*Pharmacology Biochemistry & Behavior*, Vol. 36, pp. 491–499. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

# The Role of D1 and D2 Receptors in Dopamine Agonist-Induced Modulation of Affective Defense Behavior in the Cat

SAFWAN SWEIDAN, HENRY EDINGER AND ALLAN SIEGEL[1]

*Departments of Physiology and Neurosciences, UMDNJ—New Jersey Medical School, Newark, NJ 07103*

Received 28 December 1989

SWEIDAN, S., H. EDINGER AND A. SIEGEL. *The role of D1 and D2 receptors in dopamine agonist-induced modulation of affective defense behavior in the cat.* PHARMACOL BIOCHEM BEHAV **36**(3) 491–499, 1990.—The role of D1 and D2 dopamine (DA) receptor subtypes in mediating DAergic modulation of affective defense behavior in the cat has been investigated in the present study. Feline affective defense, characterized mainly by autonomic arousal, ear retraction, hissing and paw striking, was elicited by electrical stimulation of the ventromedial hypothalamus. Following the establishment of a stable threshold current for eliciting the hissing response of the behavior, the effect of systemic (IP) administration of various DAergic agonists and antagonists on the hissing threshold was determined. The injection of the nonselective DA agonist apomorphine (1.0, 0.3 and 0.1 mg/kg) facilitated hissing in a dose-related manner. This effect was mimicked by the D2-selective agonist LY 171555 (0.1, 0.03 and 0.01 mg/kg) but not by the D1-selective agonist SKF 38393 (1.0, 5.0 and 10.0 mg/kg), and was blocked by the nonselective and the D2-selective antagonists haloperidol (0.1 and 0.5 mg/kg) and spiperone (0.2 mg/kg), respectively. The D1-selective antagonist SCH 23390 blocked apomorphine-induced facilitation only at a high dose (0.5 mg/kg). In addition, the injection of haloperidol (1.0 mg/kg), spiperone (0.2 mg/kg) or SCH 23390 (0.1 mg/kg) alone inhibited the behavior. It was therefore concluded that DAergic facilitation of affective defense behavior is mainly mediated by the D2 receptors, but that activation of the D1 receptors may play a "permissive" role. The interaction between the D1 and D2 receptors in mediating this facilitation and the behavioral specificity of the effect are discussed.

Cat Dopamine receptors Affective defense Electrical stimulation of brain Ventromedial hypothalamus

FELINE affective defense behavior is a fully integrated aggressive reaction expressed naturally whenever a cat is in a situation which is perceived to be threatening. It occurs intraspecifically, when a cat invades another cat's territory, or interspecifically, when a cat is confronted by a dog in the absence of an escape route (31). The behavioral pattern is characterized by autonomic arousal, crouching, ear retraction, growling, hissing, and an attack directed at the threatening object (1,31). Affective defense behavior can be reproducibly elicited by electrical stimulation of the ventromedial hypothalamic nucleus (VMH) and the adjacent area of the medial hypothalamus (19,22). The behavioral expression of the electrically elicited affective defense is identical to the naturally occurring reaction, and is similarly characterized by autonomic arousal, ear retraction, growling, hissing and paw striking directed at an appropriate object (10, 11, 20–22, 24).

The activation of dopaminergic neurotransmission in the central nervous system (CNS) has been thought to facilitate the expression of different forms of defensive aggressive reactions in many animal species including the cat (3, 6, 33). Systemic injections of the nonselective dopamine (DA) receptor agonist apomorphine (APO), the DA precursor L-DOPA, and the indirectly acting DA agonist methamphetamine have all been shown to facilitate the expression of the hypothalamically elicited affective defense in the cat via a dopamine receptor-mediated mechanism (34,46).

The subdivision of dopamine receptors into D1 and D2 subtypes has been biochemically established (28,29). The D1 receptors are characterized by their ability to stimulate the enzyme adenylate cyclase, whereas the D2 receptors are either inhibitory or unlinked to this enzyme (49). Pharmacologically, the exact role of each receptor subtype in mediating DA agonist-induced behaviors has not been examined until recently due to the lack of selective drugs for these receptors. However, the recent development of highly selective agonists and antagonists for D1 and D2 receptors has provided the tool to study the role of each subtype in dopaminergic neurotransmission (27). Both D1 and D2 receptors have been shown to mediate many of DA agonist-induced behaviors either separately or synergistically (9, 14, 30, 32, 38, 43). However, the role of these subtypes in mediating dopaminergic facilitation of defensive aggressive behavior has not been fully characterized. Therefore, the objective of the present study was to investigate the effects of systemic injections of selective D1 and D2 agonists and antagonists on the hypothalamically elicited affective defense behavior in the cat in order to characterize the involvement of each receptor subtype.

[1]Requests for reprints should be addressed to Dr. Allan Siegel, Department of Neurosciences, MSB H-506, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, 185 South Orange Avenue, Newark, NJ 07103.

## METHOD

### *Animals*

Fourteen adult cats of either sex (Barton Farms, Oxford, NJ), weighing 2.5–4.5 kg and not spontaneously aggressive in the presence of another cat or a rat, were used in these studies. They were housed individually with free access to food and water and maintained on a 12-hr light–12-hr dark cycle. All experiments were carried out during the light period.

### *Surgical Preparation*

Animals were anesthetized with sodium pentobarbital (45 mg/kg) and were then placed in a Kopf 1404 stereotaxic apparatus. Under aseptic procedures, eight stainless steel guide tubes (18 ga, 10 mm long) were mounted over holes drilled through the skull overlying the ventromedial hypothalamic area bilaterally. The coordinates for placement of the guide tubes were: 10.5–12.0 mm anteroposteriorly and 1.0 mm laterally (27). Three restraining bolts and two stainless steel indifferent electrodes were also mounted. The guide tubes, bolts, and indifferent electrodes were secured to the skull with small screws and acrylic self-polymerizing resin. The whole assembly was covered with a protective plastic cap.

### *Elicitation of Affective Defense Behavior*

After a postoperative period of 7–10 days, each animal was placed in a behavioral observation chamber. While the cat was awake and moving freely, a calibrated and insulated monopolar electrode (RNE-300, Rhodes Medical Instruments) was lowered vertically through a guide tube overlying the VMH in 0.5 mm steps. At each step, electrical stimulation (100–400 μA) was applied to elicit affective defense behavior. Once the behavior was consistently elicited from a site at a depth corresponding to the VMH, the electrode was cemented in place with the acrylic resin.

Monopolar electrical stimulation of the ventromedial hypothalamus consisted of trains of balanced, biphasic, rectangular, 2 msec duration, 62.5 Hz pulses. Stimuli were generated by a Grass S-88 stimulator and were led through a pair of Grass PSIU6 photoelectric constant current stimulus isolation units. Simulation current intensity was monitored by a Tektronix 502A dual-beam oscilloscope. The current intensity for eliciting the behavior ranged from 100–400 μA. The duration of each trial of electrical stimulation was 20 sec unless the behavior under examination was elicited, in which case the stimulation was terminated immediately after the response. Upon identification of an affective defense site, the electrode was cemented in place as mentioned above.

