Pharmacology Biochemistry & Behavior, Vol. 36, pp. 613–617. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Scopolamine Produces Locomotor Stereotypy in an Open Field But Apomorphine Does Not

KATHYRNE MUELLER[1] AND JENNIFER L. PEEL

Department of Psychology and Chemistry of Behavior Program
Texas Christian University, Forth Worth, TX 76129

Received 31 July 1989

MUELLER, K. AND J. L. PEEL. *Scopolamine produces locomotor stereotypy in an open field but apomorphine does not.* PHARMACOL BIOCHEM BEHAV **36**(3) 613–617, 1990.—Both dopaminergic and nondopaminergic drugs produce hyperlocomotion in rats. Dopaminergic drugs also produce focused stereotypy (absence of locomotion and intense sniffing or licking/biting of a restricted area of the environment). Some drugs produce repetitive routes of locomotion; this phenomenon might represent a combination of hyperlocomotion and stereotypy. Scopolamine (an acetylcholine antagonist) and apomorphine (a dopamine agonist) both produce hyperlocomotion in rats; apomorphine also produces focused stereotypy but scopolamine does not. This research determines whether these drugs also produce locomotor stereotypy as measured by $\hat{\gamma}$. Scopolamine (0.5 and 2.0 mg/kg) produced locomotor stereotypy at both doses. Apomorphine (1.0, 2.0, and 3.0 mg/kg) failed to reliably produce locomotor stereotypy. Thus, there is not necessarily a relationship between the ability of a drug to produce focused stereotypy and the ability of the drug to produce locomotor stereotypy.

Locomotor stereotypy Locomotor behavior Scopolamine Apomorphine Open field

AMPHETAMINE, an indirect dopaminergic agonist, is well documented for its ability to produce focused stereotypy (absence of locomotion and intense sniffing and licking/biting of a restricted area of the environment) and to increase locomotion in rats. Amphetamine also produced patterned locomotion (locomotor stereotypy) in an open field. The relationship between locomotor stereotypy and dopamine, hyperlocomotion, and focused stereotypy is as yet unclear. Many other drugs in addition to amphetamine also produce hyperlocomotion. In the research described below, two of these drugs, apomorphine and scopolamine (an acetylcholine antagonist), are tested for their ability to produce locomotor stereotypy. Since apomorphine is a dopamine agonist, and like amphetamine, produces focused stereotypy, it is expected to produce dramatic locomotor stereotypy. Since scopolamine does not produce focused stereotypy and produces hyperlocomotion by a different mechanism than amphetamine it is expected to produce little or no focused stereotypy.

Amphetamine produces dramatic behavioral changes in rats. At lower doses, amphetamine produces hyperlocomotion and locomotor stereotypy (as measured by $\hat{\gamma}$); at higher doses amphetamine produces focused stereotypy. Several studies support the conclusion that amphetamine produces hyperlocomotion and focused stereotypy by enhancing the release of dopamine in the caudate and in the nucleus accumbens (6,15). Locomotor stereotypy has been less well studied and has only recently become a focus of attention. In previous research using $\hat{\gamma}$, 1.0, 2.0, 3.0 and 4.0 mg/kg amphetamine all produced locomotor stereotypy; however, maximal locomotor stereotypy occurred at 2.0 mg/kg (12). A logical hypothesis is that locomotor stereotypy is somehow related to dopamine, hyperlocomotion and focused stereotypy. Haloperidol, a dopaminergic antagonist, reduced locomotor stereotypy at doses that failed to affect locomotion per se (13).

Like amphetamine, apomorphine produces hyperlocomotion and focused stereotypy (1); these effects are also mediated by dopamine receptors in the caudate and nucleus accumbens (6). If dopamine receptor stimulation is intimately involved in locomotor stereotypy, one would expect apomorphine to also produce locomotor stereotypy in an open field.

Like amphetamine, scopolamine produces dose-dependent hyperlocomotion (5,14). Scopolamine-induced locomotion is clearly different from amphetamine-induced locomotion (14) and appears to be mediated by different mechanisms. For example, lesions of dopamine terminals block amphetamine-induced hyperlocomotion but not scopolamine-induced hyperlocomotion (5). Scopolamine has no known action on dopamine receptors and does not produce focused stereotypy.

