomotor sensitization effect (Hoffman & Wise 1992, 1993; Wise & Carlezon 1994; Szechtman et al. 1994). Moreover, bromocryptine-induced sensitization cross-sensitizes to quinpirole (Hoffman & Wise 1993), and quinpirole-induced sensitization has been shown to cross-sensitize to apomorphine (Mattingly et al. 1993). The development of behavioral sensitization to bromocryptine or quinpirole, however, may be prevented by the co-administration of the selective dopamine D_1-type antagonist, SCH 23390 (Mattingly et al. 1993; Wise & Carlezon 1994). Thus, some minimal level of D_1 receptor stimulation appears to be necessary for the development of behavioral sensitization to D_2-type dopamine agonists.

Although it is clear that dopamine D_2-type receptors play a role in the development of behavioral sensitization, attempts to study the involvement of specific receptor subtypes within the D_2 family have been hampered by the absence of sufficiently selective compounds. Bromocryptine, for example, is generally considered to be a selective dopamine D_2 agonist, but it has less than a 2-fold higher affinity for dopamine D_2 receptors than D_3 receptors (see Schwartz et al. 1992; Sokoloff et al. 1992). Similarly, although quinpirole has approximately a 100-fold greater affinity for dopamine D_3 receptors than D_2 receptors, its affinity for dopamine D_4 receptors is only slightly lower than its affinity for D_3 receptors (Sokoloff et al. 1992; Levesque et al. 1992). Consequently, bromocryptine and quinpirole, in doses that result in locomotor sensitization, probably stimulate more than one receptor subtype within the D_2 family.

The objective of the present study was to further evaluate the involvement of dopamine D_2-type receptors in the development of behavioral sensitization using the putative selective dopamine D_3 agonist, 7-hydroxy-dipropylaminotetralin (7-OH-DPAT). 7-OH-DPAT has been reported to have a 100- and 1000-fold greater affinity for dopamine D_3 than D_2 and D_4 receptors, respectively (Levesque et al. 1992), and like bromocryptine and quinpirole, it acutely decreases locomotor activity, dopamine synthesis and release (Ahlenius & Salmi 1994; Aretha et al. 1994; Damsma et al. 1993a,b; Meller et al. 1993; Svensson et al. 1994; Yamada et al. 1994).

Experiment 1

As noted, the dopamine D_2/D_3 agonist, quinpirole, decreases locomotor activity when administered acutely, but repeated administration results in the development of behavioral sensitization (Szechtman et al. 1994). Moreover, repeated quinpirole treatments significantly enhance the locomotor activating effects of apomorphine (Mattingly et al. 1993). The purpose of Exp. 1, therefore, was to determine the effects of repeated 7-OH-DPAT treatments on locomotor activity and subsequent sensitivity to apomorphine. Consequently, groups of rats were injected daily with various doses of 7-OH-DPAT or vehicle and tested for locomotor activity for 10 days. Then, on Day 11, all rats were tested for

locomotor activity following a challenge injection of apomorphine.

Materials and methods

Subjects. Forty-eight male Wistar albino rats (Harlan Industries, Indianapolis, IN) weighing between 250 and 350 g served as subjects. All rats were housed individually in hanging wire-mesh cages in a colony room with a 12-h light-dark cycle and food and water available continuously. All behavioral testing was conducted during the light phase of the cycle.

Apparatus. Activity measures were taken in two BRS/Lehigh Valley cylindrical activity drums (Model 145-03) that were 60 cm in diameter and 43 cm high. The interior of each drum was painted flat black, and the floor was made of 4 cm diamond-shaped wire mesh. Each drum was located in a separate sound-attenuated experimental cubicle that was kept dark during testing.

Two banks of three infrared photocells were mounted on the outside of each drum. The photocells were approximately 12 cm apart and 2.5 cm above the drum floor. The photocell banks were connected to back-path eliminator diodes. Movement of the rat through a photocell beam sent a single pulse to the counters. Simultaneous pulses (i.e., pulses spaced less than 0.05 s apart) such as might occur when two beams are broken at their intersection were recorded as a single count by this method. Thus, locomotor activity was defined as the cumulative number of photocell interruptions per unit time.

Drugs. Apomorphine hydrochloride (Sigma) and (±)-7-hydroxy-dipropylaminotetralin hydrobromide (7-OH-DPAT; Research Biochemicals) were dissolved daily in distilled H_2O and injected SC in a volume of 1.0 ml/kg. Doses of both drugs were calculated based upon the salt form of each drug. Vehicle injections were given using the same route and volume as the corresponding drug injection.

Design and procedure. At the beginning of testing, the rats were randomly assigned in equal numbers to one of four treatment groups: 0 (vehicle), 0.01, 0.10, or 1.00 mg/kg 7-OH-DPAT. On each of the first ten days of the experiment (pretreatment phase), the rats were injected with the appropriate dose of 7-OH-DPAT and then tested for locomotor activity for 20 min, 15 min after the

injection. On day 11 of the experiment all rats were given a challenge injection of apomorphine (1.0 mg/kg) and then tested for activity 15 min later.

Data analysis. Significant differences among the groups in mean activity counts during the pretreatment phase (Days 1 - 10) were determined with a mixed two-factor analysis of variance (ANOVA) using drug treatment group as a between factor and daily test session as a repeated measure. Significant interactions were analyzed with additional ANOVAs performed on individual day or group data, followed by Neuman-Keuls post hoc tests. Mean activity counts of the groups on the apomorphine challenge test (Day 11) were analyzed using a one-way between groups ANOVA. For the ANOVAs and multiple comparisons, the alpha level was constrained to $p \leq 0.05$.

Results

Pretreatment Days 1 -10. Mean Activity counts per 20 min session for the four groups across the 10 pretreatment days are displayed in Fig. 1. As may be seen in this Figure, 7-OH-DPAT treatments inhibited locomotor activity relative to vehicle control rats on the first treatment day. With repeated treatments, however, the effects of 7-OH-DPAT on activity changed in a dose-dependent manner. Specifically, the 0.10 and 1.0 mg/kg doses of 7-OH-DPAT produced progressively greater increases in activity across days, whereas the lower dose group (0.01 mg/kg) continued to remain less active than the vehicle-treated rats [drug effect, $F(3, 44) = 9.50$, $P<0.0001$; Drug X Day interaction, $F(9, 396)=12.10$, $P< 0.0001$]. Subsequent analysis of groups' activity on Day 1 indicated that all three 7-OH-DPAT dose groups were significantly less active than the vehicle control group (Ps < 0.05). The two highest dose groups (0.10 and 1.0 mg/kg) did not significantly differ in activity (P > 0.05), but both groups were significantly less active than the 0.01 mg/kg dose group (Ps < 0.05). Analysis of the groups' activity on Day 10 indicated that only the 0.01 mg/kg dose group was significantly less active than the vehicle control group (P < 0.05). The activity of two higher dose groups (0.10 and 1.0 mg/kg) did not significantly differ from that of the vehicle group (Ps > 0.05).

Apomorphine Challenge Test - Day 11. The mean activity counts of the four

pretreatment groups following an apomorphine challenge injection are shown in Fig. 2. As suggested by inspection of Fig. 2, the ANOVA performed on these data revealed no significant activity differences among the groups [drug effect, $F(3, 44) = 0.97$, $P > 0.05$]. Thus, pretreatment with 7-OH-DPAT did not affect subsequent sensitivity to apomorphine.

Experiment 2

Consistent with previous findings, 7-OH-DPAT produced an acute dose-dependent decrease in locomotor activity (Svensson et al. 1994). The locomotor inhibition produced by the two higher doses of 7-OH-DPAT (0.10 and 1.0 mg/kg), however, dissipated with repeated treatments. This pattern of activity observed with repeated 7-OH-DPAT treatments is almost identical to that observed previously with repeated quinpirole treatments under the same test conditions (cf., Mattingly et al. 1993). However, repeated quinpirole treatments significantly increase the locomotor response to a subsequent apomorphine (1.0 mg/kg) challenge injection (Mattingly et al. 1993), whereas repeated 7-OH-DPAT treatments in Exp. 1 did not affect subsequent behavioral sensitivity to the same challenge dose of apomorphine.