One day after the placement of the electrode, each behavioral site was stimulated electrically in the presence of food, an anesthetized rat, and another cat to fully characterize the behavioral response and to determine the current intensity threshold for eliciting the behavior.

### *Determination of Hissing Threshold*

The hissing component of electrically elicited affective defense was selected as an index for the behavior because it is elicited consistently in all animals and in each trial even in an impoverished environment (21,24). It is associated with threat-related motivational changes that result from the stimulation (2), and it is an all-or-none response whose occurrence depends primarily on stimulation current parameters. Therefore, drug-induced changes in the response can be readily determined by examining the changes in stimulation current parameters required to elicit hissing.

The threshold current intensity for eliciting the hissing component was used in these experiments as the dependent variable under examination. It was defined as the current intensity at which the hissing component was elicited in 50% of the trials. This threshold was determined according to the "up-and-down" method (8,54). During threshold determination, the current was periodically on for 20 seconds and off for two minutes. When a hissing response was not elicited within 20 sec for a given trial, the current intensity for the next trial was increased by a fixed amount of current (step). When a trial did yield a response, the current intensity was decreased by the same step. This procedure was repeated until 10 response changes (from hiss to no hiss or vice versa) were recorded. An estimate of the threshold current that elicited the response in 50% of the trials was calculated by taking the mean of intensities of all response changes. The size of the step was 10 μA when the hissing response was elicited with current intensities less than 200 μA and 20 μA when the response was elicited with current intensities between 200 and 400 μA.

### *Systemic Injections of Dopaminergic Drugs*

Cats were placed in a wooden behavioral observation chamber and were stimulated according to the up-and-down method to determine the preinjection (baseline) hissing threshold. They were then injected intraperitoneally (IP) with nonselective, D1-selective, and D2-selective dopaminergic agonists and antagonists. Postinjection hissing thresholds were determined during the first, second, third, fourth, and sixth hour after the injection. If hissing threshold did not return to the preinjection value by the sixth hour, a further determination was made 12 hours following injection.

Most animals received more than one treatment. A control group of five different animals also received injections of the vehicle alone. The type and order of these treatments were randomly selected. A minimum of 5 days separated consecutive injections in the same animal to prevent the development of tolerance or receptor up or down regulation. Although the use of animals more than once might be viewed as a violation of independence for statistical analysis, the completely randomized selection of animals make this violation an insignificant one (16).

### *Effect of Apomorphine on Circling Behavior*

In this experiment, the behavioral specificity of apomorphine treatment was assessed by comparing its effects on affective defense to its effects on circling behavior, an unrelated electrically elicited response.

Stimulating electrodes were implanted unilaterally in sites within the lateral hypothalamic area (10.5–12.0 mm, AP; 2.5 mm, L) in two cats. Electrical stimulation at these sites elicited contralateral circling behavior. The current intensity threshold for eliciting circling behavior was defined as the current intensity that elicited one full circle in 50% of the trials. It was determined according to a similar "up-and-down" method that was used to determine hissing threshold.

### *Histological Verification of Stimulation Sites*

Upon completion of the experiments, animals were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.9% saline followed by 10% formalin. Brains were removed, blocked, and sectioned. The sections (40 μm thick) were mounted and stained with cresyl violet to identify the loci of the tips of the electrodes. The sites were mapped on frontal sections taken from the stereotaxic atlas of the cat's hypothalamus (26).

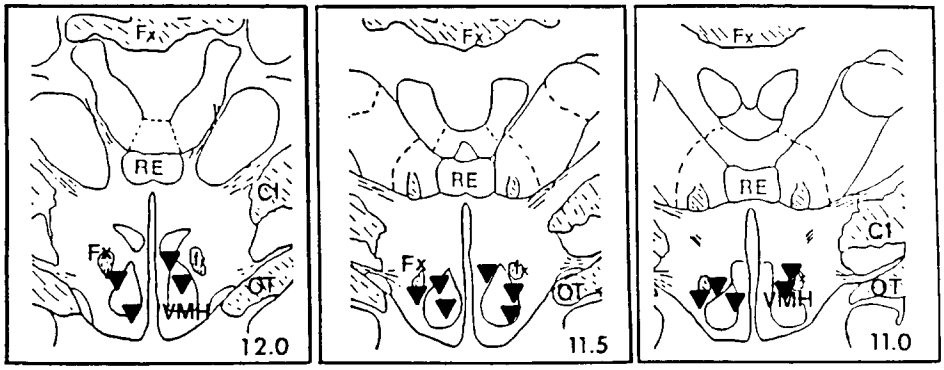


FIG. 1. Maps of stimulation sites within the ventromedial hypothalamic area, from which affective defense was elicited. Triangles indicate tips of stimulating electrodes. Abbreviations: CI, internal capsule; FX, fornix; OT, optic tract; RE, nucleus reuniens; VMH, ventromedial hypothalamic nucleus.

*Statistical Analysis*

Drug-induced changes in hissing thresholds were expressed as percentages relative to pretreatment baseline threshold. All percent changes were expressed as means ± SEM. The number of treated animals (N) was 5 in most cases. Drug-induced changes in hissing threshold at the 5 different postinjection periods were compared to vehicle-induced changes at the same periods by a two-way ANOVA, in which the two sources of variance were between treatments and between time periods (within treatment). At each postinjection time period, drug-induced changes were compared to vehicle-induced changes by the Fisher Significant Difference (FSD) test for preset pairwise multiple comparisons of means (16). Differences were considered statistically significant at $p<0.05$. The between-subject variance, when analyzed for all sets of experiments, was not statistically significant, therefore, it was not considered in the results.

*Drugs*

The following drugs were used in these experiments: apomorphine hydrochloride (Sigma Chemical Co., St. Louis, MO), quinpirole hydrochloride (LY 171555) (gift of Eli Lilly Laboratories, Indianapolis, IN), RS-SKF 38393 hydrochloride (Research Biochemical Inc., Natick, MA), haloperidol (Sigma Chemical Co., St. Louis, MO), spiperone (Research Biochemical Inc., Natick, MA), and R(+)-SCH 23390 maleate (gift of Schering Co., Bloomfield, NJ).

