ELSEVIER

Pharmacology, Biochemistry and Behavior 71 (2002) 569–580

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

# Subthalamic 5-$HT_{1A}$ and 5-$HT_{1B}$ receptor modulation of RU 24969-induced behavioral profile in rats

Diana L. Martinez-Price[1], Mark A. Geyer*

*Graduate Program in Neuroscience and Department of Psychiatry-0804, University of California-San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0804, USA*

Received 1 June 2001; received in revised form 24 August 2001; accepted 7 September 2001

**Abstract**

The effects of systemic administration of the serotonin (5-HT)$_{1A/1B}$ agonist 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)1*H*-indole (RU 24969) on locomotor and investigatory behavior in rats have been well characterized using the behavioral pattern monitor (BPM). To elucidate the neural circuitry underlying this behavioral profile, intracerebral dose–response studies were conducted at two sites with high densities of 5-$HT_{1B}$ receptors, the subthalamic nucleus (STN) and substantia nigra. Infusion of RU 24969 into the STN produced systemic RU 24969-like changes in locomotor activity and patterns but an uncharacteristic *increase* in investigatory holepokes. Intra-STN administration of the selective 5-$HT_{1A}$ receptor agonist 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT) produced RU 24969-like changes in locomotor patterns only, while the 5-$HT_{1B}$ receptor agonist 3(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one dihydrochloride (CP-93,129) increased locomotor activity, produced no change in locomotor patterns and nonsignificantly increased holepokes. Intranigral infusion of RU 24969 produced systemic and intra-STN RU 24969-like increases in locomotor activity. Intranigral RU 24969, however, failed to produce any changes in locomotor patterns or investigatory holepokes. Intranigral infusions of CP-93,129 or 8-OH-DPAT had no effects on locomotor activity, locomotor patterns or investigatory holepokes. These results provide evidence for multiple-site mediation of the locomotor-activating effects of RU 24969 and for a dissociation of the neural substrates underlying locomotor and investigatory components of the RU 24969-induced behavioral profile. 

*Keywords:* Locomotion; Subthalamic nucleus; 5-$HT_{1A}$; 5-$HT_{1B}$; RU 24969; CP-93,129; 8-OH-DPAT

## 1. Introduction

The roles of recognition sites for central neurotransmitters in the mediation of the behavioral effects of the putative 5-$HT_1$ receptor agonist 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)1*H*-indole (RU 24969) in the rat have previously been investigated using various pharmacologic depletion and anatomic lesioning techniques, as well as antagonist studies. Depletion of serotonin (5-HT) with 5,7-dihydroxytryptamine (5,7-DHT) potentiates RU 24969-induced hyperlocomotion compared to intact animals, suggesting an involvement of postsynaptic 5-HT receptors in the mediation of this response (Oberlander et al., 1986; Tricklebank et al., 1986). Early studies using antagonists with a high affinity for 5-$HT_1$ receptors revealed that RU 24969-induced hyperlocomotion was inhibited by the (−) isomers of pindolol and propranolol, consistent with an involvement of 5-$HT_{1A}$ or 5-$HT_{1B}$ receptors (Cheetham and Heal, 1993; Rempel et al., 1993). The RU 24969-induced response was not altered by the 5-$HT_{2A/2C}$ receptor antagonist ritanserin, the selective 5-$HT_3$ receptor antagonist ondansetron or the nonselective 5-HT receptor antagonists methysergide and metergoline, suggesting that neither 5-$HT_2$ nor 5-$HT_3$ receptor activation is necessary for RU 24969-induced hyperlocomotion. In attempts to elucidate specific receptor subtype contributions to the RU 24969-induced behavioral profile, antagonist studies using more selective 5-$HT_{1A}$ and 5-$HT_{1B}$ receptor antagonists provided different conclusions and seemingly conflicting results with regard to 5-HT receptor involvement in the production of the RU 24969-induced behavioral profile. RU 24969-induced changes in locomotor

* Corresponding author. Tel.: +1-619-543-3582; fax: +1-619-543-2493.

*E-mail address*: mgeyer@ucsd.edu (M.A. Geyer).

[1] Current address: National Center for Microscopy and Imaging Research, Department of Neuroscience, University of California-San Diego, La Jolla, CA 92093-0608, USA.

0091-3057/02/$ – see front matter 
PII: S0091-3057(01)00704-3

and investigatory behaviors might be attributable to the activation of a single 5-HT receptor subtype but is more likely the result of the activation of several 5-HT receptor subtypes. Indeed, recent evidence suggests that activation of *both* 5-$HT_{1A}$ and 5-$HT_{1B}$ receptors may contribute to the overall behavioral profile produced by RU 24969 administration (O'Neill and Parameswaran, 1997; Martinez and Geyer, 1999; O'Neill and Sanger, 1999).

Using the behavioral pattern monitor (BPM), previous studies in this laboratory have addressed the hypothesis that 3,4-methylenedioxymethamphetamine (MDMA) and other 5-HT releasers produce locomotor-activating effects via activation of postsynaptic 5-$HT_{1B}$ receptors (Callaway and Geyer, 1992; Callaway et al., 1992). Systemic administration of RU 24969 produces MDMA-like increases in locomotor activity and decreases in investigation in the rat (Rempel et al., 1993). As with RU 24969, examination of the qualitative aspects of the locomotor patterns reveals a tendency for MDMA-treated rats to ambulate around the perimeter of the BPM chamber with a tendency to avoid the center of the chamber (Paulus and Geyer, 1992; Rempel et al., 1993). In similar paradigms, both 5-$HT_{1A}$ and 5-$HT_2$ receptor agonists decrease locomotor activity (Mittman and Geyer, 1989; Wing et al., 1990). MDMA exhibits behavioral tolerance and cross-tolerance with RU 24969 but not with a direct 5-$HT_{1A}$ receptor agonist 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT), a 5-$HT_2$ agonist or amphetamine (Callaway et al., 1992). Hence, MDMA may be producing behavioral effects via activation of the same neural substrates as RU 24969, possibly by indirect action at the 5-$HT_{1B}$ receptor. The mechanisms subserving the decreases in investigatory behaviors and changes in locomotor patterns produced by RU 24969 and MDMA, however, remain unclear.

An alternative approach for identifying the neural substrates of a particular drug involves identifying potential receptor substrates and then directly infusing the drug into areas of the brain known to possess high concentrations of that receptor. Central studies of RU 24969 have applied this approach to the question of 5-$HT_{1B}$ receptor involvement in producing RU 24969-induced behaviors. 5-$HT_{1B}$ receptors are terminal autoreceptors on serotonergic neurons and terminal heteroreceptors on nonserotonergic neurons. 5-$HT_{1B}$ receptor are widespread throughout the brain but are most dense in basal ganglia nuclei known to be components of neuronal circuitry involved in locomotion, principally the substantia nigra pars reticulata (SNr), subthalamic nucleus (STN) and ventral pallidum (VP). Of these structures, the SNr has received the most attention in studies attempting to localize the locomotor-activating effects of systemic RU 24969. Intranigral administration of RU 24969 produces rotational behavior in rats, implicating the substantia nigra as a possible site of action (Blackburn et al., 1984; Higgins et al., 1991; Martinez and Geyer, 1999).

This series of intracerebral studies was conducted in an attempt to dissociate the neural substrates underlying distinct locomotor and investigatory components of the systemic RU 24969-induced behavioral profile. We predicted that both intra-STN and intranigral administration of RU 24969 would produce systemic RU 24969-like hyperlocomotion. Based on the hypothesized neural circuitry involving 5-$HT_{1B}$ receptor modulation of afferent GABAergic projections, we predicted that intracerebral RU 24969-induced hyperlocomotion would be reproduced by infusion of a 5-$HT_{1B}$ but not a 5-$HT_{1A}$ receptor agonist. No specific predictions were made regarding intra-STN or intranigral 5-$HT_1$ agonist-induced changes in either qualitative aspects of locomotor activity or investigatory holepokes. It was also predicted, however, that infusion of RU 24969 into either site might produce systemic RU 24969-like changes in locomotor activity and investigatory behaviors in the rat. Taken together, the experimental data presented in this report support a model of multiple-site mediation of components of the systemic RU 24969-induced behavioral profile.

## 2. Materials and methods

### 2.1. Subjects

Male Sprague–Dawley rats (275–300 g; Harlan, San Diego, CA) were housed in pairs and maintained on a reversed 12-h light/dark schedule (lights off at 07:00 h) in compliance with AAALAC guidelines. Food and water were provided ad libitum. All testings occurred during the dark phase between 09:00 and 15:00 h. Animals were handled and weighed upon arrival and allowed to acclimate for 1 week prior to surgery. All surgical subjects were housed in pairs, observed postoperatively for 10 days for infection and pain and allowed to recover for 1 week prior to testing. The experimental protocol followed in all studies was approved by the University of California-San Diego Animal Subjects Committee.