The development of behavioral sensitization to apomorphine and quinpirole is accompanied by an increase in basal dopamine synthesis in striatal and mesolimbic tissue, which has been attributed to the development of autoreceptor subsensitivity (Rowlett et al. 1991, 1995). Dopamine D_3 receptors are thought to function as autoreceptors (e.g., Meller et al. 1993), and consistent with this view, 7-OH-DPAT has been reported to acutely decrease dopamine synthesis and release (Aretha et al. 1994; Damsma et al. 1993a). The purpose of Experiment 2, therefore, was to determine whether repeated 7-OH-DPAT treatments, like quinpirole and apomorphine, would also produce an increase in basal dopamine synthesis. Consequently, in Experiment 2, groups of rats were injected with 7-OH-DPAT and tested for locomotor activity for ten days as in Exp. 1. On Day 11, basal dopamine synthesis was assessed by measuring the accumulation of 3,4-Dihydroxyphenylalanine (DOPA) in striatal and mesolimbic (nucleus accumbens-

olfactory tubercle, NAOT) tissue, after administration of a DOPA decarboxylase inhibitor. In addition, a preliminary experiment was conducted to ensure that the doses of 7-OH-DPAT used in this experiment decrease dopamine synthesis when administered acutely.

Materials and methods

Subjects and design. Seventy-two male Wistar albino rats (Harlan Sprague Dawley, Indianapolis, IN) weighing between 250 - 300 g served as subjects. In both experiments, the rats were randomly assigned, in equal numbers, to one of four treatment groups: 0.00 (vehicle), 0.01, 0.10, or 1.00 mg/kg 7-OH-DPAT. All rats were housed and maintained as in Exp. 1. Behavioral testing and brain tissue collection were conducted during the light phase of the light-dark cycle. Locomotor activity was measured the same as previously described.

Tissue dissections and assay for DOPA. For tissue dissections, rats were killed by rapid decapitation and the brains were removed and placed on an ice-cold dissection plate. Striatal and NAOT samples were dissected from a coronal slice that extended approximately 2-3 mm anterior to bregma. Each sample was weighed and placed in 0.1 M $HCLO_4$ (100 mg/ml) and stored at -70$_o$ C.

On the day of the assay, the tissue samples were thawed and sonicated (Vibracell, setting 80). The tissue homogenates were then centrifuged at 30,000*g* for 15 min (4°C). Supernatants (20 ul) were assayed for DOPA using a high-performance liquid chromatograph system consisting of a Bioanalytical Systems LC4B electrochemical detector (working electrode = +750 mV against the Ag/AgCl reference electrode), a PM-11 pump, and a temperature-controlled column (35°C, 3 um). The mobile phase consisted of 50 mM Na_2HPO_4, 124mM citric acid, .1 mM EDTA, and 10% methanol (pH 3.0). The amount of DOPA was determined by comparison with the peak heights of DOPA standards, which were assayed daily. Peak identity was verified by retention times and by sometimes spiking a tissue sample with a small amount of DOPA standard.

Drugs. 7-OH-DPAT was prepared and administered as previously described. The DOPA decarboxylase inhibitor, NSD-1015 (M-hydroxybenzylhydrazine dihydrochloride, Sigma), was dissolved daily in distilled water and injected IP in a volume of 1.0

ml/kg. DOPA standards (Research Biochemicals) were mixed in 0.1 M $HCLO_4$.

Procedures. In the preliminary experiment, rats in the four groups (n=6/group) were first injected with the appropriate dose of 7-OH-DPAT and 15 min later injected with NSD-1015 (100 mg/kg). All rats were then killed 30 min later. In the basal dopamine synthesis experiment, rats in the four groups (n=12/group) were injected daily with the appropriate dose of 7-OH-DPAT and tested for locomotor activity for 10 days as in Experiment 1. Twenty-four hours after the last drug treatment, all rats were given NSD-1015 (100 mg/kg) and were sacrificed 30 min later.

Results

Preliminary dopamine synthesis experiment. Mean DOPA levels in striatal and mesolimbic (NAOT) tissue for the four groups given various doses of 7-OH-DPAT are presented in Figure 3. As may be seen, DOPA accumulation was less in mesolimbic than striatal tissue [region effect, $F(1,20) = 22.36$, $P < 0.0001$]. More important, DOPA accumulation was significantly decreased by 7-OH-DPAT [drug effect, $F(3, 20) = 5.15$, $P < 0.01$], in both striatal and mesolimbic regions (Drug X Region interaction, $F(3, 20) = 0.23$, $P > 0.05$). Subsequent analysis of the significant drug effect indicated that DOPA levels were significantly reduced for rats given 1.0 mg/kg 7-OH-DPAT compared to rats given vehicle [Neuman-Keuls test, $P < 0.05$]. DOPA accumulation in rats injected with either 0.01 or 0.10 mg/kg 7-OH-DPAT, however, did not differ significantly from that of rats injected with vehicle [$P > 0.05$].

Locomotor activity - Days 1 - 10. The effect of daily 7-OH-DPAT treatments on locomotor activity was the same as in Exp. 1 (data not shown). Briefly, all doses of 7-OH-DPAT inhibited locomotor activity after the first injection, but this inhibition progressively decreased for the 0.10 and 1.0 mg/kg dose groups across the ten activity test sessions [drug effect, $F(3, 44) = 5.00$, $P < 0.01$; Drug x Day interaction, $F(9, 396) = 11.22$, $P < 0.0001$]. On Day 10, the 0.01 mg/kg 7-OH-DPAT group remained significantly less active than the vehicle control group ($P < 0.05$), but the activity of the two higher dose groups (0.10 and 1.00 mg/kg) did not significantly differ from that of the vehicle rats ($P > 0.05$).

DOPA Accumulation. Mean DOPA levels in the two brain regions for rats previously treated with 7-OH-DPAT or vehicle are shown in Figure 4. A mixed factor ANOVA performed on these data revealed a significant main effect of region [$F(1, 44) = 7.98$, $P < 0.01$]. However, neither the main effect of drug nor the Drug X Region interaction were significant [$F(3,44) = 0.81$, $P > 0.05$ and $F(1,44) = 0.86$, $P > 0.05$, respectively]. Thus, although the highest dose of 7-OH-DPAT acutely decreased dopamine synthesis, repeated 7-OH-DPAT treatments did not result in an increase basal dopamine synthesis.

Experiment 3

The results of Experiment 2 suggest that dopamine D_3 autoreceptors do not become subsensitive with repeated 7-OH-DPAT treatments. This lack of change in the sensitivity of autoreceptors with repeated 7-OH-DPAT treatment may account for the differential effect of repeated quinpirole and 7-OH-DPAT treatments on subsequent sensitivity to apomorphine (cf., Mattingly et al. 1993; Exp. 1).

The development of autoreceptor subsensitivity has also been suggested to be one of several mechanisms mediating the development of behavioral sensitization to the indirect agonist, cocaine (see Henry et al. 1989). Consistent with this view, quinpirole has been reported to cross-sensitize to cocaine (Horger & Schenk 1991), and rats sensitized to cocaine display cross-sensitization to apomorphine (Kityatkin 1994). The purpose of Experiment 3, therefore, was to determine the effect of repeated 7-OH-DPAT treatments on subsequent sensitivity to cocaine. If the development of autoreceptor subsensitivity is responsible for the cross-sensitization observed among quinpirole, apomorphine, and cocaine, then repeated 7-OH-DPAT treatments should not affect subsequent behavioral sensitivity to cocaine.

