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Mesolimbic 7-OH-DPAT Affects Locomotor Activities in Rats

MERLE E. MEYER

Department of Psychology, University of Florida, Gainesville, FL 32611-2250

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MEYER, M. E. *Mesolimbic 7-OH-DPAT affects locomotor activities in rats.* PHARMACOL BIOCHEM BEHAV **55**(2) 209–214, 1996.—This study tested the hypotheses that the dopamine D_3 receptor is both an autoreceptor and a postsynaptic receptor and has an affinity for dopamine at the nanomolar level. The effect of bilateral microinjections of a dopamine D_3-like agonist, 7-OH-DPAT, into the nucleus accumbens and into the ventral tegmental area (VTA) was tested with rats in activity monitors. Horizontal movement, rearing, and stereotypy times in seconds were automatically measured during 12 consecutive 10-min time blocks. Intraaccumbens 7-OH-DPAT (0.0001–10.0 μg/side) resulted in a highly significant dose by time block interactions. The dose of 0.0001 μg/side resulted in the potentiation of horizontal movement time during the time blocks 10–40 min; whereas, 0.001–1.0 μg/side potentiated locomotion during the early blocks following the 10-min interval. However, 10.0 μg/side resulted in a biphasic effect, attenuation followed by potentiation. 7-OH-DPAT (0.0001–1.0μg/side) potentiated rearing time in the early time blocks and (0.001–10.0μg/side) attenuated stereotypy time during the first 20 min time blocks. On the other hand, intra-VTA 7-OH-DPAT (10.0 μg/side) attenuated horizontal movement time during the first 20-min time blocks and (0.01 and 0.0001 μg/side) potentiated movement time at the 20-min time block. Intraventral tegmental area 7-OH-DPAT had no effects on rearing and stereotypy times. These data support the hypothesis that the D_3 receptor has an affinity for dopamine at the nanomolar level and question the hypothesis that the D_3 receptors are both autoreceptors and postsynaptic receptors.

Nucleus accumbens Ventral tegmental area Dopamine Locomotion Rearing Stereotypy behavior
D_3 receptor 7-OH-DPAT Rat

DOPAMINE receptors within the central nervous system have been of significant interests because of their possible involvement in schizophrenia and Parkinson's disease. As the result of five distinct genes and splice variants, the existence of at least five subtypes of dopamine receptors is now accepted. These subtypes have been classified into two subfamilies where the intronless genes encode D_1 and D_5 receptors associated with adenylate cyclase and where intron genes encode D_2, D_3, and D_4 receptors (22,23,27,30).

Recently, 7-OH-DPAT (7-hydroxy-N,N-di-n-propyl-2-aminotetralin) has been identified as a partial selective probe for the cloning of the dopamine D_3 receptor (11). The 7-OH-DPAT binding of this dopamine D_3-like agonist had >100-, >1000-, and >10,000-fold selectivity for the D_3 over D_2, D_4, and D_1 receptors, respectively. In addition, from the autoradiography studies, it has been shown that the D_3 receptors have restricted distribution of 7-OH-DPAT binding sites associated primarily to the Islands of Calleja, the 9 and 10 lobes of the cerebellum, the shell of the nucleus accumbens, and olfactory bulb. The D_3 receptor mRNA has also been shown to be present in the same areas, but also in the substantia nigra and the ventral tegmental area (2,9).

There is pharmacological evidence to support the hypothesis that the D_3 receptor is both a presynaptic and a postsynaptic receptor (14,26,28,31). However, from the localization evidence, it is not clear whether these pharmacological effects involve the presynaptic and postsynaptic receptors. It has been suggested that the effects of 7-OH-DPAT in the nucleus accumbens would act primarily on the postsynaptic D_3 receptors and in the ventral tegmental area (VTA) primarily on the D_3 autoreceptors (8). At the behavioral level, attenuation of locomotor activities in response to low dosages of dopamine agonists has been classically interpreted as resulting from selective stimulation of dopamine autoreceptors. On the other hand, potentiation of locomotor activities by intermediate dosages of dopamine agonists and the induction of stereotypy with high doses has been interpreted as resulting from postsynaptic effects (4,7,25).