### 2.2. Drugs and solutions

RU 24969 (Tocris Cookson, Ballwin, MO), 3(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one dihydrochloride (CP-93,129; Pfizer, Groton, CT) and (±) 8-OH-DPAT (Research Biochemicals, Natick, MA) were dissolved in 0.9% saline solution. Control animals received equivalent infusions of vehicle. All solutions were prepared fresh daily.

### 2.3. Behavioral pattern monitors

The BPM is a 30.5 × 61-cm black Plexiglas chamber, which uses the combined features of activity and holeboard chambers to allow the measurement and sequential analysis of locomotor and investigatory behaviors (refer to Geyer et al., 1986 for details). The chamber is enclosed for sound isolation, well ventilated and uses red light bulbs for dark phase testing within a darkened room. A microcomputer

records the animal's successive holepokes, rearings and position. Holepokes are measured at 2.5-cm holes placed along the walls (3 along each long wall and 1 along back short wall) and floor (3) of each chamber. Each hole is equipped with a photocell that detects when the animal pokes its nose into the hole. The chamber is criss-crossed by a $4 \times 8$ array of photobeams, which detects the position of the animal in an $x-y$ plane. Activity within each chamber is monitored continuously by computer and data are collected and stored for later analysis.

### 2.4. Surgical procedures

For each experiment, surgeries were conducted by implanting bilateral chronic indwelling cannulae using coordinates obtained from a stereotaxic atlas (Paxinos and Watson, 1986). The coordinates for the STN (Experiments 1–3) were anteroposterior (AP) −3.8 mm from bregma, lateromedial (LM) ±2.6 mm from midline and dorsoventral (DV) −5.0 mm from skull (level skull). The coordinates for the control (Experiment 4; aimed medial and dorsal to STN coordinates) were AP −3.8 mm from bregma, LM ±2.2 mm from midline and DV −4.4 mm from skull (level skull). The coordinates for the substantia nigra (Experiments 5–7) were AP −5.3 mm from bregma, LM ±2.0 mm from midline and DV −5.0 mm from skull (level skull). Animals were anesthetized using Nembutal (pentobarbital; 50 mg/kg) and secured in the stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Aseptic surgical procedures were followed according to AAALAC guidelines. Bilateral 25-G stainless-steel cannulae were implanted 3 mm above the target structure. The cannulae were secured in place using stainless-steel skull screws (Small Parts, Miami Lakes, FL) and a UV light-curable dental material (Henry Schein, Port Washington, NY). The cannulae were kept patent by inserting a 30-G stylet wire into each cannula. During these daily health checks, cannulae were checked and missing or dirty stylets were replaced as necessary.

### 2.5. Drug infusions

Testing started no sooner than 7 days postoperatively, at which time the stylets were removed and replaced by 13-mm 30-G stainless-steel injector tubes. These injectors protruded 3 mm past the ventral tip of each cannula to reach the target site. There was a 30-min presession habituation after which the appropriate treatment was infused using a Harvard microinfusion pump (model 22, South Natick, MA). A volume of 0.5 μl/side was infused into the appropriate target site. Vehicle or drug solution was delivered bilaterally through the injectors from 10-μl Hamilton microsyringes via lengths of P.E. tubing at a rate of 0.2 μl/1 min. Movement of small (<1 μl) air bubbles in the tubing confirmed fluid flow. The injectors were left in place for 1 min postinfusion to allow for the diffusion of the drug. The injectors were then removed and clean stylets were replaced within the cannulae prior to the animal being placed into the testing chamber. The animal was then placed into the BPM chamber for a 3-h test session.

### 2.6. Dependent variables

Locomotor activity was quantified by the number of transitions between eight equal $15.25 \times 15.25$-cm regions in the BPM (crossings; Callaway et al., 1990). The number of holepokes was calculated for each time interval in 60-min blocks (Geyer et al., 1986).

### 2.7. Nonlinear measures of behavioral patterns

In addition to traditional quantitative measures of locomotor activity, data were assessed using scaling measures to quantify sequential patterns of behavioral organization, a property that is not assessed by traditional measures of locomotor and investigatory behaviors (Geyer and Paulus, 1992). The derivation of these scaling measures has been described previously by Geyer et al. (1986) and Paulus and Geyer (1991a,b, 1992, 1993). The spatial scaling exponent (spatial $d$) quantifies the geometrical structure of motor activity by assessing the "straightness" of consecutive movements and is independent of the amount of locomotor activity. A low $d$ value indicates a smoother locomotor path, while a high $d$ value indicates a rougher, more circumscribed locomotor path. This measure was utilized to allow the further parsing of drug-induced behavioral profiles.

### 2.8. Confirmation of cannulae placement

Upon completion of each experiment, dye (Evan's Blue; 0.5 μl/side) was infused into the cannulae and an overdose of pentobarbital was administered to all animals. The brain was extracted and stored in 10% formalin (Fisher, Pittsburgh, PA) until sectioning. Coronal sections with a thickness of 80 or 150 μm were prepared using a sliding microtome (Leica, Deerfield, IL). Sections (150-μm thick) were positioned between two clean glass slides and examined carefully, observing and noting dye placement and cannulae tracts to confirm placement of the cannulae. Additionally, the sections were scanned (ScanJet 5p; Hewlett-Packard, Palo Alto, CA) into image files for future examination. Sections (80-mm thick) were stained with cresyl violet. Then, cannulae tracts and injector tip placements were examined. Animals with injector placements outside of the targeted area were excluded from analyses of behavioral data. At the time of examination of injector tip placements, the experimenter was blind to the pharmacological treatment as well as to the behavioral data for each rat.

### 2.9. Data analysis

Measures of locomotor activity and investigation were examined in 60-min blocks. Statistical comparisons were

made at the various time resolutions using a statistical package (Dixon, 1993). For drug and drug–time interaction studies, two-way ANOVAs were performed. Specific post hoc comparisons were done using Tukey's Studentized Range Method. The criterion for significance was set at $P<.05$.

## 3. Results

### 3.1. Experiment 1: infusion of mixed 5-HT$_{1A/1B}$ agonist RU 24969 into STN

Figs. 1 and 2 illustrate the behavioral effects of intra-STN infusion of RU 24969 (0, 1.5 and 5.0 μg/side; $n=11$ animals/group). Intra-STN RU 24969 increased locomotor activity, as indicated by a significant main effect of drug on crossings [$F(2,30)=3.32$, $P<.05$]. An interaction between time block and drug for crossings [$F(4,60)=4.65$, $P<.005$] confirmed that the increase in locomotor activity produced by RU 24969 occurred during the first 60-min interval (0–60 min). Therefore, data presented are representative of this time period (Fig. 1A). Post hoc comparisons revealed that both 1.5- and 5.0-μg/side RU 24969 increased the number of crossings relative to the vehicle control group. RU 24969 increased investigatory holepokes, as indicated by a significant main effect of drug on the total number of holepokes [$F(2,30)=5.08$, $P<.02$]. Post hoc comparisons showed that both doses (1.5 and 5.0 μg/μl) of RU 24969 increased the number of holepokes relative to the vehicle control group (Fig. 1B). Visual examination of the geometrical characteristics of rat locomotor patterns revealed that the locomotor paths of the animals receiving RU 24969 tended to be straighter, smoother and less circumscribed than animals infused with vehicle (Fig. 2). This change in the visually observed locomotor patterns was confirmed by a significant main effect of drug treatment on the descriptive statistic spatial $d$ [$F(2,30)=3.32$, $P<.05$], and post hoc comparisons showed that RU 24969 significantly decreased spatial $d$ (Fig. 2).

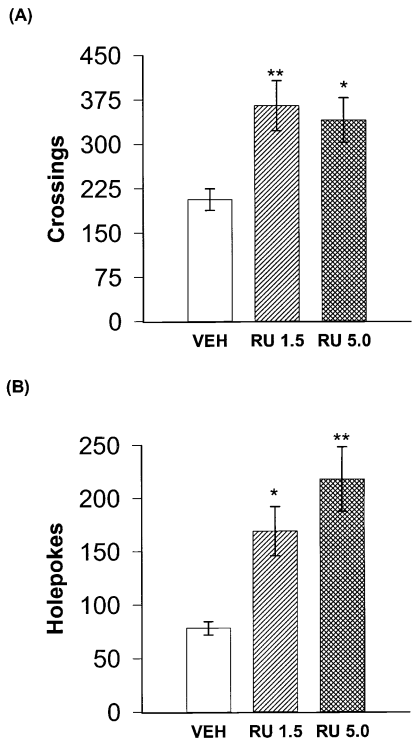


Fig. 1. (A) Effects of intra-STN infusion of (0.5 μl/side) vehicle or RU 24969 (1.5 and 5.0 μg/side) on Crossings. Data are presented as group means±S.E.M. Both doses of RU 24969 produced significant increases in the number of Crossings. An asterisk (*) denotes $P<.05$ significantly different from vehicle control group, while a double asterisk (**) denotes $P<.01$ significantly different from vehicle control group. (B) Effects of intra-STN infusion of vehicle or RU 24969 (1.5 and 5.0 μg/side) on investigatory holepokes. Data are presented as group means±S.E.M. Unlike systemic RU 24969, intra-STN RU 24969 produced a significant *increase* in the number of investigatory holepokes. An asterisk (*) denotes $P<.05$ significantly different from vehicle control group, and a double asterisk (**) denotes $P<.01$ level of significance.