The behavioral results of Experiments 1 and 2 indicate that 7-OH-DPAT acutely inhibits locomotor activity. This locomotor inhibition, however, dissipates with repeated treatments of higher doses of 7-OH-DPAT. As mentioned, this pattern of activity changes is similar to that produced by quinpirole when short duration activity test intervals are used (cf., Mattingly et al. 1993; Rowlett et al. 1995). With longer test intervals, however, quinpirole has been

reported to produce a biphasic locomotor response acutely and locomotor sensitization with repeated treatment (Eilam & Szechtman 1989; Szechtman et al. 1994). In Experiment 3, therefore, we extended the daily activity test interval from 20 minutes to two hours to determine whether 7-OH-DPAT treatments would produce a similar pattern of activity.

Materials and methods

Subjects, design, drugs, and procedure. Twenty-three male Wistar rats (Harlan Sprague-Dawley) weighing between 250-300 g served as subjects. At the beginning of the experiment, the rats were randomly assigned to one of four dose groups (N = 5-6/group): 0.00 (vehicle), 0.01, 0.10, or 1.00 mg/kg 7-OH-DPAT. The rats were tested daily for locomotor activity after the appropriate drug injection for ten days under the same conditions as in previous experiments except the test duration was extended to 120 minutes. On Day 11, all rats were tested for activity after a challenge injection of cocaine hydrochloride (Sigma, 10 mg/kg). Cocaine hydrochloride was dissolved in distilled H_2O and injected IP in a volume of 1.0 ml/kg 5 min prior to activity testing.

Results

Pretreatment Days 1-10. Mean Activity counts per 120 min session for the four groups across the 10 pretreatment days are displayed in Fig. 5. As may be seen in this Figure, 7-OH-DPAT treatments inhibited locomotor activity relative to vehicle control rats on the first treatment day. With repeated treatments, however, the effects of 7-OH-DPAT on activity changed in a dose-dependent manner. Specifically, the 1.0 mg/kg dose of 7-OH-DPAT produced progressively greater increases in activity across days, whereas the two lower dose groups (0.01 and 0.10 mg/kg) continued to remain less active than the vehicle-treated rats [drug effect, $F(3, 19)=53.99$, $P<0.0001$; Drug X Day interaction, $F(9, 171)=6.58$, $P< 0.0001$]. Additional ANOVAs performed on individual group data across the 10 test days indicated that activity of the 1.0 mg/kg dose group significantly increased, [day effect, $F(9, 45)=4.96$, $P< 0.0001$]. In contrast, the activity counts of the vehicle and 0.01 mg/kg dose groups significantly decreased across days [$F(9,36)=22.22$, $P<0.0001$ and $F(9,45)=9.45$, $P<0.0001$, respectively], and the total

activity counts of the 0.10 mg/kg dose group did not significantly change [$F(9,45)=0.36$, $P>.05$]. These drug-induced changes in activity across days, however, were not constant across time-blocks within the 10 test sessions, [block effect, $F(5,95)=12.50$, $P<0.0001$; Drug X Block interaction, $F(15, 95)=18.89$, $P<0.0001$; Day X Block interaction, $F(45, 855)=3.06$, $P< 0.0001$; Drug X Day X Block interaction, $F(135, 855)=1.63$, $P< 0.0001$.

The nature of these interactions is depicted in Fig. 6 which presents the within session activity of the groups on treatment Day 1 and Day 10. Subsequent analyses of the groups' activity on Day 1 indicated that all 7-OH-DPAT doses significantly inhibited activity on the first two 20 min blocks (Ps <0.05). On blocks 4 and 5, only the 0.10 mg/kg group was significantly less active than vehicle rats (Ps < 0.05). In contrast, on block 6 the rats treated with the 1.0 mg/kg dose of 7-OH-DPAT were significantly more active than vehicle-treated rats ($P < 0.05$). Thus, the 1.0 mg/kg dose of 7-OH-DPAT produced a weak biphasic effect on locomotor activity. However, whether this finding represents a true biphasic effect or a simply a drug-induced disruption of habituation processes is not clear.

By treatment Day 10, the initial inhibitory effect of the 0.10- and 1.00-mg/kg doses of 7-OH-DPAT had dissipated. In contrast, rats receiving the 0.01 mg/kg dose of 7-OH-DPAT remained significantly less active than vehicle control rats during the first 20 min of this session ($P < 0.05$). The 0.10 mg/kg dose group did not significantly differ in activity from the vehicle rats at any time-block on this treatment day (Ps > 0.05). Further, consistent with the results of Experiments 1 and 2, the 1.0 mg/kg rats did not significantly differ from vehicle rats on the first two 20 min time-blocks (Ps > 0.05). However, as may be seen in Fig. 6, these rats displayed progressively greater increases in activity across the last four 20 min blocks relative to vehicle-treated rats (Ps < 0.05). Thus, the daily increase in activity observed in the 1.0 mg/kg dose group across days (Fig. 5) was largely due to progressive increases in activity in the second hour of testing.

Cocaine Challenge Test - Day 11. Figure 7 presents the mean activity counts of

the four pretreatment groups across the six 20 min time-blocks following a challenge injection of cocaine. The cocaine challenge injection produced a large increase in activity in rats previously treated with vehicle for 10 days (cf., Fig. 6, Day 10). As expected, this cocaine-induced increase in activity decreased across the two hour test session [block effect, $F(5, 95)=144.28$, $P< 0.0001$]. More important, rats previously treated with 7-OH-DPAT for 10 days did not significantly differ from vehicle-pretreated rats in this cocaine-induced increase in activity [drug effect, $F(3, 19)=1.81$, $P > 0.05$; Drug X Block interaction, $F(15, 95)=1.28$, $P > 0.05$].

Discussion

Consistent with previous studies, the acute administration of 7-OH-DPAT produced an initial decrease in locomotor activity (Ahlenius & Salmi 1994; Damsma et al. 1993a; Svensson et al. 1994). The acute locomotor inhibition produced by 7-OH-DPAT has generally been attributed to the stimulation of dopamine autoreceptors (e.g., Ahlenius & Salmi 1994). Whether this 7-OH-DPAT-induced decrease in activity is related specifically to the selective stimulation of D_3 autoreceptors is not known. However, doses of 7-OH-DPAT below the affinity of D_2 receptors have been reported to produce a decrease in locomotor activity and a maximal decrease in extracellular dopamine within 20 minutes after treatment (Damsma et al. 1993a). Thus, the locomotor inhibition produced by the lowest dose of 7-OH-DPAT (0.01 mg/kg) used in the present study may reflect selective D_3 autoreceptor stimulation. However, this dose of 7-OH-DPAT did not significantly affect dopamine synthesis in the current study. Moreover, others have reported a reduction in locomotor activity with low doses of 7-OH-DPAT that do not significantly affect dopamine synthesis or release (e.g., Svensson et al. 1994). These findings suggest the possibility that the locomotor inhibition produced by low doses of 7-OH-DPAT may be due to stimulation of a postsynaptic D_3 receptor, rather than to D_3 autoreceptors (see also, Waters et al. 1993).

With repeated administration, the initial inhibitory effects of the 0.10 and 1.0 mg/kg doses of 7-OH-DPAT, but not the 0.01 dose, progressively declined. This finding is similar to that observed with repeated quinpirole treatments

(Mattingly et al. 1993; Rowlett et al. 1995), and may be related to the development of autoreceptor subsensitivity (Rowlett et al. 1995). Consistent with a role of dopamine autoreceptors, acute treatment with the 1.0 mg/kg dose of 7-OH-DPAT significantly decreased dopamine synthesis in both striatal and mesolimbic tissue, and the 0.10 mg/kg dose tended to decrease (although not significantly) dopamine synthesis in the mesolimbic region in the present study. Thus, these doses may stimulate dopamine autoreceptors. However, with repeated treatment, quinpirole results in an increase in basal dopamine synthesis (Rowlett et al. 1995), presumably due to the development of autoreceptor subsensitivity. In contrast, repeated 7-OH-DPAT treatments did not significantly affect basal dopamine synthesis in the present study. Hence, autoreceptor subsensitivity may not account for the tolerance observed to the inhibitory effects of the two higher doses of 7-OH-DPAT.