All drugs were dissolved in 1.5 ml of 0.1% ascorbic acid solution and the pH was adjusted appropriately immediately before injection. Doses were chosen to be within the range that does not elicit marked changes in locomotor behavior, and that are equipotent in their agonistic (apomorphine, LY 171555 and SKF 38393) or antagonistic activity at their respective receptors. For DA agonists the doses were 0.1, 0.3 and 1.0 mg/kg for APO; 0.01, 0.03 and 0.1 mg/kg for LY 171555; and 1.0, 5.0 and 10.0 mg/kg for SKF 38393. The DA antagonists were used at doses that have been shown to block apomorphine-induced activation of DAergic receptors; 0.1 mg/kg for SCH 23390; 0.2 mg/kg for spiperone; and 1.0 mg/kg for haloperidol (HAL) (15,55). Haloperidol, however, was injected at additional doses of 0.1 mg/kg and 0.5 mg/kg to establish that its blocking activity is dose-dependent, and therefore, is receptor-mediated.

## RESULTS

The locations of the electrode tips that elicited affective defense behavior upon electrical stimulation are shown in Fig. 1. All stimulation sites were located within the ventromedial hypothalamic nucleus, or in the area of the medial hypothalamus surrounding it. Hypothalamically elicited affective defense behavior was typically characterized by marked pupillary dilatation, piloerection, arching of the back, ear retraction, growling, hissing, and paw striking directed at a conspecific.

*Effects of DA Agonists Apomorphine, Quinpirole and SKF 38393*

The injection of 1 mg/kg (3.3 μmol/kg) of the nonselective DA agonist apomorphine induced a small increase in locomotor and stereotyped behavior, mild pupillary dilatation, retraction of the nicitating membrane, little increase in responsiveness to external stimuli, and a hyperdefensive state. The hyperdefensive effect was manifested as a significant decrease in the threshold current intensity for eliciting the hissing response as compared to the effect of vehicle, $F(1,40)=22.37$, $p<0.001$ (Fig. 2). The magnitude of the decrease was significantly different from vehicle effect ($p<0.05$, FSD) during the first (22.6 ± 6.6%) and second (18.6 ± 6.1%) hour following injection. Hissing threshold then returned towards baseline value, and was not significantly different from vehicle effect ($p>0.05$).

Lower doses of apomorphine, 0.3 and 0.1 mg/kg (1.0 and 0.3 μmol/kg), did not induce any apparent increase in locomotor or stereotyped behavior. Both doses, however, decreased the hissing threshold significantly as compared to vehicle, $F(1,40)=6.26$, $p<0.01$ and $F(1,40)=2.5$, $p<0.05$, respectively. The magnitude of threshold reduction was significantly different from vehicle effect only during the first hour after the injection of 0.3 mg/kg (11.8 ± 2.4%) and 0.1 mg/kg (10.4 ± 3.4%).

Quinpirole (LY 171555), a selective D2-DA receptor agonist, was injected at three different doses: 0.1, 0.03, and 0.01 mg/kg (0.4, 0.12, and 0.04 μmol/kg). At the highest dose (0.1 mg/kg), quinpirole induced a mild increase in locomotor behavior, pupillary dilatation, retraction of the nicitating membrane, increased responsiveness to external stimuli, and a hyperdefensive state. Most cats often assumed a threatening defensive posture in response to gentle patting on the back by the experimenter following injection at this dose. The injection of the intermediate dose (0.03 mg/kg) induced similar but much weaker effects, whereas the injection of the lowest dose (0.01 mg/kg) was not associated with any apparent change in the behavior.

Furthermore, the injection of 0.1 mg/kg and 0.03 mg/kg of LY

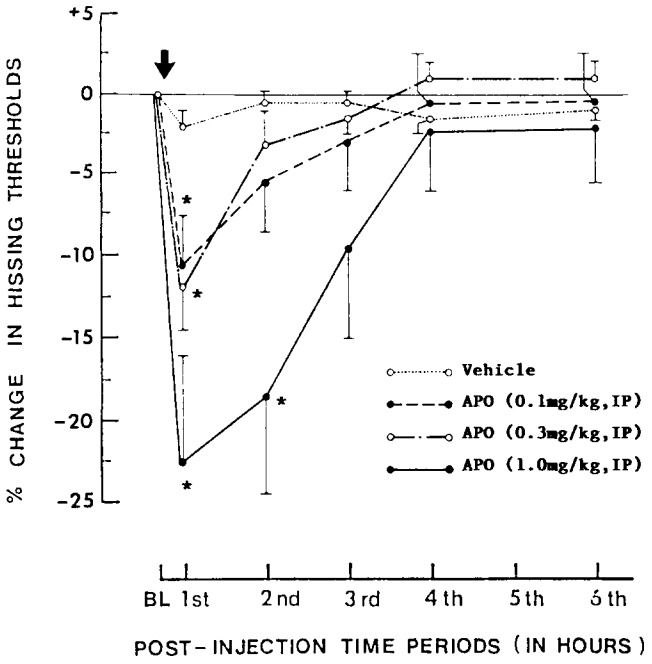


FIG. 2. Time course of the effect of IP injection of apomorphine (APO) or vehicle on affective defense. Each point represents average change in hissing threshold expressed as percentage relative to preinjection baseline (BL). Arrow indicates time of injection. Bars = SEM. Number of animals (N) = 5 for each treatment. *$p<0.05$.

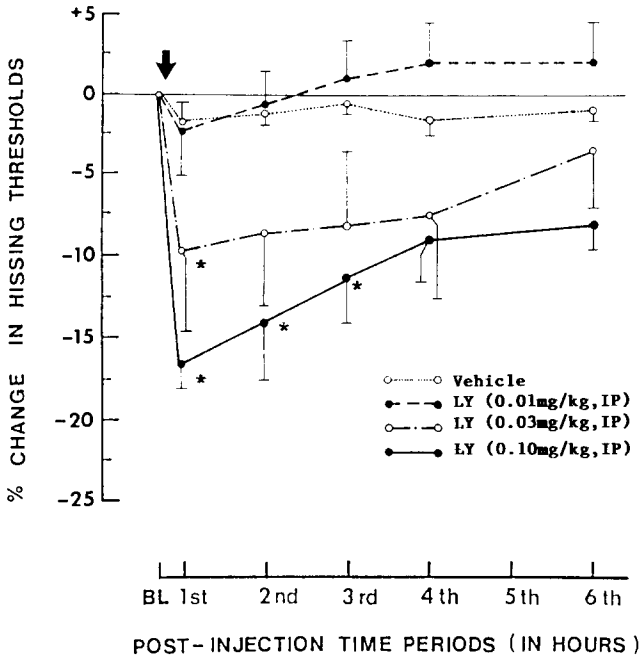


FIG. 3. Time course of the effect of IP injection of LY 171555 or vehicle on affective defense. Each point represents average change in hissing threshold relative to preinjection baseline (BL). N = 5 for each treatment. Bars = SEM. *$p<0.05$.