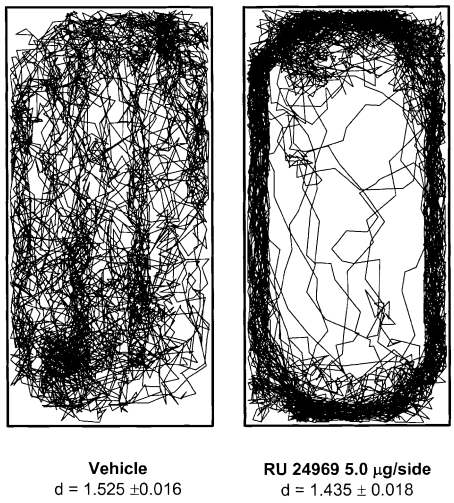


Fig. 2. Representative plots of individual rats and treatment group spatial $d$ values for intra-STN vehicle (left) and RU 24969 5.0 μg/side (right). The reconstructed plot consists of plotted $x$–$y$ positions over the first 60 min of the test session. The vehicle-treated animal showed no particular preference for any area of the test chamber. In contrast, the RU 24969-treated animal ambulated around the periphery of the chamber (while avoiding the center). Spatial $d$ values are presented as group means ± S.E.M. RU 24969 (5.0 μg/side) produced a significant decrease in spatial $d$. The asterisks (**) denote $P<.01$ significantly different from vehicle control group. The spatial $d$ value for the RU 24969 1.5-μg/side dose (plot not presented) is 1.47 ± 0.013.

### 3.2. Experiment 2: infusion of a selective 5-HT$_{1B}$ agonist CP-93,129 into STN

Effects of intra-STN infusion of the selective 5-HT$_{1B}$ agonist CP-93,129 (0, 0.5 and 1.5 μg/side; $n=6$, 9 and 8 animals/group, respectively) are shown in Figs. 3 and 4.

An analogue of RU 24969, CP-93,129, is 200 times more selective for the 5-$HT_{1B}$ receptor versus the 5-$HT_{1A}$ receptor and 100 times more selective for the 5-$HT_{1D}$ receptor (Macor et al., 1990). Like RU 24969, intra-STN CP-93,129 increased locomotor activity, as indicated by a significant main effect of drug on crossings [$F(2,19)=5.04$, $P<.02$; Fig. 3A). A significant interaction between time block and drug for crossings [$F(2,19)=5.04$, $P<.05$] revealed that the increase in locomotor activity produced by CP-93,129 occurred during the first 60-min interval (0–60 min). Hence, data presented are representative of this time period. Post hoc comparisons revealed that 1.5-μg/side CP-93,129 increased crossings (Fig. 3A). Examination of investigatory measures demonstrated that CP-93,129 produced changes in investigatory activity, as indicated by a significant main effect of drug on the total number of holepokes [$F(2,19)=4.94$, $P<.02$; Fig. 3B). Although post hoc comparisons revealed that the highest dose of CP-93,129 was not significantly different from saline, there was a nonsignificant trend toward CP-93,129 increasing holepokes ($P=0.1$). In contrast to intra-STN RU 24969, administration of CP-93,129 into the STN produced no changes in spatial *d*, as evidenced by a lack of main effect of treatment and absence of interactions (Fig. 4).

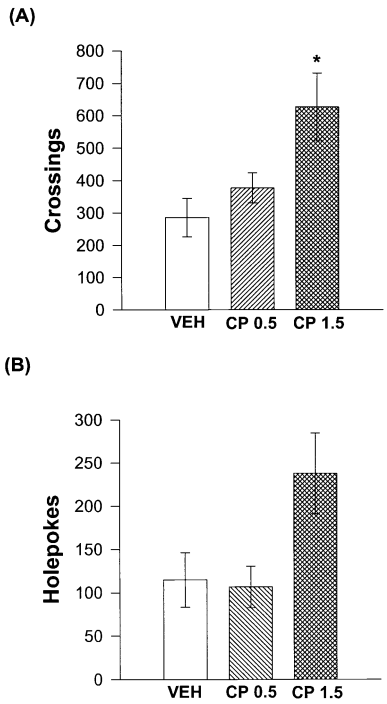


Fig. 3. (A) Effects of intra-STN infusion of vehicle or CP-93,129 (0.5 and 1.5 μg/side) on Crossings. Data are presented as group means ± S.E.M. CP-93,129 (1.5 μg/side) produced a significant increase in the number of Crossings. An asterisk (*) denotes $P<.05$ significantly different from vehicle control group. (B) Effects of intra-STN infusion of vehicle or CP-93,129 (0.5 and 1.5 μg/side) on investigatory holepokes. Data are presented as group means ± S.E.M. CP-93,129 (1.5 μg/side) produced a nonsignificant increase in the number of holepokes.

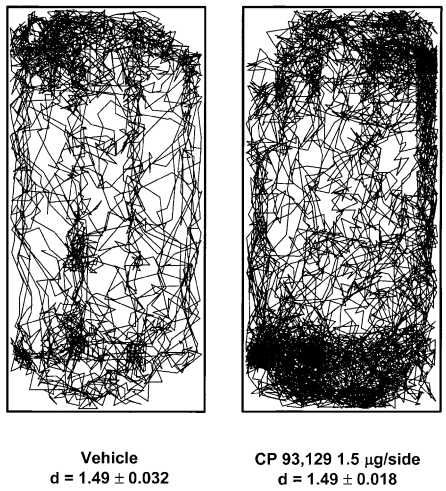


Fig. 4. Representative plots of rats treated with intra-STN vehicle or CP-93,129 (1.5 μg/side). The reconstructed plot consists of plotted *x*–*y* positions over the first 60 min of the test session. Upon visual inspection, there are no apparent qualitative differences between the plots shown. The spatial *d* values are presented as group means ± S.E.M. The spatial *d* value for the 0.5-μg/side dose (plot not presented) is 1.46 ± 0.018.

### *3.3. Experiment 3: infusion of a selective 5-$HT_{1A}$ agonist 8-OH-DPAT into STN*

Infusion of 8-OH-DPAT (0, 0.04, 0.2 and 1.0 μg/side; $n=4$, 7, 6 and 7 animals, respectively) into the STN produced no changes in the amount of locomotor activity or the frequency of investigatory holepokes, as indicated by

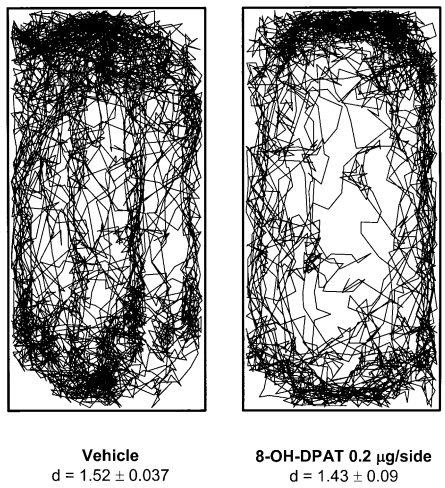


Fig. 5. Representative plots of rats and group values for spatial *d* of rats treated with intra-STN infusion of vehicle or 8-OH-DPAT (0.2 μg/side). The reconstructed plot consists of plotted *x*–*y* positions over the first 60 min of the test session. Upon visual examination of the plots, it was noted that the locomotor paths of 8-OH-DPAT-treated animals tended to avoid the center of the chamber and were smoother. Spatial *d* values are presented as group means ± S.E.M. 8-OH-DPAT (0.2 μg/side) produced a significant decrease in spatial *d*. The values for the other doses of 8-OH-DPAT (0.04 and 1.0 μg/side; plots not presented) were 1.48 ± 0.025 and 1.48 ± 0.013, respectively.