When a long duration test interval was used in Exp.3, the 1.0 mg/kg dose of 7-OH-DPAT, but not the 0.01 or 0.10 mg/kg doses, resulted in the development of behavioral sensitization in a manner similar to that produced by repeated bromocryptine (Hoffman & Wise 1992, 1993; Wise & Carlezon 1994) and quinpirole (Szechtman et al. 1989) treatments. In contrast, the locomotor inhibition produced by the 0.01 mg/kg dose of 7-OH-DPAT, the dose most likely to be selective to D_3 receptors, did not significantly change across the ten day test period. Taken together, these findings suggest that the development of behavioral sensitization to dopamine D_2-type receptor agonists may require some minimal level of dopamine D_2 receptor stimulation. It is possible, however, that the effects of D_2 and D_3 receptor stimulation are additive and that the development of behavioral sensitization may result from some minimal level of combined D_2 and D_3 receptor activity.

Despite the fact that the high dose (1.0 mg/kg) of 7-OH-DPAT produced a pronounced behavioral sensitization effect, repeated 7-OH-DPAT treatments at any dose did not increase subsequent sensitivity to the locomotor-activating effects of cocaine or apomorphine. As noted previously, rats sensitized to quinpirole display cross-sensitization to cocaine and apomorphine (Horger & Schenk 1991;

Mattingly et al. 1993), and cross-sensitization between cocaine and apomorphine has also been reported (Kityakin 1994). The lack of cross-sensitization between 7-OH-DPAT and apomorphine and cocaine may be related to the apparent inability of repeated 7-OH-DPAT treatments to induce autoreceptor subsensitivity. Repeated apomorphine, quinpirole, and cocaine treatments have been reported to produce autoreceptor subsensitivity (Rebec & Lee 1983; Jeziorski & White 1989; Rowlett et al. 1995), which appears to contribute indirectly to the development of behavioral sensitization (see Henry et al. 1989). In the present study, however, no evidence of autoreceptor subsensitivity following repeated 7-OH-DPAT treatments was observed. Indeed, even doses of 7-OH-DPAT that acutely decreased dopamine synthesis did not significantly affect basal dopamine synthesis with repeated administration. Thus, the development of behavioral sensitization to high doses of 7-OH-DPAT, like tolerance to the initial locomotor inhibition, does not appear to be related to changes in autoreceptor sensitivity. Interestingly, it has recently been reported that rats sensitized to the locomotor-activating effects of the D_2-type agonist, bromocryptine, like 7-OH-DPAT, are not cross-sensitized to cocaine (Hoffman & Wise 1993). At present, it is not clear why differences in cross-sensitization occur following repeated 7-OH-DPAT, quinpirole, and bromocryptine treatments.

The differential effects of selective dopamine receptor agonists in producing locomotor sensitization/cross-sensitization and changes in basal dopamine synthesis are summarized in Table 1. As may be seen in this table, the available information suggests that despite similar D_2/D_3 receptor profiles, quinpirole, but not 7-OH-DPAT, closely resembles apomorphine. That is, quinpirole, like apomorphine, produces locomotor sensitization and cross-sensitization to cocaine as well as acute decreases in dopamine synthesis and enhanced basal dopamine synthesis after repeated treatments. In contrast, 7-OH-DPAT does not produce cross-sensitization to apomorphine or cocaine and does not result in enhanced basal dopamine synthesis after repeated treatments. As mentioned, the lack of an enhanced basal dopamine synthesis effect after repeated 7-OH-DPAT may reflect the absence of autoreceptor subsensitivity. Interestingly,

comparison of apomorphine, quinpirole, and 7-OH-DPAT reveals a potential pattern; the absence of enhanced basal dopamine synthesis may be correlated with the lack of cross-sensitization to other dopamine agonists. This potential correlation may be consistent with an hypothesis developed by Henry et al. (1989), which suggests that repeated cocaine treatment results in subsensitivity to impulse-regulating autoreceptors followed by terminal field D_1 receptor supersensitivity. We previously have postulated that repeated quinpirole treatments may result in autoreceptor subsensitivity, consequently increasing stimulation of D_1 receptor via increased dopamine release (Mattingly et al. 1993; Rowlett et al. 1995). Because repeated stimulation of D_1 receptors is sufficient to produce a sensitized response to apomorphine (see Table 1), we propose that cross-sensitization to apomorphine and cocaine was not observed after repeated 7-OH-DPAT treatments because of a lack of D_1 receptor stimulation in the absence of autoreceptor subsensitivity.

As may be seen in the Table, several inconsistencies are evident with this hypothesis. For example, 7-OH-DPAT did produce locomotor sensitization. Since repeated 7-OH-DPAT treatments did not affect autoreceptor sensitivity, it is not clear how such treatments could result in D_1 receptor supersensitivity. Perhaps locomotor sensitization to 7-OH-DPAT may be directly related to the selective stimulation of D_3 receptors. Further, although the non-selective D_2-type agonist bromocryptine resembles apomorphine and quinpirole in some respects (see Table 1), it does not produce cross-sensitization to cocaine (Wise & Carlezon 1994). Based upon receptor selectivity, we would predict that repeated bromocryptine treatments would result in enhanced basal dopamine synthesis. In contrast, because bromocryptine does not produce cross-sensitization to cocaine, we may also predict that repeated treatments with this compound would *not* result in enhanced basal dopamine synthesis. Resolution of these inconsistencies awaits further study. Moreover, it should be noted that a variety of other properties of these drugs may account for the discrepant findings. For example, early studies with bromocryptine suggested that this compound has an extremely long duration of action and may act in an irreversible fashion (Bannon et al. 1980).

In summary, the behavioral effects of repeated 7-OH-DPAT treatments are similar to the reported effects of other dopamine D_2-type agonists. Like quinpirole and bromocryptine, 7-OH-DPAT acutely inhibits locomotor activity, but with repeated treatment, results in the development of behavioral sensitization. Unlike quinpirole, however, repeated 7-OH-DPAT treatment does not result in cross-sensitization to apomorphine or cocaine, and does not affect basal dopamine synthesis. These differences suggest that the development of behavioral sensitization to dopamine agonists is not mediated by a common unitary neurochemical mechanism (cf., Hoffman & Wise 1993; Mattingly et al. 1994).

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Figure Captions

Fig. 1. Mean activity counts (±SEM) across 10 daily 20 min test sessions for rats treated with either vehicle (0.00 mg/kg) or 7-OH-DPAT (0.01, 0.10, or 1.00 mg/kg).

Fig. 2. Mean activity counts (± SEM) during the 20 min test session following an acute injection of apomorphine (1.0 mg/kg, SC) on Day 11 for rats previously treated for 10 days with either vehicle (0.00 mg/kg) or various doses of 7-OH-DPAT (0.01, 0.10, or 1.00 mg/kg).

Fig. 3. Mean DOPA levels (ug/g ± SEM) following an injection of vehicle or various doses of 7-OH-DPAT (0.01, 0.10, or 1.00 mg/kg). All rats were treated with NSD-1015 (100 mg/kg) prior to dissection of the striatum or NAOT (nucleus accumbens-olfactory turbercle).

* $P < 0.05$ vs vehicle group, Neuman-Keuls test.

Fig. 4. Mean DOPA levels (ug/g ± SEM) for rats given 10 daily injections of vehicle or various doses of 7-OH-DPAT (0.01, 0.10, or 1.00 mg/kg). All rats were treated with NSD-1015 (100 mg/kg) on Day 11 prior to dissection of the striatum or NAOT (nucleus accumbens-olfactory turbercle).