The primary function of the present study investigated the possibility that the D_3 receptors in the mesolimbic system were both presynaptic and postsynaptic receptors. We studied the behavioral effects of 7-OH-DPAT microinjected into the nucleus accumbens and ventral tegmental area on various locomotor activities. A secondary function was to explore the effects of nanomolar dosages on locomotor activity.

METHOD

Subjects

Long–Evans rats (Charles River, Wilmington, MA), weighing between 250–275 g, were used in this study. The rats were individually housed in stainless steel cages, had food and water ad lib, and were maintained on a 12 L:12 D (0700–1900 h) cycle. The animals were tested in the light phase between 1000–1600 h. The room in which the animals were maintained was at a constant temperature (21°C ± 2). This study was carried out in compliance with the rules set forth in the NIH Guide for the Care and Use of Laboratory Animals.

Surgery

The animals, while under equithesin anesthesia, were cannulated bilaterally with the use of a stereotaxic instrument. Guide cannulae, fabricated from 23 g stainless steel hypodermic needles were permanently fixed to the skull with microscrews and dental cement. The guide cannulae were implanted following the coordinates from Paxinos and Watson (17) with references to bregma, lateral, from the midline and skull surface, respectively: nucleus accumbens, +1.7 mm, 1.4 mm, −2.5 mm (injection cannulae, −7.0 mm) and ventral tegmental area, −4.8 mm, 1.0 mm, −2.5 mm (injection cannulae, −8.0 mm). The surgical procedures followed the University of Florida's aseptic rodent surgery guidelines. The animals were allowed a 2-week recovery before behavioral testing. During recovery, the animals were not handled or transported except for routine cleaning.

Histology

After the completion of behavioral testing, the rats were given an overdose of sodium pentobarbital and were perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brains were frozen, sectioned, mounted on slides, stained with cresyl violet, and the locations of the injection cannulae verified by two independent observers. Only those rats with bilateral placements in the nucleus accumbens or ventral tegmental area were used in the data analyses.

Drug and Drug Administration

The dopamine receptor D_3-like agonist, 7-OH-DPAT (7-OH-N,N-di-n-propyl-2-aminotetralin; Mol.Wt. 328.3) was obtained from Research Biochemicals Inc. (Natick, MA). The drug was dissolved in distilled water. Distilled water was also given for the vehicle control injections (0.00 μg/side). The drug solutions were made up daily to the concentrations of 0.0001, 0.001, 0.01, 0.1, 1.0, or 10.0 μg. The 0.25 μl of solution was microinjected bilaterally through 30 g injection cannulae over a period of 60 s and the cannulae remained in place for an additional 60 s.

Apparatus

The animals were not habituated to the test apparatus but immediately following intraaccumbens or intraventral tegmental area microinjections, each rat was placed in an Omnitech Digiscan Animal Activity Monitor (Columbus, OH) for 120 min. The acrylic cage within the monitor measured 41.91 × 41.91 × 30.54 cm. The monitor was equipped with 16 beams 2.54 cm apart from front to back and from side to side, as well as 16 beams 2.54 cm apart from side to side on the upper level. Every 100 ms, the computer sampled the status of all of the beams. The Digiscan analyzer converted the patterns of beams broken into different measures of locomotor activity. In this study, the measures automatically analyzed were the horizontal movement time in seconds (as long as the animal moved, movement time was incremented); rearing time in seconds (as long as the animal was rearing and activated the upper sensors, rearing time was incremented); and stereotypy time in seconds (as long as the animal was repeatedly breaking the same beam or set of beams, the monitor considered the animal was emitting stereotypy behaviors; this measure corresponded to grooming, head bobbing, weaving, chewing, etc.). These measurements were made during 12 consecutive 10-min time blocks.

Statistical Analyses

Each independent treatment group consisted of 8 to 12 animals chosen at random. There were 74 animals in the nucleus accumbens study and 42 animals in the VTA study. Animals were treated only once.