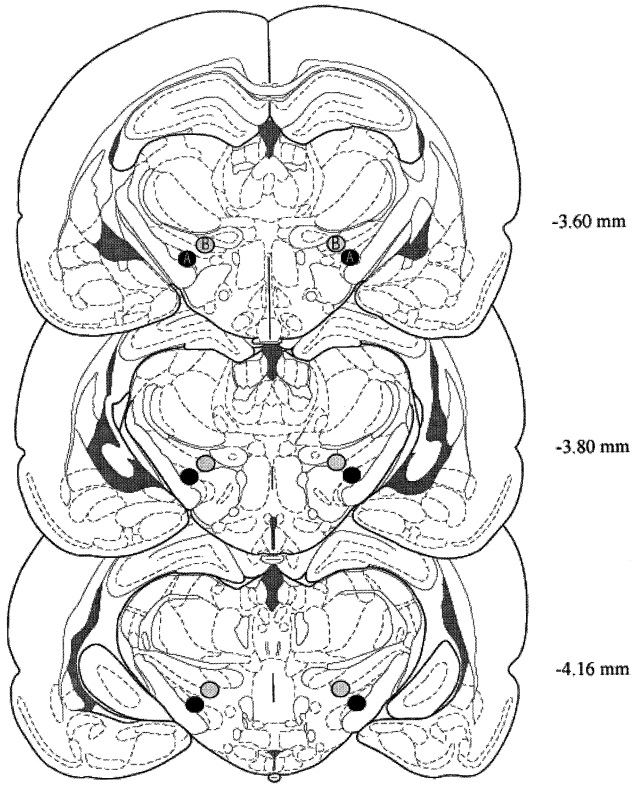


Fig. 6. Coronal cross-sections depicting the area encompassing injector tip placements (as indicated by dye infusion) within (A) the STN (from animals in Experiments 1–3) and (B) a control experiment (Experiment 4) targeting an area medial and dorsal to STN coordinates. Numbers denote distance (in mm) from bregma. Sketches derived from the atlas of Paxinos and Watson (1986).

a lack of main effect of treatment and absence of interactions (data not shown). Nevertheless, examination of the locomotor paths of rats infused with 8-OH-DPAT (Fig. 5) revealed straighter, smoother and less circumscribed paths, as confirmed by a significant main effect on spatial *d* [$F(3,20) = 6.60$, $P < .005$; Fig. 5). Post hoc comparisons revealed that 0.2-μg/side 8-OH-DPAT decreased spatial *d* (Fig. 5).

### 3.4. Experiment 4: infusion of CP-93,129 or RU 24969 into a control site adjacent to STN to assess anatomical specificity of increase in locomotor activity

In order to assess the site specificity of the effects observed in Experiment 1, vehicle or CP-93,129 was infused (0.15, 0.5 or 1.5 μg/side; $n = 8$, 9, 8 and 9 animals/group) into an area adjacent to the STN (see Fig. 6 for placement). In addition, these animals were retested following infusion with vehicle or a 5.0-μg/side dose of RU 24969. This dose of RU 24969 has been shown to produce increases in both crossings and holepokes and a decrease in spatial *d*. As shown in Tables 1 and 2, there were no effects of intra-STN CP-93,129 or RU 24969 on crossings, holepokes or spatial *d* when infused into this area.

### 3.5. Experiment 5: intranigral infusion of the mixed 5-$HT_{1A/1B}$ agonist RU 24969

Experiment 5 was designed to examine the effects of simultaneous bilateral intranigral infusions of RU 24969 (0, 1.5 and 5.0 μg/side) on locomotor activity, locomotor patterns and investigatory behaviors of the rat ($n = 6$, 8 and 5 animals/treatment group). These doses of RU 24969 were chosen in accordance with pilot data (not shown) as well as with the results of a previous study demonstrating that these doses produce a systemic RU 24969-like effect when infused into the STN (Experiments 1–3). It was hypothesized that administration of intranigral RU 24969 would produce a systemic RU 24969-like behavioral profile con-

Table 1
Infusion of vehicle or CP-93,129 (0.5 and 1.5 μg/side) into site adjacent to STN

| Treatment | Vehicle ($n = 8$) | CP-93,129 0.15 μg/side ($n = 9$) | CP-93,129 0.5 μg/side ($n = 8$) | CP-93,129 1.5 μg/side ($n = 9$) |
|---|---|---|---|---|
| Crossings | | | | |
| 0–60 min | 365.62 ± 22.98 | 246.55 ± 23.27 | 255.87 ± 50.12 | 279.33 ± 32.83 |
| 61–120 min | 232.62 ± 24.36 | 170.77 ± 31.97 | 154.75 ± 15.87 | 169.00 ± 18.74 |
| 121–180 min | 172.00 ± 29.61 | 151.33 ± 30.43 | 139.37 ± 10.87 | 177.11 ± 17.41 |
| Total holepokes | | | | |
| 0–60 min | 179.62 ± 37.05 | 166.55 ± 40.82 | 229.50 ± 48.64 | 203.88 ± 41.60 |
| 61–120 min | 227.00 ± 40.72 | 213.44 ± 53.97 | 253.37 ± 72.70 | 250.00 ± 56.78 |
| 121–180 min | 186.87 ± 43.41 | 208.33 ± 51.89 | 185.00 ± 33.89 | 304.33 ± 87.43 |
| Spatial *d* | | | | |
| 0–60 min | 1.483 ± 0.016 | 1.479 ± 0.014 | 1.474 ± 0.020 | 1.486 ± 0.015 |
| 61–120 min | 1.529 ± 0.021 | 1.518 ± 0.023 | 1.499 ± 0.022 | 1.474 ± 0.022 |
| 121–180 min | 1.543 ± 0.020 | 1.523 ± 0.015 | 1.524 ± 0.017 | 1.486 ± 0.016 |

Effects of infusion of vehicle or CP-93,129 (0.5 and 1.5 μg/side) into site adjacent to STN (Experiment 4) on crossings, holepokes and spatial *d*. Data are presented as group means ± S.E.M. in 60-min blocks.

Table 2
Infusion of vehicle or RU 24969 (5.0 μg/side) into site adjacent to STN

| Treatment | Vehicle ($n=8$) | RU 24969 5.0 μg/side ($n=8$) |
|---|---|---|
| Crossings | | |
| 0–60 min | 287.0 ± 11.24 | 296.3 ± 54.05 |
| 61–120 min | 143.0 ± 31.00 | 181.3 ± 24.50 |
| Total holepokes | | |
| 0–60 min | 151.0 ± 41.21 | 144.3 ± 36.79 |
| 61–120 min | 108.0 ± 46.18 | 124.3 ± 43.67 |
| Spatial *d* | | |
| 0–60 min | 1.460 ± 0.021 | 1.517 ± 0.033 |
| 61–120 min | 1.551 ± 0.034 | 1.582 ± 0.006 |

Effects of infusion of vehicle or RU 24969 (5.0 μg/side) into site adjacent to STN (Experiment 4) on crossings, holepokes and spatial *d*. Data are presented as group means ± S.E.M. in 60-min blocks.

sisting of an increase in locomotor activity and decreases in spatial *d* and investigatory holepokes.

The effects of intranigral infusion of RU 24969 are presented in Table 3 and depicted in Figs. 7–9. There was a main effect-treatment [RU 24969 $F(2,20)=10.11$, $P<.001$]. Specific post hoc comparisons revealed that the highest dose of RU 24969 (5.0 μg/side) significantly increased the amount of locomotor activity, as measured by crossings in comparison to the vehicle-infused animals during the first two 60-min blocks of the test session (Table 3 and Fig. 7). An absence of a main effect of treatment or interactions of any kind indicated that intranigral infusion of RU 24969 did not produce any changes in the number of investigatory holepokes (Table 3 and Fig. 8). There was a Treatment × Time interaction [RU 24969 × Time $F(4,40)=2.64$, $P<.05$] for spatial *d*. Post hoc analyses failed to reveal the source of the interaction, although

Table 3
Intranigral infusion of RU 24969, a mixed 5-$HT_{1A/1B}$ agonist

| Treatment | Vehicle ($n=6$) | RU 24969 1.5 μg/side ($n=8$) | RU 24969 5.0 μg/side ($n=5$) |
|---|---|---|---|
| Crossings | | | |
| 0–60 min | 218.37 ± 30.3 | 331.37 ± 62.7 | 552.28 ± 108.9* |
| 61–120 min | 176.37 ± 35.69 | 108.62 ± 15.89 | 399.71 ± 74.9** |
| 121–180 min | 151.12 ± 27.26 | 85.25 ± 25.43 | 206.14 ± 51.91 |
| Total holepokes | | | |
| 0–60 min | 94.12 ± 44.51 | 118.12 ± 21.24 | 82.57 ± 25.48 |
| 61–120 min | 140.0 ± 60.17 | 150.00 ± 47.13 | 132.42 ± 32.63 |
| 121–180 min | 141.6 ± 64.88 | 100.62 ± 37.07 | 115.00 ± 35.97 |
| Spatial *d* | | | |
| 0–60 min | 1.532 ± 0.021 | 1.549 ± 0.034 | 1.553 ± 0.057 |
| 61–120 min | 1.564 ± 0.030 | 1.544 ± 0.033 | 1.587 ± 0.049 |
| 121–180 min | 1.540 ± 0.018 | 1.667 ± 0.045 | 1.685 ± 0.064 |

Data are presented as group mean ± S.E.M.
* $P<.05$ relative to vehicle control group.
** $P<.01$ relative to vehicle control group.