Fig. 5. Mean total activity counts (± SEM) across the 10 daily 2 hr test sessions for rats treated with either vehicle (0.00 mg/kg) or 7-OH-DPAT (0.01, 0.10, or 1.00 mg/kg).

Fig. 6. Mean activity counts (± SEM) across the 20 min time-blocks on Day 1 (top panel) and Day 10 (bottom panel) of testing for groups of rats injected daily with either vehicle (0.00 mg/kg) or various doses of 7-OH-DPAT (0.01, 0.10, or 1.00 mg/kg).

* $P < 0.05$ 7-OH-DPAT dose group vs vehicle group, Neuman-Keuls test.

Fig. 7. Mean activity counts (± SEM) across the 20 min time-blocks following an acute injection of cocaine (10 mg/kg, IP) on Day 11 for rats previously treated for 10 days with either vehicle (0.00 mg/kg) or various doses of 7-OH-DPAT (0.01, 0.10, or 1.00 mg/kg).

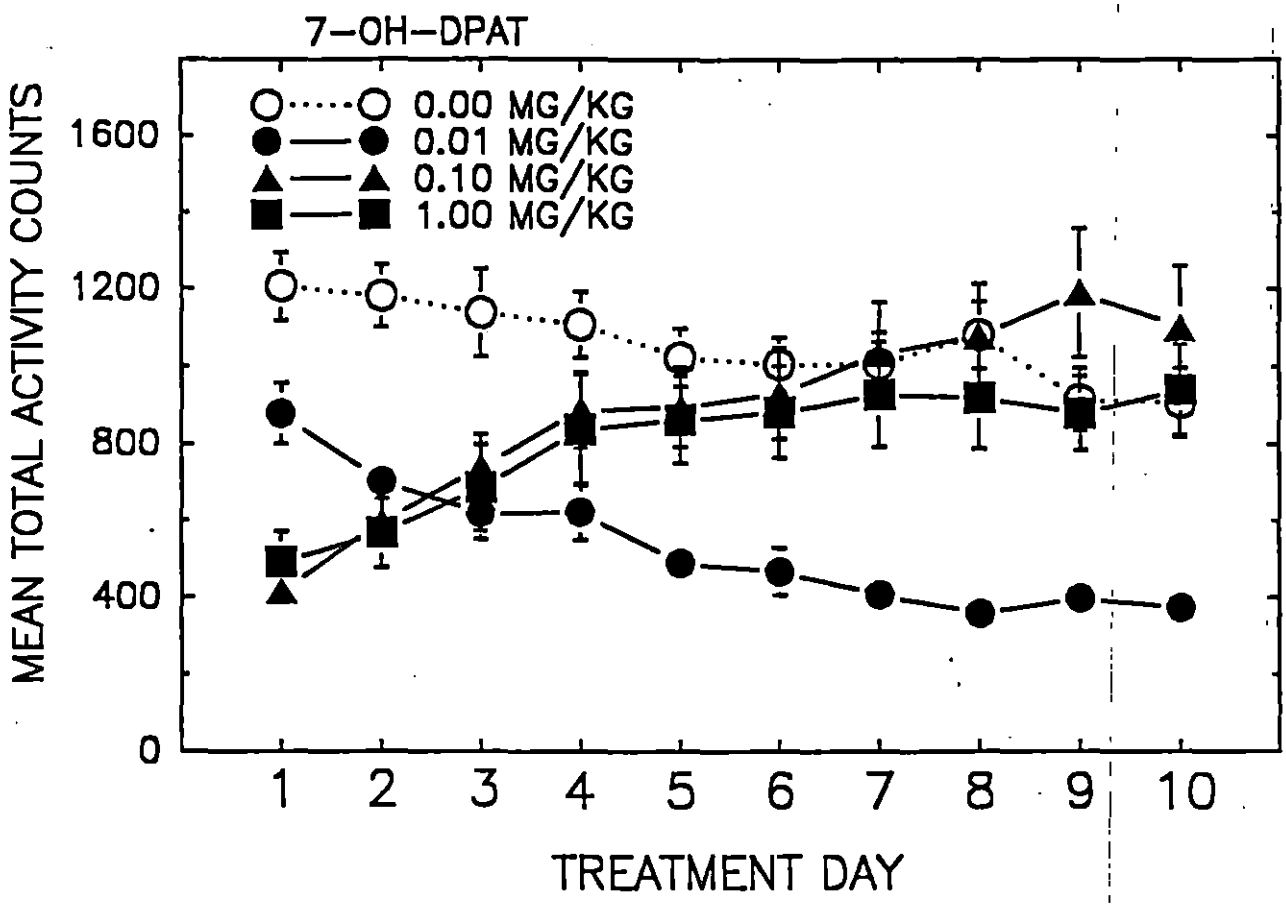
7-OH-DPAT
0.00 MG/KG
0.01 MG/KG
0.10 MG/KG
1.00 MG/KG
MEAN TOTAL ACTIVITY COUNTS
1600
1200
800
400
0
1
2
3
4
5
6
7
8
9
10
TREATMENT DAY

FIGURE 1

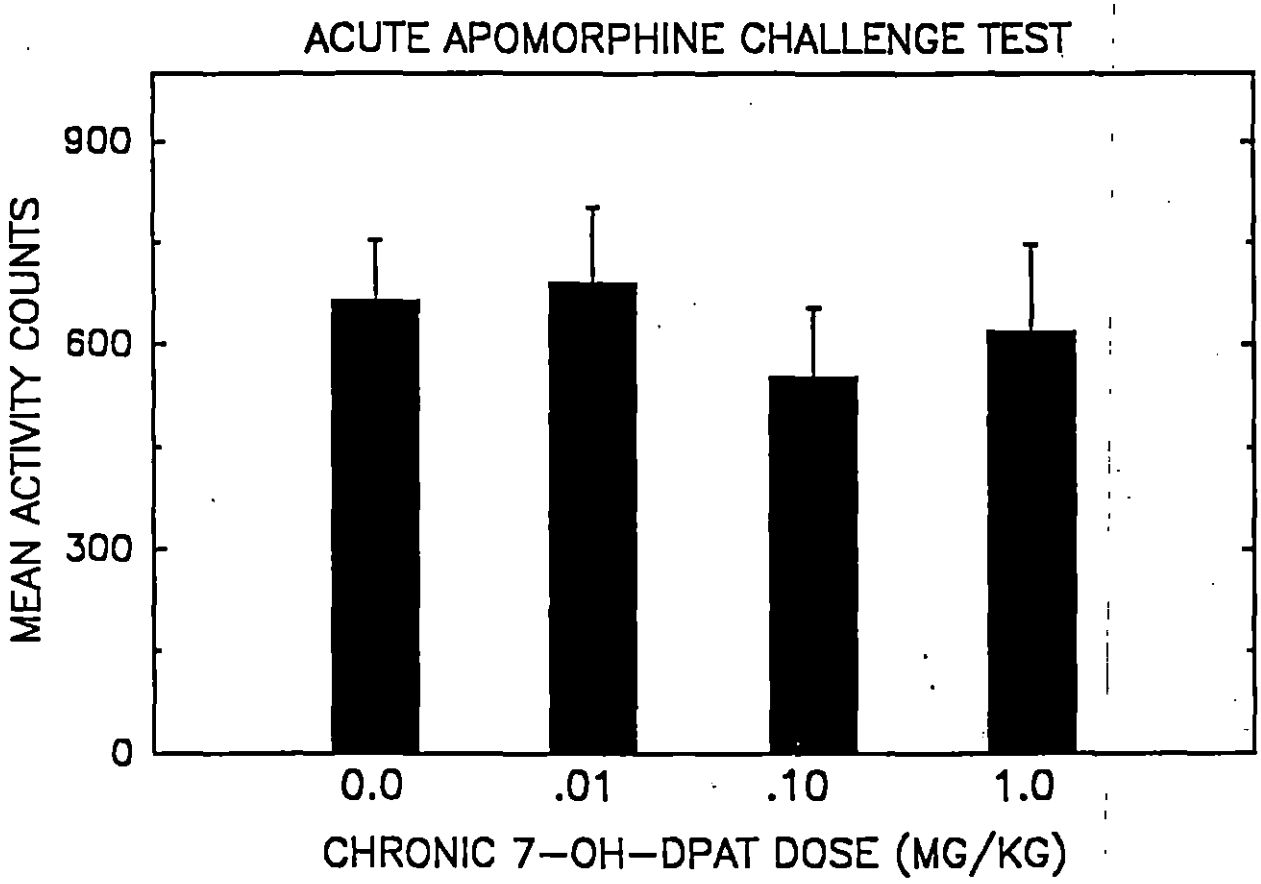
ACUTE APOMORPHINE CHALLENGE TEST
MEAN ACTIVITY COUNTS
900
600
300
0
0.0
.01
.10
1.0
CHRONIC 7-OH-DPAT DOSE (MG/KG)