171555 decreased the threshold current intensity for eliciting the hissing response significantly as compared to vehicle effect, $F(1,40) = 114.0$, $p<0.001$ and $F(1,40) = 11.3$, $p<0.01$, respectively. The magnitude of decrease in hissing threshold was significantly different from vehicle effect ($p<0.05$, FSD) during the first (16.6 ± 1.4%), second (14.0 ± 3.4%) and third (11.2 ± 2.7%) hour following the injection of 0.1 mg/kg, and during the first hour (9.6 ± 5.2%) following the injection of 0.03 mg/kg of the drug. At the lowest dose tested, 0.01 mg/kg, however, LY 171555 injection did not induce any significant change in hissing threshold, $F(1,40) = 2.17$, $p>0.05$. These results are shown in Fig. 3.

The selective D1-DA agonist, SKF 38393, was injected at three doses. The injection of the lower dose, 1 mg/kg (3.4 μmol/kg), induced mild sedation, whereas the injection of 5 mg/kg (17 μmol/kg) induced emesis, mild pupillary dilatation, retraction of nicitating membrane and salivation in addition to sedation. The effect of injection of 1 mg/kg SKF 38393 on the hissing threshold was not statistically significant relative to vehicle control, $F(1,40) = 3.19$, $p>0.05$. However, when the effect of injection of 5 mg/kg was analyzed, there was a statistically significant treatment effect, $F(1,40) = 5.43$, $p<0.05$. The injection of 5 mg/kg significantly decreased hissing threshold ($p<0.05$, FSD) during the first (7.8 ± 3.0%), and second (7.0 ± 3.0%) hour following injection. The results are depicted in Fig. 4.

Because of the relative insolubility of higher doses of the drug in the 0.1% ascorbic acid vehicle, a dose of 10 mg/kg was dissolved in dimethyl sulfoxide and injected in two cats. In both animals, however, the effect of injection of this dose of SKF 38393 on the hissing threshold was not significantly different from the effect of vehicle injection at any time period following injection ($p>0.05$, FSD).

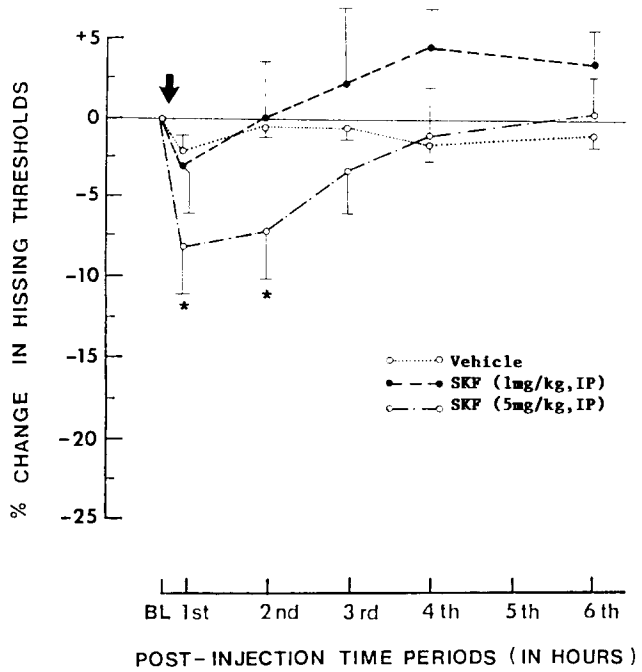


FIG. 4. Time course of the effect of IP injection of SKF 38393 or vehicle on affective defense. Each point represents average change in hissing threshold relative to preinjection baseline (BL). N = 5 for each treatment. Bars = SEM. *$p<0.05$.

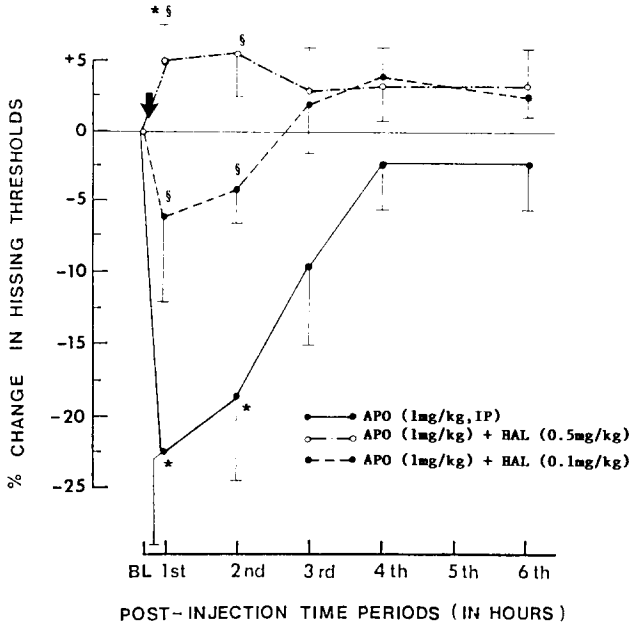


FIG. 5. Effect of IP pretreatment with haloperidol (HAL) 30 min prior to IP injection of apomorphine (APO) on affective defense. Each point represents average change in hissing threshold expressed as percentage relative to preinjection baseline (BL). Arrow indicates time of APO injection. N = 5 for each treatment. Bars = SEM. *$p<0.05$ compared to vehicle. §$p<0.05$ compared to APO injection alone.

*Effects of Pretreatment With the DA Antagonists Haloperidol, Spiperone and SCH 23390*

The nonselective DA antagonist haloperidol was injected 30 minutes prior to the injection of 1 mg/kg apomorphine at two doses. Pretreatment with the lower dose, 0.1 mg/kg (0.26 μmol/kg), markedly attenuated the facilitatory effect of apomorphine on the hissing response. Hissing thresholds following haloperidol-pretreated apomorphine injection were not significantly different from those measured following vehicle injections, $F(1,40) = 0.30$, $p>0.05$. However, the effect of haloperidol-pretreated apomorphine was significantly different from the effect of apomorphine injection alone, $F(1,40) = 18.22$, $p<0.001$. Pretreatment with a higher dose of haloperidol, 0.5 mg/kg (1.3 μmol/kg), blocked the facilitatory effect of apomorphine and induced a small increase in hissing threshold. This increase was, nevertheless, significantly different from both vehicle, $F(1,40) = 21.41$, $p<0.001$, and the effect of apomorphine injection alone, $F(1,40) = 41.47$, $p<0.001$. These results are depicted in Fig. 5.

Spiperone, a selective D2-DA receptor blocker, was injected at a dose of 0.2 mg/kg (0.5 μmol/kg) 30 minutes prior to the injection of 1 mg/kg of apomorphine. As shown in Fig. 6, spiperone pretreatment blocked the facilitatory effect of apomorphine on the hissing response. When compared to vehicle injection, spiperone-pretreated apomorphine injection did not induce any significant change in hissing threshold at any postinjection time period ($p>0.05$, FSD). However, the effect of spiperone pretreatment was significantly different from the results obtained with apomorphine treatment alone, $F(1,40) = 28.5$, $p<0.001$.