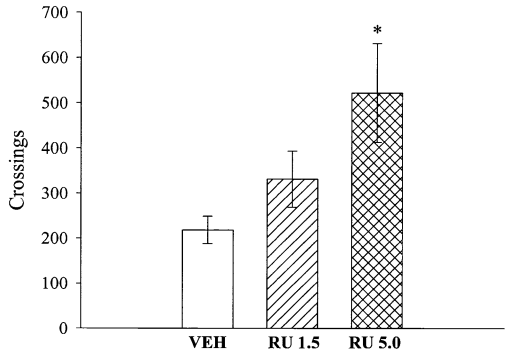


Fig. 7. Effects of intranigral administration of vehicle or RU 24969 (1.5 and 5.0 μg/side) on Crossings. Data are representative of the first 30 min of the test session and are presented as group means ± S.E.M. The 5.0-μg/side dose of RU 24969 produced a significant increase in the number of crossings. The asterisk (*) indicates a significant difference from the vehicle control group, with the criterion for significance set at $P<.05$.

the data seem to show that intranigral RU 24969 caused a small, nonsignificant increase in spatial *d* relative to the control animals (Table 3 and Fig. 9).

### 3.6. Experiment 6: intranigral infusion of the specific 5-$HT_{1B}$ agonist CP-93,129

This experiment tested the effects of intranigral administration of a selective 5-$HT_{1B}$ agonist CP-93,129 (0, 0.15, 0.5 and 1.5 μg/side) on locomotor activity, locomotor patterns and investigatory holepokes in the rat ($n=8$, 5, 7 and 7 animals/treatment group). The doses of CP-93,129 were chosen in accordance with a previous study in which doses of 0, 0.5 and 1.5 μg/side were used and demonstrated that 1.5-μg/side CP-93,129 produces significant increases in crossings (Experiment 2). Systemic 5-$HT_1$ antagonist studies of RU 24969 have demonstrated that increases in locomotor activity are antagonized by 5-$HT_{1B}$ or 5-$HT_{1A}$ receptor antagonists, while decreases in spatial *d* and investigatory activity are mediated via 5-$HT_{1A}$ receptor activation (Martinez and Geyer 1997). In addition, intra-STN admin-

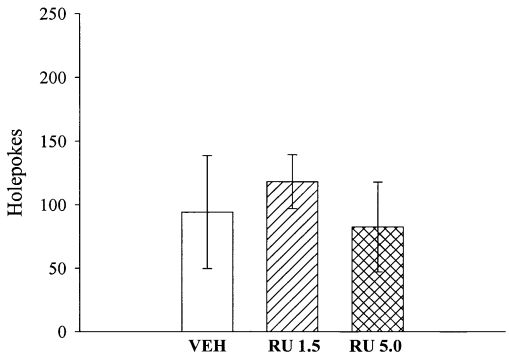


Fig. 8. Effects of intranigral infusion of vehicle or RU 24969 (1.5 and 5.0 μg/side) on investigatory holepokes. There were no significant effects of RU 24969 administration on holepokes at any time during the 90-min test session. Data presented here are representative of the first 30-min block of the test session and are presented as group means ± S.E.M.

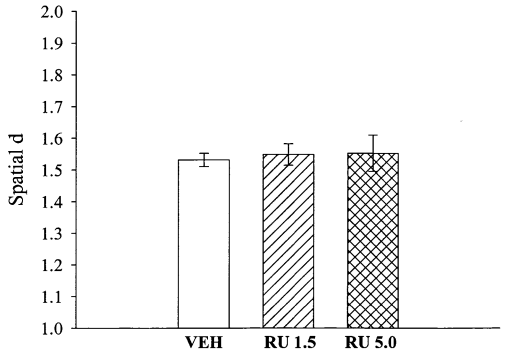


Fig. 9. Effects of intranigral administration of vehicle or RU 24969 (1.5 and 5.0 μg/side) on locomotor patterns. There were no observable effects of RU 24969 treatment on spatial *d* values at any time during the 90-min test session. Data depicted are representative of the first 30-min block of the test session and are presented as group means ± S.E.M.

istration of CP-93,129 produces increases in locomotor activity and holepokes but no changes in spatial *d*. Thus, it was predicted that intranigral administration of CP-93,129 would produce an RU 24969-like increase in locomotor activity and possibly holepokes while having no effects on rat locomotor patterns as measured by spatial *d*.

The results from Experiment 5 showed that intranigral infusion of RU 24969 produces increases in locomotor activity in rats, without additional effects on locomotor patterns or investigatory holepokes. In contrast, intranigral administration of the specific 5-$HT_{1B}$ agonist CP 93,129 resulted in no discernable changes in locomotor activity, patterns or investigatory holepokes, as evidenced by a lack of main effects or interactions (Table 4).

### *3.7. Experiment 7: intranigral infusion of the specific 5-$HT_{1A}$ agonist 8-OH-DPAT*

Experiment 7 was conducted to address the possibility that intranigral administration of RU 24969 produces

Table 4
Intranigral infusion of CP-93,129, a selective 5-$HT_{1B}$ agonist

| Treatment | Vehicle ($n=8$) | CP-93,129 0.15 μg/side ($n=5$) | CP-93,129 .5 μg/side ($n=7$) | CP-93,129 1.5 μg/side ($n=7$) |
|---|---|---|---|---|
| Crossings | | | | |
| 0–60 min | 206.3 ± 36.1 | 233.8 ± 41.1 | 198.0 ± 37.0 | 238.4 ± 51.5 |
| 61–120 min | 130.6 ± 16.4 | 129.6 ± 20.5 | 137.8 ± 28.2 | 87.5 ± 29.2 |
| 121–180 min | 123.8 ± 25.3 | 133.4 ± 31.3 | 96.1 ± 15.7 | 104.0 ± 18.0 |
| Total holepokes | | | | |
| 0–60 min | 45.5 ± 5.7 | 80.4 ± 20.8 | 61.2 ± 18.2 | 89.5 ± 32.8 |
| 61–120 min | 70.5 ± 16.5 | 61.6 ± 18.1 | 71.4 ± 22.4 | 92.4 ± 40.5 |
| 121–180 min | 68.2 ± 12.7 | 133.2 ± 37.0 | 54.7 ± 17.2 | 157.4 ± 61.3 |
| Spatial *d* | | | | |
| 0–60 min | 1.507 ± 0.022 | 1.487 ± 0.022 | 1.467 ± 0.014 | 1.484 ± 0.018 |
| 61–120 min | 1.566 ± 0.025 | 1.535 ± 0.029 | 1.509 ± 0.018 | 1.580 ± 0.049 |
| 121–180 min | 1.549 ± 0.017 | 1.477 ± 0.055 | 1.520 ± 0.022 | 1.541 ± 0.028 |

Data are presented as group mean ± S.E.M.