A two-factor mixed-design analysis of variance was used to analyze the 12 10 min time blocks by dose levels interaction effect for each behavioral measure. Significant interaction effects were followed up within time blocks by Dunnett's multiple comparison tests between the control group and the treatment groups. p-Values equal to or less than 0.05 were judged statistically significant.

RESULTS

Locomotor Effects of Intraaccumbens 7-OH-DPAT

Horizontal Movement Time. The seven dose levels (0.00, 0.0001, 0.001, 0.01, 0.1, 1.0, and 10.0 μg/side of 7-OH-DPAT) by 12 10-min time block interaction effect was highly significant, $F(66, 737) = 3.37, p < 0.001$, and is shown in Fig. 1A. The subsequent analyses revealed significant behavioral effects of the D_3 agonist upon horizontal movement time in seconds. During the time blocks of 10–40 min, the 0.0001 μg/side of 7-OH-DPAT elicited significant potentiation of locomotion ($ps < 0.01$). For the treatment groups with dose levels from 0.001 to 10.0 μg/side, they were all significantly potentiated movement time at time blocks of 20 to 40 min postinjection time; and only the 10.0 μg/side group was significant at 50 and 60 min ($ps < 0.05$ and 0.01). However, at time block 10 min, the horizontal movement time of the 10.0 μg/side group was significantly attenuated ($p < 0.01$). All other comparisons were not statistically significant ($ps > 0.05$).

Rearing Time. The dose by time block interaction was highly significant, $F(66, 737) = 1.94, p < 0.01$, and is shown in Fig. 1B. The subsequent analyses resulted in significant potentiation of rearing time in groups 0.0001–1.0 μg/side during time blocks 10 and 20 min and at 30 min for the 0.0001 μg/side group ($ps < 0.05$ and 0.01). All other comparisons were not statistically significant ($ps > 0.05$).

Stereotypy Time. The dose by time block interaction for stereotypy time was highly significant, $F(66, 737) = 2.09, p < 0.001$, and is shown in Fig. 1C. The subsequent analyses revealed significant attenuation of stereotypy time for the 0.001 to 10.0 μg/side groups during the 10-min time block and during the 20-min time block for the 10.0 μg/side group ($ps < 0.05$ and 0.01). All other comparisons were not statistically significant ($ps > 0.05$).