The effect of pretreatment with selective D1-DA receptor blocker SCH 23390 on the facilitatory effect of apomorphine was

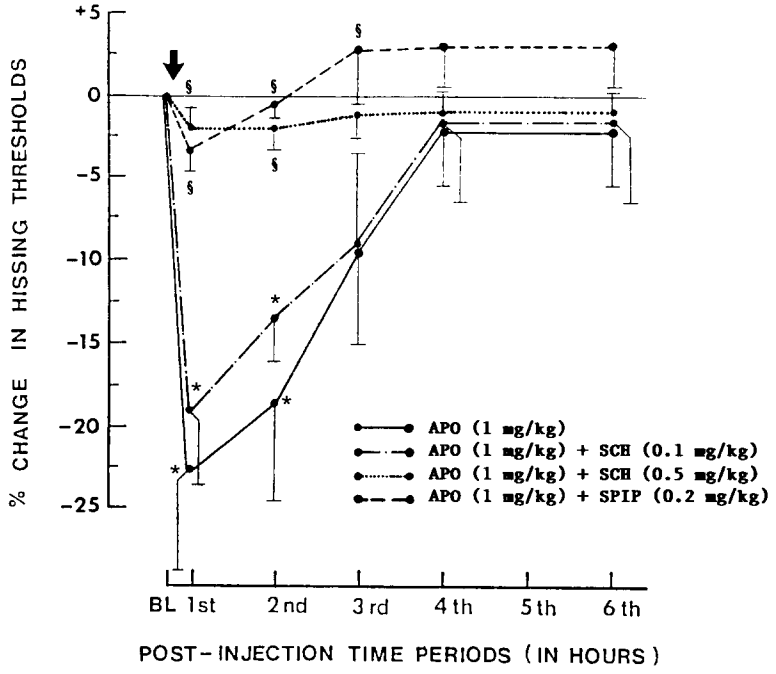


FIG. 6. Effect of IP pretreatment with SCH 23390 (SCH) or spiperone (SPIP) 30 min prior to the IP injection of APO on affective defense. Each point represents average change in hissing threshold expressed as percentage relative to preinjection baseline (BL). Arrow indicates time of APO injection. N = 5 for each treatment. Bars = SEM. *$p<0.05$ compared to vehicle. §$p<0.05$ compared to APO injection alone.

examined at two doses. The injection of 0.1 mg/kg (0.3 μmol/kg) SCH 23390 30 min prior to administration of 1 mg/kg apomorphine did not block apomorphine-induced facilitation of the hissing response. When compared to vehicle injection, apomorphine injection following SCH 23390 pretreatment resulted in a statistically significant decrease in hissing threshold, $F(1,40) = 18.68$, $p<0.001$. The magnitude of this facilitation was significantly different from vehicle treatment ($p<0.05$, FSD) during the first ($18.8 \pm 4.4\%$) and second ($13.4 \pm 2.4\%$) hour following injection. When the effect of SCH 23390 pretreatment was compared to the effect of apomorphine injection alone, there was no statistically significant difference between the two treatments, $F(1,40) = 0.43$, $p>0.05$. In contrast, pretreatment with a dose of 0.5 mg/kg (1.5 μmol/kg) of SCH 23390 resulted in a marked attenuation of apomorphine-induced facilitation of the hissing response. The changes in hissing threshold following this pretreatment were not significantly different from the effect of vehicle injection ($p>0.05$), but were significantly different from the effect of apomorphine injection alone ($p<0.05$). These results are depicted in Fig. 6.

*Effects of DA Antagonists Haloperidol, Spiperone and SCH 23390*

Haloperidol was injected at a dose of 1 mg/kg (2.6 μmol/kg). Qualitatively, this injection induced a decrease in locomotor behavior, sedation, mild state of catalepsy (characterized by the tendency of the cat to remain fixed in unusual postures for less than one min), decreased responsiveness to environmental stimuli, and a suppression of affective defense. Postinjection hissing thresholds were increased significantly relative to vehicle treatment, $F(1,40) = 30.18$, $p<0.001$. The magnitude of increase was statistically significant ($p<0.05$, FSD) during the second ($9.8 \pm 5.4\%$), third ($8.8 \pm 3.8\%$), and fourth ($7.6 \pm 2.7\%$) hour following injection. Figure 7 shows these results.

The injection of spiperone at a dose of 0.2 mg/kg (0.5 μmol/kg) induced little decrease in locomotor behavior, sporadic mewing responses, and a suppression of the hissing response. Postinjection hissing thresholds increased significantly relative to vehicle treatment, $F(1,40) = 39.57$, $p<0.001$. As shown in Fig. 7, the magnitude of the threshold increase was significantly different from vehicle ($p<0.05$, FSD) during the first ($7.8 \pm 2.2\%$), second ($8.8 \pm 1.9\%$) and fourth ($5.8 \pm 2.5\%$) hours following injection.

In contrast to haloperidol or spiperone injections, the injection of SCH 23390 at a dose of 0.1 mg/kg (0.3 μmol/kg) did not induce any noticeable change in locomotor behavior. However, postinjection hissing thresholds increased significantly as compared to vehicle effect, $F(1,40) = 18.49$, $p<0.001$. The magnitude of increase was significantly different from vehicle effect ($p<0.05$, FSD) during the first hour following injection ($11.2 \pm 4.4\%$). Figure 7 depicts these results.

*Effect of Apomorphine on Circling Behavior*

The purpose of this control experiment was to investigate the possibility that apomorphine-induced facilitation of hypothalamically elicited hissing can be attributed to a nonspecific facilitation of all locomotor responses. In two cats, 0.3 mg/kg (1.0 μmol/kg) of apomorphine, a dose that did not induce noticeable increase in locomotor behavior but facilitated hissing, was injected systemically. In both cats, no significant change was observed in the threshold current required to induce circling.

## DISCUSSION

In this study an attempt was made to substantiate the hypotheses that CNS dopaminergic neurotransmission regulates the expression of feline affective defense behavior elicited by electrical stimulation of the ventromedial hypothalamus, and to identify the DA receptor subtype involved in this modulation.