Table 5
Intranigral infusion of 8-OH-DPAT, a selective 5-$HT_{1A}$ agonist

| Treatment | Vehicle ($n=8$) | 8-OH-DPAT 0.04 μg/side ($n=9$) | 8-OH-DPAT 0.2 μg/side ($n=9$) | 8-OH-DPAT 1.0 μg/side ($n=8$) |
|---|---|---|---|---|
| Crossings | | | | |
| 0–60 min | 272.3 ± 34.9 | 283.8 ± 48.1 | 307.5 ± 45.5 | 322.3 ± 23.2 |
| 61–120 min | 131.2 ± 15.5 | 134.0 ± 24.2 | 130.3 ± 22.2 | 193.2 ± 18.9 |
| 121–180 min | 95.7 ± 22.1 | 93.5 ± 21.8 | 89.5 ± 27.7 | 128.3 ± 31.7 |
| Total holepokes | | | | |
| 0–60 min | 94.5 ± 19.4 | 100.1 ± 21.7 | 131.5 ± 36.8 | 120.1 ± 20.2 |
| 61–120 min | 103.3 ± 27.9 | 113.8 ± 27.0 | 93.2 ± 27.4 | 199.2 ± 55.5 |
| 121–180 min | 76.5 ± 26.9 | 122.5 ± 27.6 | 88.5 ± 38.4 | 194.1 ± 74.75 |
| Spatial *d* | | | | |
| 0–60 min | 1.457 ± 0.008 | 1.488 ± 0.033 | 1.470 ± 0.016 | 1.451 ± 0.015 |
| 61–120 min | 1.522 ± 0.018 | 1.569 ± 0.030 | 1.558 ± 0.018 | 1.513 ± 0.017 |
| 121–180 min | 1.535 ± 0.018 | 1.588 ± 0.039 | 1.585 ± 0.051 | 1.571 ± 0.037 |

Data are presented as group mean ± S.E.M.

increases in locomotor activity (crossings) via activation of 5-$HT_{1A}$ receptors. The effects of intranigral infusion of the selective 5-$HT_{1A}$ agonist 8-OH-DPAT (0, 0.04, 0.2 and 1.0 μg/side) were assessed on measures of locomotor activity, locomotor patterns and investigatory activity in the rat ($n=8–9$ animals/treatment group). The dose range of 8-OH-DPAT used in this central study can be approximated to systemic doses of 0-, 0.012-, 0.06- and 0.3-mg/kg 8-OH-DPAT, which well encompasses the doses of 8-OH-DPAT used in previous systemic studies and shown to produce decreases in locomotor activity, spatial *d* and investigatory holepokes (Mittman and Geyer, 1989;

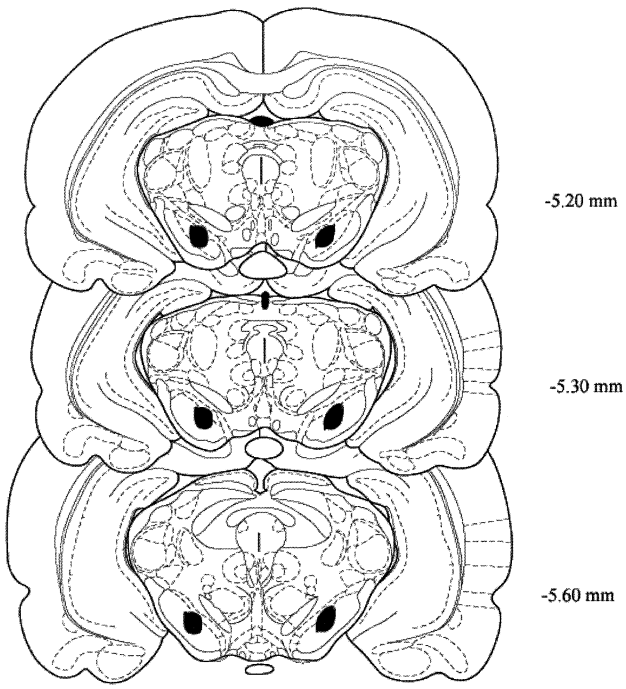


Fig. 10. Coronal cross-sections depicting the area encompassing injector tip placements in the substantia nigra (as indicated by dye infusion). Numbers denote distance (in mm) from bregma. Sketches derived from the atlas of Paxinos and Watson (1986).

Krebs-Thomson and Geyer, 1996). In addition, intra-STN administration of 8-OH-DPAT produces no changes in locomotor activity levels or holepokes but does decrease spatial *d*. Thus, it was hypothesized that intranigral administration of 8-OH-DPAT would produce a decrease (or no change) in locomotor activity and decreases in holepokes and spatial *d*.

As with intranigral CP-93,129 (Experiment 6), intranigral 8-OH-DPAT produced no discernable changes in crossings, spatial *d* or investigatory holepokes, as demonstrated by a lack of main effects of treatment or interactions (Table 5).

### *3.8. Confirmation of cannulae placements*

The locations of injector tip placements in the STN, control site and substantia nigra experiments are depicted in Fig. 10. Atlas coronal cross-section plates depict areas encompassing cannulae placements within each area. Rats were to be excluded from statistical analysis a priori due to placement of injector tips outside of the targeted areas, as indicated by dye infusion placements and cannulae tracts. The placements, however, were consistent for each individual experiment.

## 4. Discussion

The current studies indicate that activation of subthalamic and/or nigral 5-$HT_1$ receptors may mediate RU 24969-induced increases in locomotor activity, thereby providing evidence for multiple-site modulation of the locomotor-activating effects of RU 24969. Furthermore, the finding of intra-STN, but not intranigral, CP-93,129 induced increases in locomotor activity suggests that different 5-HT receptor substrates underlie the hyperlocomotion resulting from infusion of RU 24969 into either site. Specifically, it appears that activation of subthalamic 5-$HT_{1B}$, but not 5-$HT_{1A}$, receptors is sufficient to produce RU 24969-like increases in locomotor activity, while intranigral activation of either 5-$HT_{1B}$ or 5-$HT_{1A}$ receptor alone is not. In addition, the lack of changes in rat locomotor patterns or investigatory holepokes induced by intranigral infusion of RU 24969, CP-93,129 or 8-OH-DPAT suggests that nigral 5-$HT_1$ receptors do not modulate these particular behaviors.

Studies of RU 24969 have provided evidence of 5-$HT_{1A}$ receptor mediation of systemic RU 24969-induced decreases in spatial *d*. Specifically, pretreatment with the selective 5-$HT_{1A}$ antagonist WAY 100,635, but not the selective 5-$HT_{1B}$ antagonist GR 127,935, prevents RU 24969-induced changes in the locomotor patterns (thigmotaxis or "wall hugging") as well as decreases in spatial *d* (Martinez and Geyer, 1997). Consistent with these previous findings, intra-STN RU 24969 produced systemic RU 24969-like changes in rat locomotor patterns and decreases in spatial *d*. Intra-STN 8-OH-DPAT, but not CP-93,129, also produced systemic RU 24969-like changes in the characteristics of the locomotor patterns and a decrease in spatial *d*, which implicates 5-$HT_{1A}$, but not 5-$HT_{1B}$, receptor involvement in RU 24969-induced changes in rat locomotor patterns.

Systemic RU 24969 administration produces reliable decreases in investigatory holepokes. Systemic RU 24969-induced decreases in holepokes are prevented by pretreatment with WAY 100,635, but not GR 127,925, in the first 30-min block of testing (Martinez and Geyer, 1997). Interestingly, the number of holepokes increased significantly during the second 30-min block of testing (Experiment 1), which could be interpreted as resulting from an "unmasking" of 5-$HT_{1B}$ receptor activity in the presence of 5-$HT_{1A}$ receptor antagonism. Taken together with the present study, these data might indicate a role for 5-$HT_{1A}$ receptor activation in the systemic RU 24969-induced reduction of investigatory holepokes while hinting at an opposite role for 5-$HT_{1B}$ receptor activation in the increase in investigatory holepokes, as observed with intra-STN RU 24969.

The role of the STN in motor function has received a great deal of interest in the context of disorder-associated neuropathology, pharmacological treatments and neurosurgical intervention (Albin et al., 1989; Bergman et al., 1990; Klockgether and Turski, 1993; Albin, 1995). Because 5-$HT_{1B}$ receptors and 5-$HT_{1B}$ receptor mRNA are localized within the STN (Bruinvels et al., 1993; Raid et al., 2000), a structure postulated to be involved in locomotor activity, a central administration study of RU 24969 was conducted at this site. One possible mechanism for 5-$HT_{1B}$ receptor modulation of the nigrostriatal system via the STN involves a GABAergic projection from the ventral pallidum to the STN (Smith and Bolam, 1990). Inhibitory 5-$HT_{1B}$ receptors are present on the GABAergic projection from the ventral pallidum to the STN, thereby providing a means of dampening the inhibitory influence over the STN. This hypothesis is supported by the observation that 5-HT increases the spontaneous activity of STN neurons in the rat (Flores et al., 1995). Additionally, removal of the GABAergic influence by infusing picrotoxin, a GABA antagonist, into the ventral pallidum increases locomotor activity in the rat (Gong et al., 1997; Fletcher et al., 1998). Furthermore, a recent report by Chandha et al. showed that infusion of CP-93,129 inhibits the release of GABA from globus pallidus slice preparations (Chadha et al., 2000). The STN projects to the substantia nigra (Kanazawa et al., 1976) (Van der Kooy and Hattori, 1980) and this projection is glutamatergic (Nakanishi et al., 1987a; Smith and Parent, 1988; Robledo and Feger, 1990) and therefore excitatory in nature. Stimulation of STN increases the spontaneous firing of nigral neurons (Nakanishi et al., 1987b; Robledo and Feger, 1990) and enhances nigral dopamine release in rats (Mintz et al., 1986). The enhancement of nigral dopamine release is mediated by activation of NMDA receptors present on nigral dopaminergic dendrites (Rosales et al., 1994). Hence, 5-$HT_{1B}$ receptor activation in this instance would result in an increased excitatory input to the sub-

stantia nigra, increased neuronal firing in the nigra and finally increased dopaminergic transmission from the nigra to the striatum. Increased dopaminergic neurotransmission of the nigrostriatal pathway has been associated with increases in locomotor activity (Sharp et al., 1987) (Kuczenski and Segal, 1989).