FIGURE 2

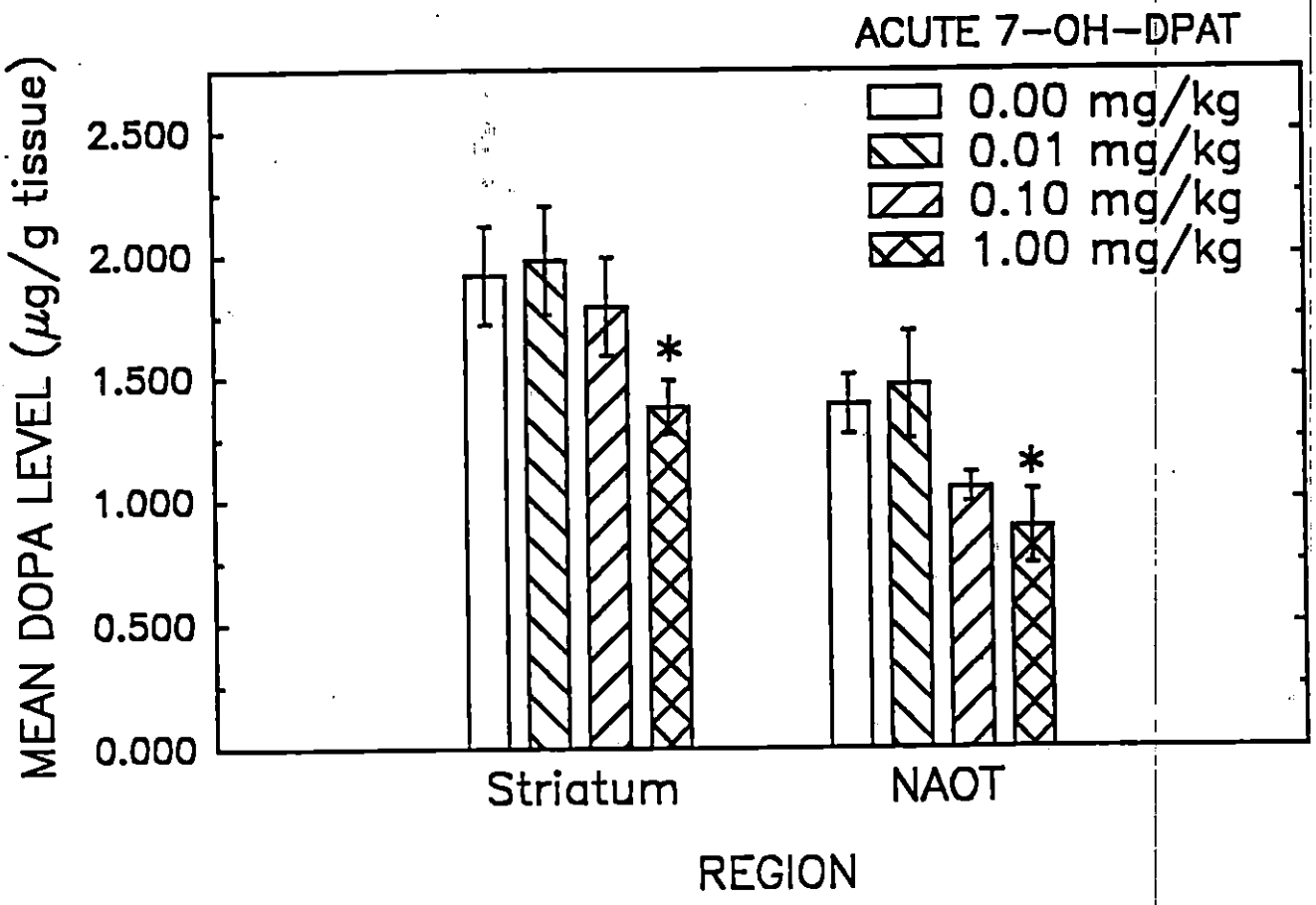
ACUTE 7-OH-DPAT
0.00 mg/kg
0.01 mg/kg
0.10 mg/kg
1.00 mg/kg
MEAN DOPA LEVEL (μg/g tissue)
2.500
2.000
1.500
1.000
0.500
0.000
*
*
Striatum
NAOT
REGION

FIGURE 3

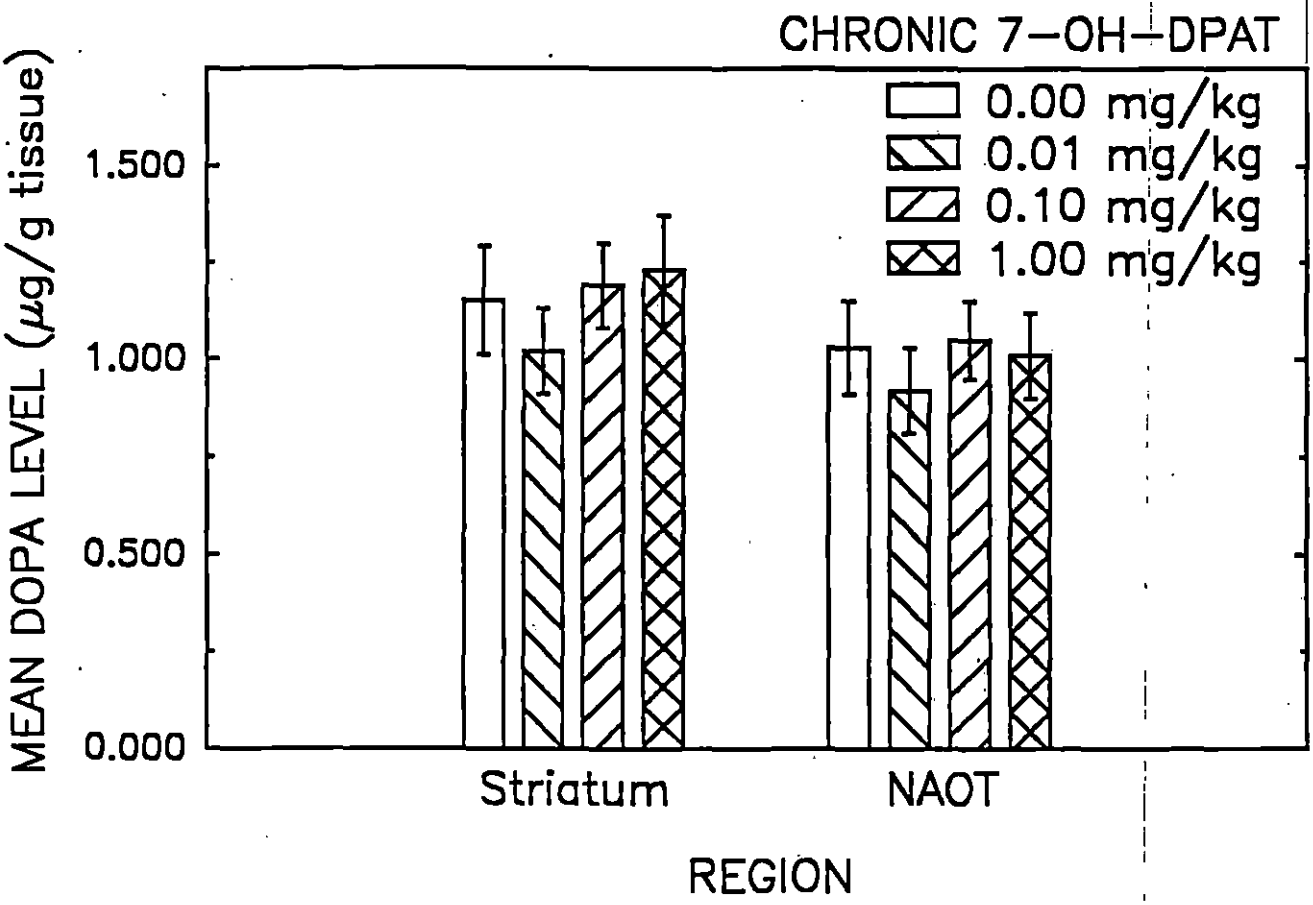
CHRONIC 7-OH-DPAT
0.00 mg/kg
0.01 mg/kg
0.10 mg/kg
1.00 mg/kg
MEAN DOPA LEVEL (µg/g tissue)
1.500
1.000
0.500
0.000
Striatum
NAOT
REGION