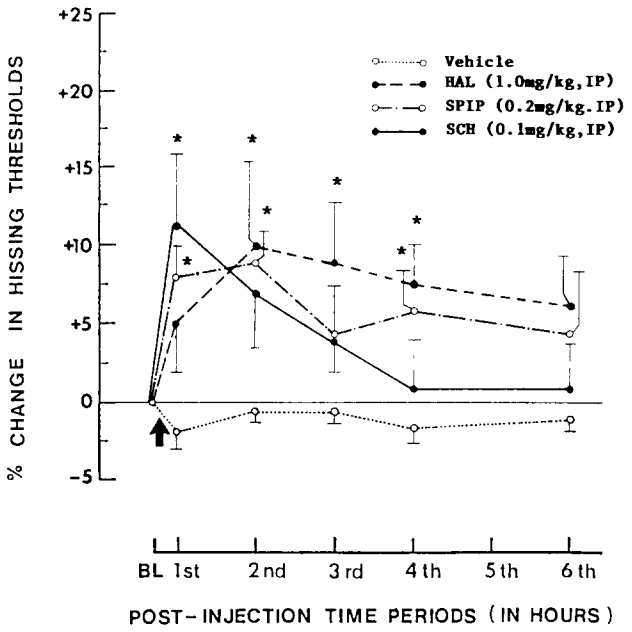


FIG. 7. Time course of the effect of IP injection of haloperidol, spiperone, SCH 23390 or vehicle on affective defense. Each point represents average change in hissing threshold expressed as percentage relative to preinjection baseline (BL). N = 5 for each treatment. Bars = SEM. *$p<0.05$.

The systemic administration of apomorphine, a nonselective, directly acting dopamine receptor agonist (3, 7, 12) has been shown to elicit a number of different responses that include hyperirritability and hyperdefensiveness (13, 34, 50, 53). In the present study, the hyperdefensive effect of apomorphine was closely examined. The results indicate that apomorphine injection decreases the threshold for eliciting the hissing component of affective defense in a dose-related manner (Fig. 2), an effect that was blocked by the DA receptor blocker haloperidol (5,52) (Fig. 5) suggesting the involvement of a DA receptor-mediated mechanism in this action. Since haloperidol injection by itself increased the hissing threshold (Fig. 7), it may be further concluded that endogenously released dopamine in the CNS is involved in regulating the expression of VMH-elicited hissing. These findings and conclusions are in agreement with the findings of Maeda *et al.* (34–36), who reported a decrease in the threshold of VMH-elicited hissing and attack responses following the systemic injection of apomorphine and methamphetamine.

Since any decrease in the current intensity for eliciting the hissing response has been interpreted as facilitation of affective defense behavior, the results of the studies presented here indicate that enhancement of dopaminergic transmission in the CNS of the cat is associated with a hyperdefensive effect. This supports the hypothesis that dopamine is involved as a facilitatory neurotransmitter in the expression of defensive aggression in animals. Similar conclusions were made by other investigators including Eichelman (17), Pradhan (41), Pucilowski (42) and Singhal and Telner (48).

Dopaminergic facilitation of VMH-elicited affective defense appears to involve both D1 and D2 receptor subtypes. The D2 receptors, however, appear to play a more significant role. This

conclusion is based on the findings that the injection of the D2 agonist LY 171555 (51) facilitated the expression of affective defense in a dose-related manner (Fig. 3), whereas the injection of the D2 antagonist spiperone (45) blocked apomorphine-induced facilitation (Fig. 6). The D1 agonist SKF 38393 (40,47), on the other hand, slightly facilitated VMH-elicited affective defense, and only at a dose of 5 mg/kg (Fig. 4). This nondose-dependent effect appears most likely to be nonspecific and associated with the autonomic arousal induced by SKF 38393. The D1 antagonist SCH 23390 (23,25) blocked apomorphine-induced facilitation only at a high dose (0.5 mg/kg).

It is, therefore, concluded that activation of D2 receptors is responsible, in large part, for the hyperdefensive effect induced by exogenously administered DA agonists. However, the involvement of D1 receptors in the apomorphine-induced effect cannot be excluded since the injection of 5 mg/kg of SKF 38393 slightly facilitated the hissing response, whereas the injection of 0.5 mg/kg of SCH 23390 blocked the facilitatory effect of 1 mg/kg of apomorphine. On the other hand, both D1 and D2 receptors appear to play a role in mediating the modulatory effect of endogenously released dopamine on affective defense. This is indicated by the findings that both spiperone and SCH 23390 inhibited affective defense when injected alone (Fig. 7).

The mechanism by which each DA receptor subtype mediates dopaminergic modulation of affective defense has not been fully examined in these studies. However, two possibilities exist. The first is that each receptor subtype mediates DA agonist-induced hyperdefensive effect via different but additive mechanisms. The D2 receptor might, for example, mediate dopaminergic facilitation of the behavior by its effect on the neural substrate mediating defensive and/or locomotor behavior (7,44), whereas the D1 receptor might mediate dopaminergic facilitation by its effect on the autonomic arousal state of the animal as was observed in this study. This possibility could be supported by the findings that injection of LY 171555 facilitated hissing only at doses that changed the locomotor behavior of the animal (0.03 and 0.1 mg/kg) but not at a dose of 0.01 mg/kg, which had no effect on locomotion, and that the D2 antagonist-induced inhibition of hissing was associated with a decrease in locomotor behavior in contrast to D1 antagonist-induced inhibition which was not. This suggestion, however, does not explain the findings that a dose of 0.5 mg/kg of SCH 23390 completely blocked apomorphine-induced facilitation of affective defense, whereas a dose of 0.1 mg/kg of SCH 23390, which inhibited the behavior when injected alone (Fig. 7), was ineffective in attenuating the effect of apomorphine. An alternative explanation for these findings can be proposed in light of the recently identified interaction between the D1 and the D2 receptors in mediating many DA agonist-induced effects including grooming, sniffing and locomotion (9, 30, 32). According to this hypothesis, D1 and D2 receptors act synergistically in mediating dopaminergic effects. D1 receptors appear to play a permissive role, in which their activation is essential for the expression of D2-mediated behavior. With regard to the hyperdefensive effect of dopaminergic stimulation, it may accordingly be speculated that this effect is principally mediated by D2 receptors, but that D1 activation is essential for the expression of the D2-mediated effect. Therefore, the finding that a high dose of SCH 23390 blocked apomorphine-induced facilitation of hissing could be a result of the absence of the D1 activation essential for the D2-mediated hyperdefensive effect of apomorphine. Similarly, SCH 23390-induced inhibition of the behavior at a dose of 0.1 mg/kg could result from an inhibition of a physiological D1 activation induced by endogenously released dopamine (physiological D1 tone). The injection of this dose of SCH 23390 might possibly have not blocked the apomorphine-induced effect because apomorphine competed with this low dose of SCH 23390 at D1 receptors and provided sufficient D1 receptor activation to sustain their permissive role.

The conclusion that the expression of affective defense in the cat is regulated by dopaminergic transmission through a D2 receptor-mediated mechanism that requires the coexistence of a "permissive" stimulation of D1 receptors is in agreement with the findings of Puglisi-Allegra and Cabib (44) that the D2 receptors play a major role in the expression of defensive behavior in the mouse. However, a dose of 0.1 mg/kg of SCH 23390, which inhibited affective defense in the cat (Fig. 7), had no effect on the defensive behavior in the mouse. This could be due to species differences or, more likely, due to the difference in the sensitivity of the methods used in quantifying the behavior. It is suggested that the determination of drug-induced behavioral changes by measuring hissing threshold currents in the cat is likely to be more sensitive than counting the number of bites or attacks encountered between two mice.