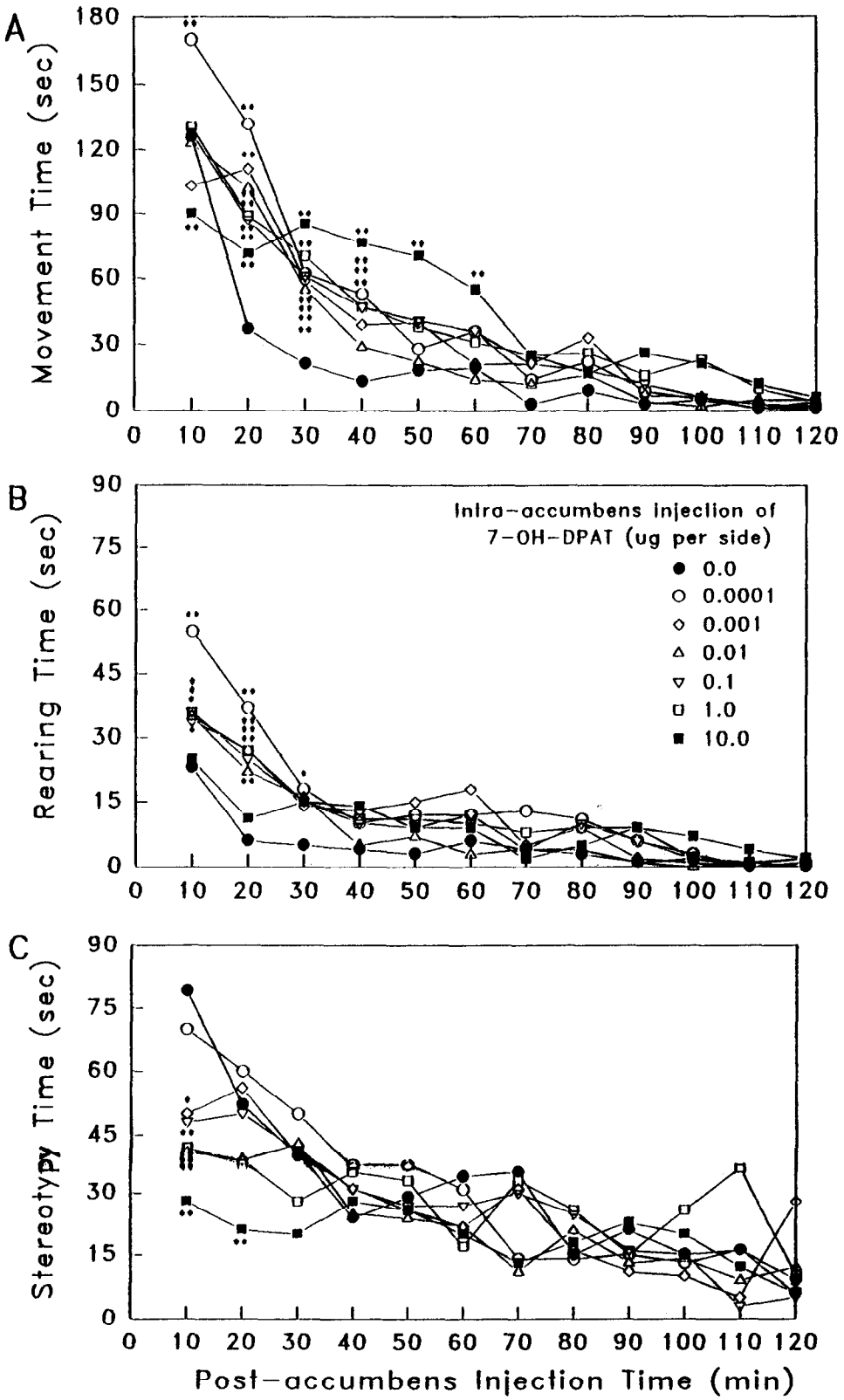

FIG. 1. Significant interaction effects of seven dosages of intra-accumbens injections of 7-OH-DPAT over 12 10-min intervals on (A) horizontal movement time in seconds; on (B) rearing time in seconds; and on (C) stereotypy time in seconds. Significant differences from the vehicle control group (0.00 μg) at each time point: $*p < 0.05$; $**p < 0.01$.