FIGURE 4

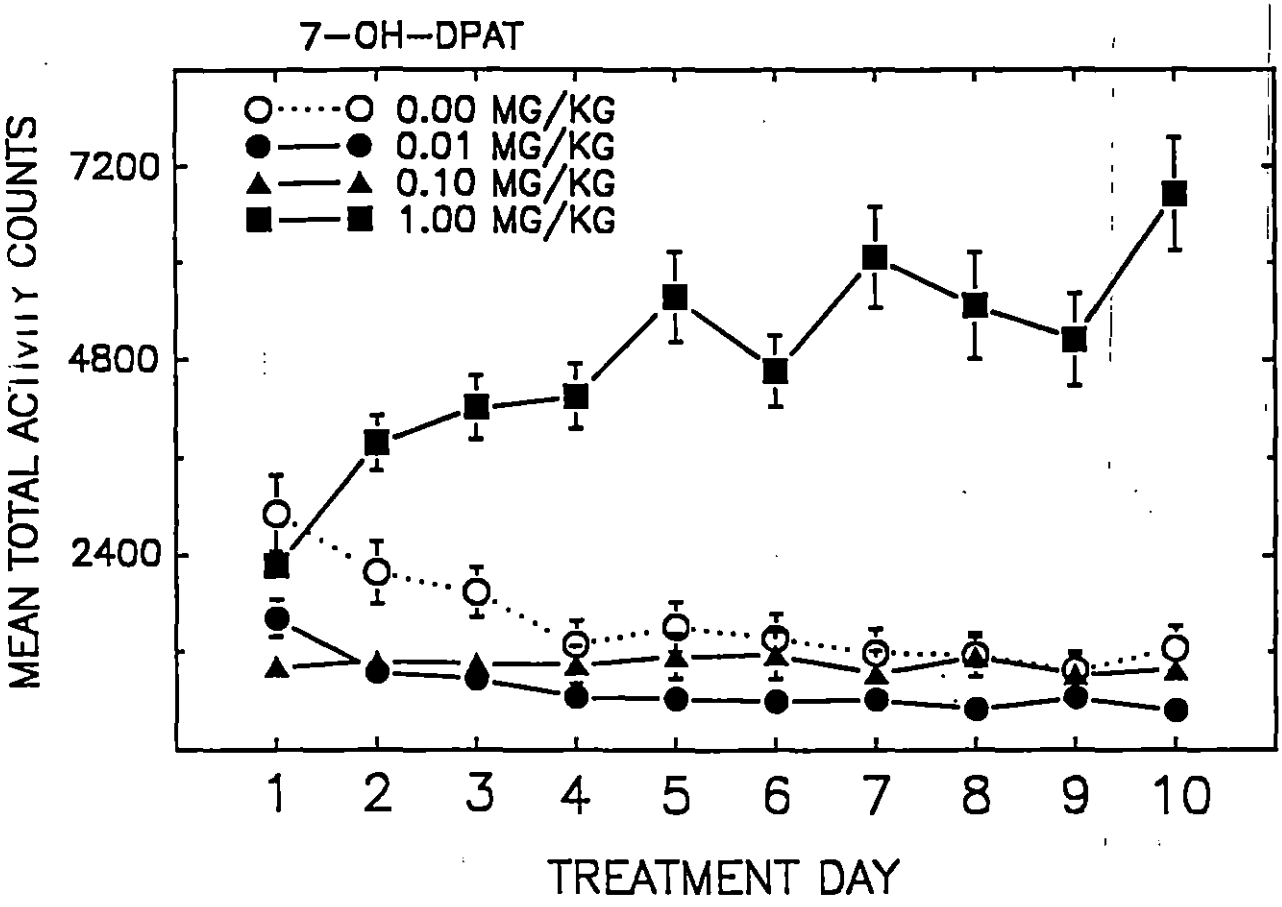
7-OH-DPAT
0.00 MG/KG
0.01 MG/KG
0.10 MG/KG
1.00 MG/KG
MEAN TOTAL ACTIVITY COUNTS
7200
4800
2400
1
2
3
4
5
6
7
8
9
10
TREATMENT DAY