The finding that both intra-STN and intranigral infusions of RU 24969 produce increases in locomotor activity indicates that multiple sites mediate systemic RU 24969-induced hyperlocomotion. Intra-STN administration of CP-93,129, but not 8-OH-DPAT, elicits a robust increase in locomotor activity, suggesting that selective activation of intra-STN 5-$HT_{1B}$, but not 5-$HT_{1A}$, receptors alone results in hyperlocomotion. In contrast, intranigral infusion of neither CP-93,129 nor 8-OH-DPAT resulted in any changes in the amount of locomotor activity in the present study. One explanation for this discrepancy is the possibility that, unlike intra-STN studies, the intranigral doses of CP-93,129 and 8-OH-DPAT used in the current study were too low to elicit a locomotor response. The amounts of locomotor activity, however, produced by infusion of RU 24969 (0, 1.5 and 5.0 μg/side) into the substantia nigra or STN were similar (Figs. 1 and 7). Since intra-STN administration of CP-93,129 produced a robust increase in locomotor activity, it is reasonable to expect that the use of the same doses of CP-93,129 would be appropriate to elicit a comparable increase in locomotor activity when infused into the substantia nigra. An alternative explanation for the lack of intranigral CP-93,129- or 8-OH-DPAT-induced changes in locomotor activity might be that the receptor substrates subserving intranigral RU 24969-induced increases in locomotor activity differ from those underlying intra-STN RU 24969-induced increases in locomotor activity.

Multiple-site mediation of the locomotor-activating effects of systemically administrated RU 24969 is demonstrated by the finding that RU 24969 infusions into either the nigra or the STN produce similar increases in locomotor activity. Intra-STN infusion of CP-93,129, but not 8-OH-DPAT, also increases locomotor activity. The results of the intra-STN studies of RU 24969, CP-93,129 and 8-OH-DPAT suggest that increases in locomotor activity produced by infusion of RU 24969 are mediated by 5-$HT_{1B}$, and not 5-$HT_{1A}$, receptor activation. In contrast, intranigral infusion of either CP-93,129 or 8-OH-DPAT failed to produce any changes in the amount of locomotor activity, suggesting that the 5-HT receptor substrates underlying intranigral and intra-STN RU 24969-induced hyperlocomotion are different.

This discrepancy might be attributable to incomplete dose–response studies of CP-93,129 and 8-OH-DPAT. However, previous systemic studies of 5-$HT_{1A}$ and 5-$HT_{1B}$ receptor agonists have demonstrated a functional interaction between these two receptors in producing increases in locomotor activity. Systemic coadministration of 5-$HT_{1A}$ and 5-$HT_{1B}$ receptor agonists results in an increase in locomotor activity greater than that produced by either compound alone, thereby demonstrating a functional synergy of activation of these two receptors in the production of hyperlocomotion (O'Neill and Parameswaran, 1997; O'Neill and Sanger, 1999). The results of Experiments 1–3 are not indicative of a functional interaction at the level of the STN. The intranigral study results, however, might be suggestive of a functional 5-$HT_{1A/1B}$ interaction. A possible 5-$HT_{1A/1B}$ receptor interaction could be addressed in future behavioral studies examining the effects of intranigral coinfusion of CP-93,129 and 8-OH-DPAT on locomotor activity. It has been hypothesized that intranigral RU 24969 induced increases in activity via activation of inhibitory 5-$HT_{1B}$ receptors located postsynaptically on an inhibitory GABAergic projection from the ventral palladium to the substantia nigra. Thus, a possible role for 5-$HT_{1A}$ receptor activation within the proposed circuitry underlying intranigral RU 24969-induced increases in locomotor activity cannot be excluded.

The results of Experiment 5 demonstrate that intranigral infusion of RU 24969 produces increases in locomotor activity but no changes in locomotor patterns or decreases in investigatory holepokes. The time course for intranigral RU 24969-induced increases in locomotor activity was similar to that observed with intra-STN RU 24969-induced increases in locomotor activity. Hence, the locomotor-activating effects of intranigral RU 24969 administration appear to be site specific rather than the result of RU 24969 diffusing from the nigra to another site. In contrast to the results of intra-STN studies, Experiments 5–7 suggest that intranigral activation of neither 5-$HT_{1A}$ nor 5-$HT_{1B}$ receptors is involved in changes in locomotor patterns or investigatory holepokes associated with systemic administration of RU 24969. The same doses of RU 24969, CP-93,129 and 8-OH-DPAT used in the intranigral study produced robust effects on locomotor activity, investigatory holepokes and spatial *d* when infused into the STN. Briefly, intra-STN administration of RU 24969 produced increases in investigatory holepokes and decreases in spatial *d*. In addition, intra-STN administration of 8-OH-DPAT, but not CP-93,129, produced decreases in spatial *d*, while CP-93,129, but not 8-OH-DPAT, produced increases in holepokes. A lack of behavioral effects resulting from intranigral infusion of either CP-93,129 or 8-OH-DPAT may indicate that the doses used in the present study were too low to produce any changes in locomotor activity, locomotor patterns or investigatory holepokes. Indeed, there is evidence to suggest that different 5-$HT_1$ receptor populations exhibit differential sensitivities to 5-HT1 receptor agonists. Electrophysiological studies have demonstrated differential effects of 8-OH-DPAT on 5-$HT_{1A}$ receptors localized to the dorsal raphe versus the hippocampus (Sprouse and Aghajanian, 1988). A similar finding was recently reported by another group examining $^3$[H] $K^+$ overflows in rat striatal and hippocampal synaptosome preparations in response to CP-93,129 (Sarhan and Fillion, 1999). Likewise, a higher intranigral dose of 8-OH-DPAT or CP-93,129 could be required to produce measurable changes in locomotor activity levels, locomotor patterns or

investigatory holepokes. Alternatively, while the data indicate that multiple sites mediate RU 24969-induced hyperlocomotion, it is possible that the sites underlying the locomotor-activating effects of systematic RU 24969 are different than those sites underlying RU 24969-induced decreases in spatial *d* and locomotor pattern changes.

The results of the intra-STN and intranigral 5-$HT_1$ receptor agonist infusions provide further evidence for dissociation of the neural substrates underlying locomotor and investigatory components of the RU 24969-induced behavioral profile. In addition to the measurement of the amount of locomotor activity, the added measurements of holepokes and spatial *d* allowed us to further dissociate the site-specific effects of centrally infused RU 24969. The present findings support the assertion that multivariate assessment of animal behavior provides a useful means to dissociate drug-induced behavioral effects. Regardless of the clinical relevance of a nonspecific drug such as RU 24969, these studies demonstrate the usefulness of intracerebral administration of pharmacological compounds in determining individual components of neural circuitry underlying animal behaviors.

## Acknowledgments

This work was supported by a grant from the National Institute on Drug Abuse (RO1 DA02925). The authors wish to thank Ms. Darlene Giracello for her invaluable assistance in conducting these experiments.

## References

Albin RL. The pathophysiology of chorea/ballism and parkinsonism. Parkinsonism Relat Disord 1995;1:3–11.

Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders (see comments). Trends Neurosci 1989;12:366–75.

Bergman H, Wichmann T, DeLong MR. Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. Science 1990;249:1436–8.

Blackburn TP, Kemp JD, Martin DA, Cox B. Evidence that 5-HT agonist-induced rotational behaviour in the rat is mediated via 5-$HT_1$ receptors. Psychopharmacology 1984;83:163–5.

Bruinvels AT, Palacios JM, Hoyer D. Autoradiographic characterisation and localisation of 5-$HT_{1D}$ compared to 5-$HT_{1B}$ binding sites in rat brain. Naunyn-Schmiedeberg's Arch Pharmacol 1993;347:569–82.

Callaway CW, Geyer MA. Tolerance and cross-tolerance to the activating effects of 3,4-methylenedioxymethamphetamine and a 5-hydroxytryptamine1B agonist. J Pharmacol Exp Ther 1992;263:318–26.

Callaway CW, Wing LL, Geyer MA. Serotonin release contributes to the locomotor stimulant effects of 3,4-methylenedioxymethamphetamine in rats. J Pharmacol Exp Ther 1990;254:456–64.