Concerning the behavioral specificity of DA agonist-induced facilitation of affective defense behavior, it has been suggested that the expression of a fully integrated behavior involves neural mechanisms that can be separated into four stages of neural processing: sensory, perceptive, motivational (response initiation) and motor (37). Drug-induced changes in the expression of a behavior can therefore be attributed to the mechanisms involved in one or more of these four stages. It is suggested that the effect of dopaminergic stimulation on the expression of affective defense may be considered behavior-specific only if motivational mechanisms are involved. Since dopaminergic stimulation has been shown to influence sensory (39), perceptive (50), and motor (7,18) mechanisms, dopamine agonist-induced facilitation of affective defense may be due to its action on any one of these mechanisms. This possibility was not investigated in detail in the present study. However, the following two findings suggest that the hyperdefensive effect of dopaminergic stimulation cannot be attributed only to motor activation. First, the systemic injections of 0.1 mg/kg and 0.3 mg/kg of apomorphine facilitated the expression of affective defense without any qualitatively noticeable increase in locomotor behavior. Secondly, apomorphine injection at a dose of 0.3 mg/kg did not change the threshold current for eliciting contralateral circling behavior. It is, therefore, concluded that dopaminergic facilitation of affective defense cannot be attributed to a general activation of locomotor behavior only, but is associated with motivational or perceptive changes. It has recently been suggested that the D2 receptor-mediated hyperdefensive effect of apomorphine in the mouse is a result of distorting the perception of environmental stimuli causing the animal to perceive danger or threat when it is absent (44). This suggestion is supported by the observation in the present study that most cats assumed a threatening defensive posture in response to gentle patting following the injection of 0.1 mg/kg of the D2 agonist LY 171555.

In conclusion, the results of this study support the hypothesis that dopaminergic transmission in the CNS facilitates the expression of affective defense behavior in the cat. This is possibly attributed to its action on the neural mechanisms involved in perception. The activation of both D1 and D2 receptors appears to be involved in dopaminergic facilitation of affective defense. While D2 receptors play the major role, D1 receptors appear to have a "permissive" effect. The specific nature of the interaction between D1 and D2 receptors in mediating the hyperdefensive effect of DA agonists has yet to be elucidated.

ACKNOWLEDGEMENTS

This study was supported by NIH grant NS07941-20 from the National Institutes of Neurological and Communicative Disorders and Stroke. This study was part of a Ph.D. dissertation submitted by S. Sweidan to UMDNJ-Graduate School of Biomedical Sciences.