FIGURE 5

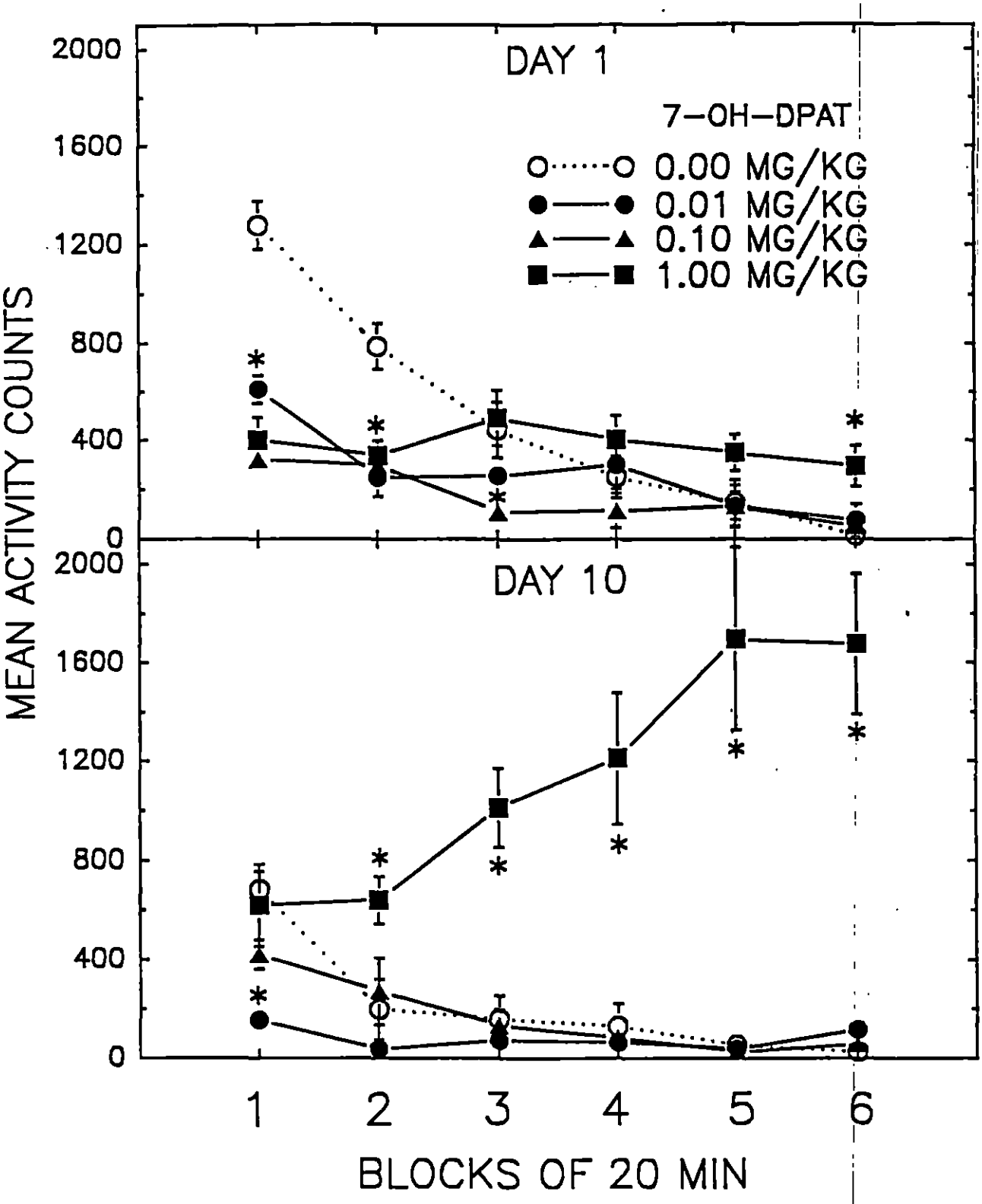
DAY 1
7-OH-DPAT
0.00 MG/KG
0.01 MG/KG
0.10 MG/KG
1.00 MG/KG
DAY 10
MEAN ACTIVITY COUNTS
2000
1600
1200
800
400
0
1
2
3
4
5
6
BLOCKS OF 20 MIN

FIGURE 6

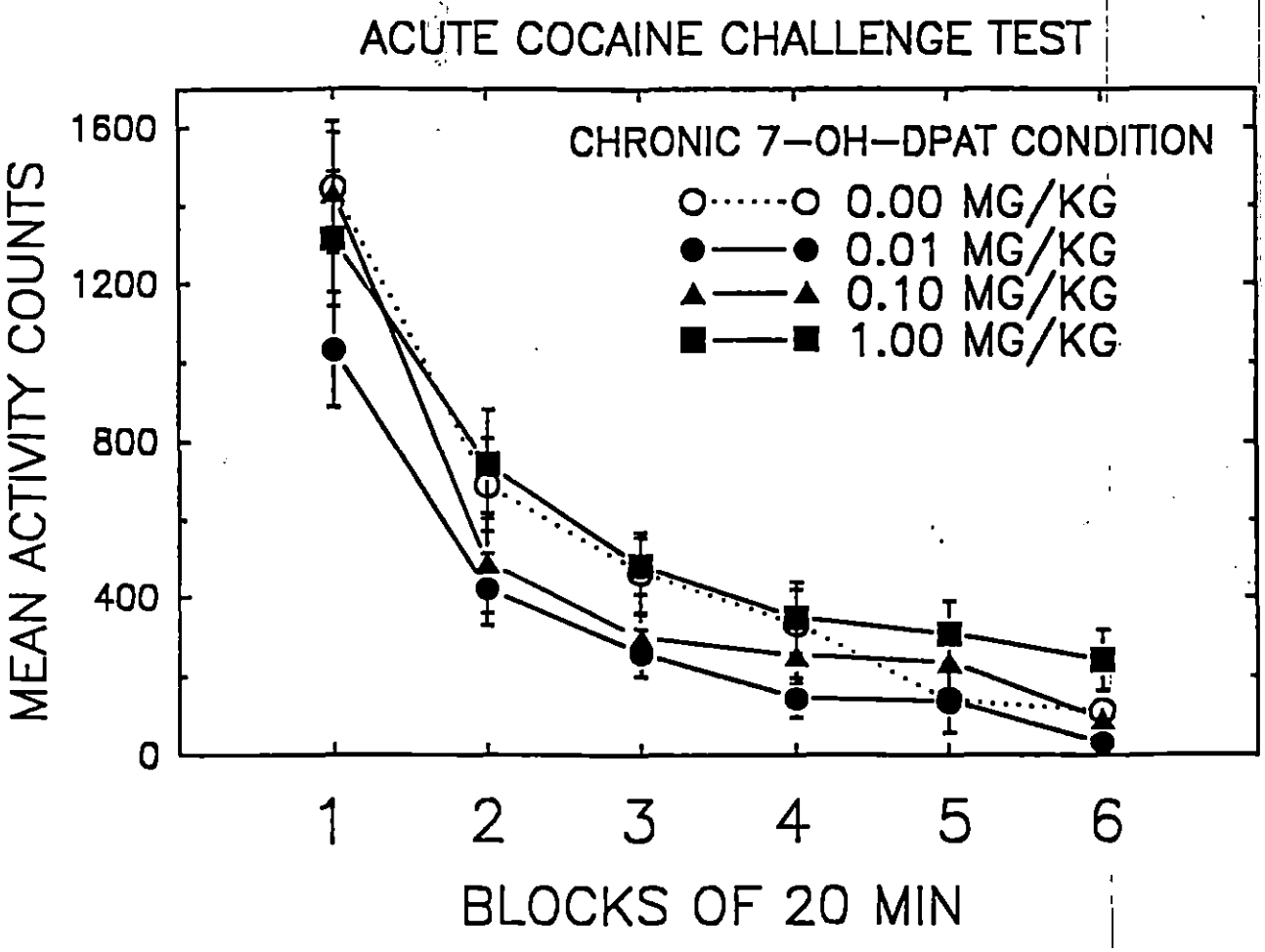
ACUTE COCAINE CHALLENGE TEST
CHRONIC 7–OH–DPAT CONDITION
0.00 MG/KG
0.01 MG/KG
0.10 MG/KG
1.00 MG/KG
MEAN ACTIVITY COUNTS
1600
1200
800
400
0
1
2
3
4
5
6
BLOCKS OF 20 MIN

FIGURE 7

Table 1. Comparison of sensitization and sensitization-related effects among apomorphine (Mixed D_1/D_2-type), quinpirole (D_3/D_2), 7-OH-DPAT (D_3), bromocryptine (D_2-type) and SKF 38393 (D_1-type) in rats.

		Cross-sensitization/ sensitization[2]			
agonist	sensitization[1]	apomorphine	cocaine	decrease DA synthesis[3]	enhance basal DA synthesis[4]
apomorphine	+	+	+	+	+
quinpirole	+	+	+	+	+
7-OH-DPAT	+	-	-	+	-
bromocryptine	+	*	-	+	*
SKF 38393	-	+	*	-	-

symbols: + effective, - not effective, * not tested. DA, dopamine.
[1]sensitization to the locomotor effects of individual drugs.
[2]cross-sensitization, as revealed by a challenge test with apomorphine or cocaine, or sensitization to apomorphine or cocaine by a drug that did not produce sensitization itself.
[3]agonist-induced decrease in striatal dihydroxyphenylalanine(DOPA) accumulation (after NSD-1015 administration).
[4]increase in basal DOPA accumulation in striatum, 24 hours after the last chronic treatment.
references:Bannon et al., 1980;Brown et al., 1985;Hoffman and Wise, 1993;Horger and Schenk, 1991; Kiyatkin, 1994; Mattingly et al., 1993; Rowlett et al., 1991,1993,1995; Szechtman et al., 1994.