Callaway CW, Rempel N, Peng RY, Geyer MA. Serotonin 5-$HT_1$-like receptors mediate hyperactivity in rats induced by 3,4-methylenedioxymethamphetamine. Neuropsychopharmacology 1992;7:113–27.

Chadha A, Sur C, Atack J, Duty S. The 5HT (1B) receptor agonist, CP-93129, inhibits [(3)H]-GABA release from rat globus pallidus slices and reverses akinesia following intrapallidal injection in the reserpine-treated rat. Br J Pharmacol 2000;130:1927–32.

Cheetham SC, Heal DJ. Evidence that RU 24969-induced locomotor activity in C57/B1/6 mice is specifically mediated by the 5-$HT_{1B}$ receptor. Br J Pharmacol 1993;110:1621–9.

Dixon W. BMDP biomedical computer programs. In: 7.01 ed. Los Angeles: University of California Press, 1993.

Fletcher PJ, Korth KM, Sabijan MS, DeSousa NJ. Injections of D-amphetamine into the ventral pallidum increase locomotor activity and responding for conditioned reward: a comparison with injections into the nucleus accumbens. Brain Res 1998;805:29–40.

Flores G, Rosales MG, Hernández S, Sierra A, Aceves J. 5-Hydroxytryptamine increases spontaneous activity of subthalamic neurons in the rat. Neurosci Lett 1995;192:17–20.

Geyer MA, Paulus MP. Multivariate and nonlinear approaches to characterizing drug effects on the locomotor and investigatory behavior of rats. NIDA Res Monogr 1992;124:203–35.

Geyer MA, Russo PV, Masten VL. Multivariate assessment of locomotor behavior: pharmacological and behavioral analyses. Pharmacol, Biochem Behav 1986;25:277–88.

Gong W, Justice JBJ, Neill D. Dissociation of locomotor and conditioned place preference responses following manipulation of GABA-A and AMPA receptors in ventral pallidum. Prog Neuro-Psychopharmacol Biol Psychiatry 1997;21:839–52.

Higgins GA, Jordan CC, Skingle M. Evidence that the unilateral activation of 5-$HT_{1D}$ receptors in the substantia nigra of the guinea-pig elicits contralateral rotation. Br J Pharmacol 1991;102:305–10.

Kanazawa I, Marshall GR, Kelly JS. Afferents to the rat substantia nigra studied with horseradish peroxidase, with special reference to fibres from the subthalamic nucleus. Brain Res 1976;115:485–91.

Klockgether T, Turski L. Toward an understanding of the role of glutamate in experimental parkinsonism: agonist-sensitive sites in the basal ganglia. Ann Neurol 1993;34:585–93.

Krebs-Thomson K, Geyer MA. The role of 5-$HT_{1A}$ receptors in the locomotor-suppressant effects of LSD: WAY-100635 studies of 8-OH-DPAT, DOI, and LSD. Behav Pharmacol 1996;6:551–9.

Kuczenski R, Segal D. Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using in vivo microdialysis. J Neurosci 1989;9:2051–65.

Macor JE, Burkhart CA, Heym JH, Ives JL, Lebel LA, Newman ME, Nielsen JA, Ryan K, Schulz DW, Torgersen LK, Koe BK. 3-(1,2,5,6-tetrahydropyrid-4yl)pyrrolo[3,2-*b*]pyrid-5-one: a potent and selective serotonin (5-HT1B) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4yl)indole. J Med Chem 1990;33:2087–93.

Martinez DL, Geyer MA. A 5-$HT_{1B}$ antagonist blocks effects of RU 24969 on locomotor, but not investigatory, activity in rats. Soc Neurosci Abstr 1997;23:519.

Martinez DL, Geyer MA. Site-specific effects and dissociation of RU 24969-induced changes in locomotor activity and investigatory holepokes. Soc Neurosci Abstr 1999;25:1124.

Mintz I, Hammond C, Guibert B, Leviel V. Stimulation of the rat subthalamic nucleus enhances the release of dopamine in the rat substantia nigra. Brain Res 1986;376:406–8.

Mittman SM, Geyer MA. Effects of 5HT-1A agonists on locomotor and investigatory behaviors in rats differ from those of hallucinogens. Psychopharmacology 1989;98:321–9.

Nakanishi H, Kita H, Kitai ST. Electrical membrane properties of rat subthalamic neurons in an in vitro slice preparation. Brain Res 1987a; 437: 35–44.

Nakanishi H, Kita H, Kitai ST. Intracellular study of rat substantia nigra pars reticulata neurons in an in vitro slice preparation: electrical membrane properties and response characteristics to subthalamic stimulation. Brain Res 1987b;437:45–55.

Oberlander C, Blaquière B, Pujol JF. Distinct functions for dopamine and serotonin in locomotor behaviour: evidence using the 5-$HT_1$ agonist RU 24969 in globus pallidus-lesioned rats. Neurosci Lett 1986;67:113–8.

O'Neill MF, Parameswaran T. RU24969-induced behavioural syndrome requires activation of both 5$HT_{1A}$ and 5$HT_{1B}$ receptors. Psychopharmacology 1997;132:255–60.

O'Neill MF, Sanger GJ. GR46611 potentiates 5-$HT_{1A}$ receptor-mediated locomotor activity in the guinea pig. Eur J Pharmacol 1999;370:85–92.

Paulus MP, Geyer MA. A scaling approach to find order parameters quantifying the effects of dopaminergic agents on unconditioned motor activity in rats. Prog Neuro-Psychopharmacol Biol Psychiatry 1991a; 15:903–19.

Paulus MP, Geyer MA. A temporal and spatial scaling hypothesis for the behavioral effects of psychostimulants. Psychopharmacology 1991b; 104:6–16.

Paulus MP, Geyer MA. The effects of MDMA and other methylenedioxy-substituted phenylalkylamines on the structure of rat locomotor activity. Neuropsychopharmacology 1992;7:15–31.

Paulus MP, Geyer MA. Quantitative assessment of the microstructure of rat behavior: I. *f*(*d*), the extension of the scaling hypothesis. Psychopharmacology 1993;113:177–86.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates Orlando, FL: Academic Press, 1986.

Raid M, Garcia S, Watkins KC, Jodoin N, Doucet E, Langlois X, El Mestikawy S, Hamon M, Descarries L. Somatodendritic localization of 5-$HT_{1A}$ and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. J Comp Neurol 2000;417:181–94.

Rempel NL, Callaway CW, Geyer MA. Serotonin1B receptor activation mimics behavioral effects of presynaptic serotonin release. Neuropsychopharmacology 1993;8:201–11.

Robledo P, Feger J. Excitatory influence of rat subthalamic nucleus to substantia nigra pars reticulata and the pallidal complex: electrophysiological data. Brain Res 1990;518:47–54.

Rosales MG, Flores G, Hernández S, Martínez-Fong D, Aceves J. Activation of subthalamic neurons produces NMDA receptor-mediated dendritic dopamine release in substantia nigra pars reticulata: a microdialysis study in the rat. Brain Res 1994;645:335–7.

Sarhan H, Fillion G. Differential sensitivity of 5-$HT_{1B}$ auto and heteroreceptors. Naunyn-Schmiedeberg's Arch Pharmacol 1999;360:382–90.

Sharp T, Zetterstrèom T, Ljungberg T, Ungerstedt U. A direct comparison of amphetamine-induced behaviours and regional brain dopamine release in the rat using intracerebral dialysis. Brain Res 1987;401:322–30.

Smith Y, Bolam JP. The output neurones and the dopaminergic neurones of the substantia nigra receive a GABA-containing input from the globus pallidus in the rat. J Comp Neurol 1990;296:47–64.

Smith Y, Parent A. Neurons of the subthalamic nucleus in primates display glutamate but not GABA immunoreactivity. Brain Res 1988;453: 353–6.

Sprouse JS, Aghajanian GK. Responses of hippocampal pyramidal cells to putative serotonin 5-$HT_{1A}$ and 5-$HT_{1B}$ agonists: a comparative study with dorsal raphe neurons. Neuropharmacology 1988;27:707–15.

Tricklebank MD, Middlemiss DN, Neill J. Pharmacological analysis of the behavioural and thermoregulatory effects of the putative 5-$HT_1$ receptor agonist, RU 24969, in the rat. Neuropharmacology 1986;25: 877–86.

Van der Kooy D, Hattori T. Single subthalamic nucleus neurons project to both the globus pallidus and substantia nigra in rat. J Comp Neurol 1980;192:751–68.

Wing LL, Tapson GS, Geyer MA. 5-$HT_2$ mediation of acute behavioral effects of hallucinogens in rats. Psychopharmacology 1990;100:417–25.