## REFERENCES


1. Abrahams, V. C.; Hilton, S. M.; Zbrozyna, A. W. The role of active muscle vasodilatation in the alerting stage of the defense reaction. J. Physiol. (Lond.) 171:189–202; 1964.
2. Adams, D. B.; Flynn, J. P. Transfer of an escape response from tail shock to brain-stimulated attack behavior. J. Exp. Anal. Behav. 9:401–408; 1966.
3. Anand, M.; Gupta, G. P.; Bhargava, K. P. Modification of electroshock fighting by drugs known to interact with dopaminergic and noradrenergic neurons in normal and brain lesioned rats. J. Pharm. Pharmacol. 29:437–439; 1977.
4. Anden, N. E.; Butcher, S. G.; Corrodi, H.; Fuxe, K.; Ungerstedt, U. Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. Eur. J. Pharmacol. 11:303–314; 1970.
5. Anden, N. E.; Rubenson, A.; Fuxe, K.; Hokfelt, T. Evidence for dopamine receptor stimulation by apomorphine. J. Pharm. Pharmacol. 19:627–632; 1967.
6. Baggio, G.; Ferrari, F. Role of brain dopaminergic mechanisms in rodent aggressive behavior: influence of N-n-propyl-norapomorphine on three experimental models. Psychopharmacology (Berlin) 70: 63–68; 1980.
7. Barone, P.; Davis, T. A.; Braun, A. R.; Chase, T. N. Dopaminergic mechanisms and motor function: Characterization of D-1 and D-2 dopamine receptor interactions. Eur. J. Pharmacol. 123:109–114; 1986.
8. Barrett, J. A.; Shaikh, M. B.; Edinger, H.; Siegel, A. The effects of intrahypothalamic injections of norepinephrine upon affective defense behavior in the cat. Brain Res. 426:381–384; 1987.
9. Braun, A. R.; Barone, P.; Chase, T. N. Interaction of D1 and D2 dopamine receptors in the expression of dopamine agonist induced behaviors. In: Breese, G. R.; Creese, I., eds. Advances in experimental medicine and biology. vol. 204. Neurobiology of central D1-dopamine receptors. New York: Plenum Press; 1986:151–166.
10. Brown, J. L.; Hunsperger, R. W.; Rosvold, H. E. Defence, attack and flight elicited by electrical stimulation of the hypothalamus of the cat. Exp. Brain Res. 8:113–129; 1969.
11. Brutus, M.; Siegel, A. Effects of the opiate antagonist naloxone upon hypothalamically elicited affective defense behavior in the cat. Behav. Brain Res. 33:23–32; 1989.
12. Bunney, B. S.; Aghajanian, G. K.; Roth, R. H. Comparison of effects of L-DOPA, amphetamine and apomorphine on firing rate of rat dopaminergic neurons. Nature 245:123–125; 1973.
13. Carlsson, S. G. Effects of apomorphine on exploration. Physiol. Behav. 9:127–129; 1972.
14. Creese, I.; Sibley, D. R.; Hamblin, M. W.; Leff, S. E. The classification of dopamine receptors: Relationship to radioligand binding. Annu. Rev. Neurosci. 6:43–71; 1983.
15. Cuomo, V.; Cagiano, R.; Colonna, M.; Renna, G.; Racagni, G. Influence of SCH 23390, a DA1-receptor antagonist, on the behavioural responsiveness to small and large doses of apomorphine in rats. Neuropharmacology 25:1297–1300; 1986.
16. Dixon, W. J.; Massey, F. J. Introduction to statistical analysis. New York: McGraw-Hill; 1983:175–177.
17. Eichelman, B. Role of biogenic amines in aggressive behavior. In: Sandler, M., ed. Psychopharmacology of aggression. New York: Raven Press; 1979:61–93.
18. Filion, M. Effects of interruption of nigrostriatal pathway and of dopaminergic agents on the spontaneous activity of globus pallidus neurons in the awake monkey. Brain Res. 178:425–441; 1979.
19. Flynn, J. P. The neural basis of aggression in cats. In: Glass, D. C., ed. Neurophysiology and emotions. New York: Rockefeller University Press; 1967:40–60.
20. Fuchs, S. A. G.; Edinger, H. M.; Siegel, A. The organization of hypothalamic pathways mediating affective defense behavior in the cat. Brain Res. 330:77–92; 1985.
21. Fuchs, S. A. G.; Edinger, H. M.; Siegel, A. The role of the anterior hypothalamus in affective defense behavior elicited from the ventromedial hypothalamus of the cat. Brain Res. 330:93–107; 1985.
22. Hess, W. R.; Brugger, M. Das subkortikale zentrum der affectiven abwehrreaktion. Helv. Physiol. Pharmacol. Acta 1:33–52; 1943.
23. Hyttel, J. SCH 23390, the first selective dopamine D1 antagonist. Eur. J. Pharmacol. 91:153–154; 1983.
24. Inselman, B. R.; Flynn, J. P. Modulatory effects of preoptic stimulation of hypothalamically-elicited attack in cats. Brain Res. 42: 73–87; 1972.
25. Iorio, L. C.; Barnett, A.; Leitz, F. H.; Houser, V. P.; Korduba, C. A. SCH 23390, a potential benzodiazepine antipsychotic with unique interaction of dopaminergic system. J. Pharmacol. Exp. Ther. 226: 462–468; 1983.
26. Jasper, H. H.; Ajmone-Marsan, C. A. A stereotaxic atlas of the diencephalon of the cat. Ottawa: Natl. Res. Council Canada; 1954.
27. Kaiser, C.; Jain, T. Dopamine receptors: Functions, subtypes and emerging concepts. Med. Res. Rev. 5:145–229; 1985.
28. Kebabian, J. W.; Greengard, P. Dopamine-sensitive adenyl cyclase: possible role in synaptic transmission. Science 174:1346–1349; 1972.
29. Kebabian, J. W.; Calne, D. B. Multiple receptors for dopamine. Nature 277:93–96; 1979.
30. Koller, W. C.; Herbster, G. D1 and D2 dopamine receptor mechanisms in dopaminergic behaviors. Clin. Neuropharmacol. 11:221–231; 1988.
31. Leyhausen, P. Cat behavior: The predatory and social behavior of domestic and wild cats. New York: Garland STPM Press; 1979.
32. Longoni, R.; Spina, L.; Di Chiara, G. Premissive role of D1 receptor stimulation for the expression of D2 mediated behavioral responses: A quantitative phenomenological study in rats. Life Sci. 41:2135–2145; 1987.
33. Maeda, H. Effects of psychotropic drugs on the hypothalamic rage response in cats. Folia Psychiatr. Neurol. Jpn. 30:539–546; 1976.
34. Maeda, H.; Sato, T.; Maki, S. Effects of dopamine agonists on hypothalamic defensive attack in cats. Physiol. Behav. 35:89–92; 1985.
35. Maeda, H.; Maki, S. Dopaminergic facilitation of recovery from amygdaloid lesions which affect hypothalamic defensive attack in cats. Brain Res. 363:135–140; 1986.
36. Maeda, H.; Maki, S. Dopamine agonist produce functional recovery from septal lesions which affect hypothalamic defensive attack in cats. Brain Res. 407:381–385; 1987.
37. Mogenson, G. J.; Jones, D. L.; Yim, C. Y. From motivation to action: Functional interface between the limbic system and the motor system. Prog. Neurobiol. 14:69–97; 1980.
38. Molloy, A. G.; Waddington, J. L. Sniffing, rearing and locomotor responses to the D1 dopamine agonist R-SKF 38393 and to apomorphine: Differential interaction with the selective D1 and D2 antagonists SCH 23390 and metoclopramide. Eur. J. Pharmacol. 108: 305–308; 1985.
39. Nieoullon, A.; Cheramy, A.; Glowinski, J. Nigral and striatal dopamine release under sensory stimuli. Nature 269:340–342; 1977.
40. O'Boyle, K. M.; Waddington, J. L. Effects of the R- and S-enantiomers of SK&F 38393 on $^{3}$H-piflutixol and $^{3}$H-spiperone binding to D1 and D2 dopaminergic sites. Br. J. Pharmacol. 80:655; 1983.
41. Pradhan, S. N. Aggression and central neurotransmitters. Int. Rev. Neurobiol. 18:213–262; 1975.
42. Pucilowski, O. Monoaminergic control of affective aggression. Acta Neurobiol. Exp. 47:213–238; 1987.
43. Pugh, M. T.; O'Boyle, K. M.; Molloy, A. G.; Waddington, J. L. Effects of putative D1 antagonist SCH 23390 on stereotyped behavior induced by the D2 agonist RU 24213. Psychopharmacology (Berlin) 87:308–312; 1985.
44. Puglisi-Allegra, S.; Cabib, S. Pharmacological evidence for a role of D2 dopamine receptors in the defensive behavior of the mouse. Behav. Neural Biol. 50:98–111; 1988.
45. Quick, M.; Iversen, L. L. Regional study of $^{3}$H-spiperone binding and the dopamine-sensitive adenylate cyclase in rat brain. Eur. J. Pharmacol. 56:323–330; 1979.
46. Sato, M.; Wada, J. A. Hypothalamically induced defensive behavior and various neuroactive agents. Folia Psychiatr. Neurol. Jpn. 28: 101–106; 1974.
47. Setler, P. E.; Sarou, H. M.; Zirkle, C. L.; Saunders, H. L. The central effect of a novel dopamine agonist. Eur. J. Pharmacol. 50:419–430; 1978.
48. Singhal, R. L.; Telner, J. I. Psychopharmacological aspects of aggression in animals and man. Psychiatr. J. Univ. Ottawa 8:

145–153; 1983.
49. Stoof, J. C.; Kebabian, J. W. Opposing roles for D1 and D2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. Nature 294:366–368; 1981.
50. Trulson, M. E.; Crisp, T. Behavioral and neurochemical effects of apomorphine in the cat. Eur. J. Pharmacol. 80:295–309; 1982.
51. Tsuruta, K.; Frey, E. A.; Grewe, C. N.; Cote, T. E.; Eskay, R. L.; Kebabian, J. W. Evidence that LY 141865 specifically stimulates the D2 dopamine receptor. Nature 292:463–465; 1981.
52. Van Rossum, J. M. The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs. Arch. Int. Pharmacodyn. Ther. 160:492–494; 1966.
53. Votava, Z.; Dyntarova, H. The effect of apomorphine on the EEG and behavior in rats. Activ. Nerv. Super. 10:303–304; 1968.
54. Wetherill, G. B. Sequential estimation of points on quantal response curves. In: Wetherill, G. B., ed. Sequential methods in statistics. London: Chapman and Hall; 1975:171–180.
55. Zetterstrom, T.; Sharp, T.; Ungerstedt, U. Effect of dopamine D1 and D2 receptor-selective drugs on dopamine release and metabolism in rat striatum in vivo. Naunyn Schmiedebergs Arch. Pharmacol. 334: 117–124; 1986.