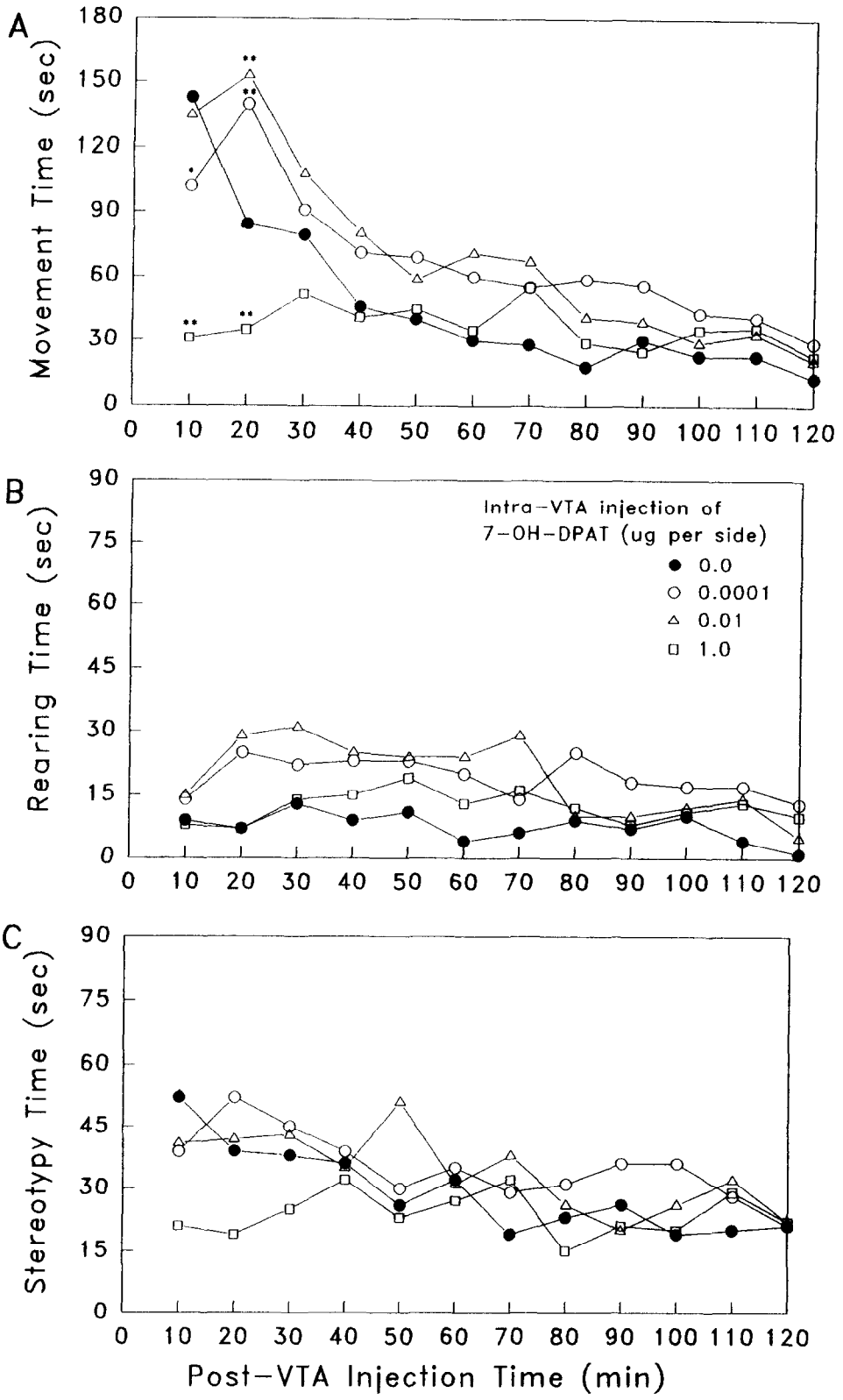

FIG. 2. Significant interaction effects of four dosages of intraventral tegmental area injections of 7-OH-DPAT over 12 10-min internals on (A) horizontal movement time in seconds; nonsignificant interactions effects on (B) rearing time in seconds and (C) stereotypy time in seconds. Significant differences from the vehicle control group (0.00 μg) at each time point: $*p < 0.05$; $**p < 0.01$.

Locomotor Effects of Intraventral Tegmental Area 7-OH-DPAT

Horizontal Movement Time. The four dose levels (0.00, 0.0001, 0.01, and 1.0 μg/side of 7-OH-DPAT) by 12 10-min time blocks interaction effect was highly significant, $F(33, 418) = 2.10$, $p < 0.001$, and is shown in Fig. 2A. Subsequent analyses showed significant attenuation for the 0.0001 and 1.0 μg/side groups during the first 10-min time block and during the 20-min time block for the 1.0 μg/side group ($ps < 0.05$ and 0.01). In time block 20 min, the 0.0001 and 0.01 μg/side groups movement times were significantly potentiated ($ps < 0.01$). All other comparisons were not significant.

Rearing Time. The dose by time block interaction for rearing time was not significant ($p > 0.05$), and is shown in Fig. 2B; no subsequent analyses were made.

Stereotypy Time. The dose by time block interaction for stereotypy behaviors was not significant ($p > 0.05$), and is shown in Fig. 2C; no subsequent analyses were made.

DISCUSSION

At the behavioral level, decreases in locomotor activities in response to low dosages of dopamine agonists have been interpreted as resulting from selective stimulation of dopamine autoreceptors followed by a reduction of dopamine synthesis and release; on the other hand, increases in locomotion elicited by higher dosages of dopamine agonists are due to postsynaptic effects (4,7,25,32). The hypothesis that autoreceptors, in response to low doses of dopaminergic agonists, mediate the attenuation of behavior is not, however, universally accepted. It has been argued that a subset of postsynaptic dopaminergic receptors are responsible for the attenuation (13,24). Recently, it has been hypothesized that the postsynaptic D_3 receptor mediate locomotor attenuation and the D_2 receptors are associated with the potentiation of locomotor activities (5,31). However, there may be no functional relationship between locomotion and autoreceptor activation (20).

Behavioral effects of 7-OH-DPAT based upon a time-sampling check-list procedure has been described (5). It was reported that systemic injections of large dosages (1.0–10.0 mg/kg) significantly potentiated sniffing, chewing, and locomotion over a total 1-h period. However, low dosages (0.01 and 0.1 mg/kg) did not result in significant attenuation of locomotion. On the other hand, a recent study described the effects of 7-OH-DPAT during a 15-min session and used comparable dosages that resulted in significant attenuation of locomotion and rearing (0.06 and 0.25 μmol kg^{-1}), whereas large dosages (4.0 and 16.0 μmol kg^{-1}) had no significant behavioral effects (1).

The D_3 receptor, from the radioligand binding studies, has been characterized within the limbic system as being both an autoreceptor and a postsynaptic receptor [i.e., (18,19,21)]. In addition, it has been shown that the dopamine neurons express D_3 receptor nRNA in the substantia nigra pars compacta and in the ventral tegmental area, which suggests that the D_3 is also an autoreceptor (22).

In the present study, the microinjections of 7-OH-DPAt directly into the nucleus accumbens resulted in the potentiation of locomotion, except for the initial attenuation with 10.0 μg/side dose. These data would support the hypothesis that the D_3 receptors are postsynaptic receptors. On the other hand, microinjections of 7-OH-DPAT directly into the ventral tegmental area did not result in the attenuation of locomotor behaviors with the 0.0001 μg/side group, but rather resulted in the potentiation of the behavior. These behavioral data would not support the hypothesis that the D_3 receptor in the ventral tegmental area functions as an autoreceptor.

These behavioral data with 7-OH-DPAT with 7-OH-DPAT were similar to the intraaccumbens D_1-family agonist effects (16) and to systemic D_1-family agonists where large dosages initially attenuated locomotor activities followed by potentiation and low dosages potentiated behavior (15). On the other hand, these present data were in contrast to the intraaccumbens quinpirole effects, where large dosages of quinpirole did not elicit a biphasic effect of attenuation followed by locomotor potentiation (29). However, quinpirole binds five times more to the D_2 receptor than to D_3 receptor (20). Low doses of the dopamine D_2/D_3 receptor agonist quinpirole attenuated locomotor activity when administrated systemically and centrally into the dorsal striatum but not in the nucleus accumbens. On the other hand, higher dosages elicited biphasic locomotor activity with attenuation followed by potentiation later in a 2-h session.

Recent studies of the 7-OH-DPAT electrophysiological effects have not differentiated between mesolimbic and nigrostriatial systems and between autoreceptors and postsynaptic receptors (6,8,12). The present intraaccumbens 7-OH-DPAT data suggest limbic behavioral functions for the D_3 receptor (3,21–23). However, without a selective D_3 antagonist, caution is needed in the interpretation of the D_3 receptor studies (10).

From the binding studies, (^{3}H) 7-OH-DPAT has been reported to bind to the D_3 receptors with subnanomolar affinity (11), as well as dopamine itself (23). The behavioral results of the present study would lend support to the hypothesis that the subnanogram level of 7-OH-DPAT elicits the potentiation of locomotor activity within both the nucleus accumbens and ventral tegmental area during relatively short durations. The relative short duration effects were similar to those reported on the effects of 7-OH-DPAT and core temperature (1).

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