Thus, amphetamine, apomorphine, and scopolamine all produce hyperlocomotion in spite of the differences between these drugs. The question is, will apomorphine and scopolamine produce locomotor stereotypy in an open field? If locomotor stereo-

[1]Requests for reprints should be addressed to Dr. Kathyrne Mueller, Department of Psychology, Box 32878, Texas Christian University, Fort Worth, TX 76129.

typy in an open field is produced by a variety of pharmacologically different drugs, then locomotor stereotypy may not be particularly useful as a behavioral tool or as a behavioral model of neuronal events. The working hypothesis is that locomotor stereotypy is related to the ability of a drug to produce focused stereotypy and is therefore related to dopamine.

METHOD

Subjects

Two experiments were conducted using male Wistar rats (Harlan Sprague-Dawley, Indianapolis, IN) housed individually on a 12-hr light/dark cycle with ad lib access to food and water. Each rat was handled for several days prior to testing. All testing was conducted 2 hr after lights-on. Each rat was tested only once; all drugs were administered subcutaneously.

Procedure

Experiment 1 (n = 10 per group) examined the effects of saline, 0.5 and 2.0 mg/kg scopolamine hydrobromide (Sigma) on lines crossed, $\hat{\gamma}$, and proportions of trip types (see below). Twenty-four hr prior to testing rats were placed in the open field (see Fig. 3) for habituation. On the day of testing, the rat was placed in the open field for 20 min, injected (the open field was washed while the rat was injected), and returned to the open field for 60 min for observation. A trained observer recorded the rat's route through the open field on a schematic of the open field.

Experiment 2 (n = 18) examined the effects of saline, 0.5, 1.0, 2.0, and 3.0 mg/kg apomorphine hydrochloride (Sigma) on lines crossed, $\hat{\gamma}$, and proportions of trip types. Otherwise the procedure was the same as Experiment 1.

Data Reduction

The procedure has been described in detail elsewhere (11,12). Briefly, the rat's route through the open field was divided into a series of trips. There are five types of trips. "C" designates a trip to the center area of the open field. Otherwise trip types are defined as the number of lines crossed during a trip. Thus, trip types range from "1" to "4" with a trip of "4" representing a complete tour of the perimeter of the open field. Gamma ($\hat{\gamma}$) is calculated from the sequence of trips as described previously (11). Gamma ($\hat{\gamma}$) is the maximum likelihood estimate of the probability that the rat will repeat the trip that it has just exhibited; thus, $\hat{\gamma}$ quantifies repetitive routes of locomotion or locomotor stereotypy. Gamma ($\hat{\gamma}$) values range from 0 to 1.0 with higher values indicating greater locomotor stereotypy.

The proportion of trip types is defined as the number of trips of a given type divided by the total number of trips. Thus, the proportion of trip types gives qualitative information about the rat's route through the open field.

RESULTS

As recommended by Kirk (7), arcsin transformations were performed on the $\hat{\gamma}$ and trip proportion data prior to statistical analysis. However, analyses of transformed and untransformed data yielded the same conclusions.

Because $\hat{\gamma}$ and the proportion of trip types convey no meaning if a rat fails to locomote, $\hat{\gamma}$ and trip proportion data were not calculated if a rat failed to exhibit four or more trips during a particular time interval. These "missing" data were replaced with the group mean; degrees of freedom were reduced accordingly in the statistical analyses. Most rats failed to locomote from 40 to 60 min after injection, therefore these data are not shown.

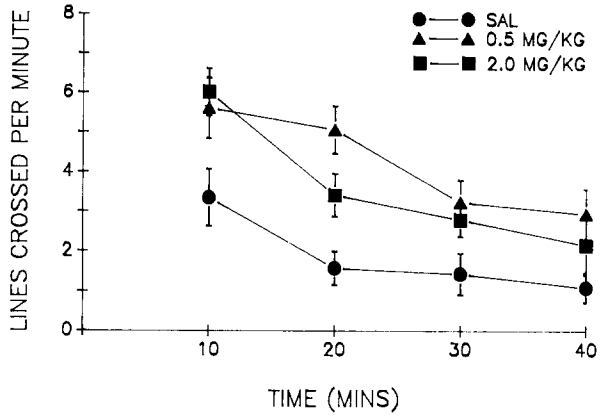

FIG. 1. Mean lines crossed exhibited by saline- and scopolamine-treated rats. (N = 10 per group.) Data are presented as lines crossed per min to facilitate comparison with the apomorphine data.

Scopolamine

As expected, scopolamine-treated rats were hyperactive with respect to controls (see Fig. 1); the main effect of dose is significant, $F(2,26) = 7.98$, $p<0.002$. In general, locomotion decreased over time; the main effect of time after injection is significant, $F(3,78) = 32.91$, $p<0.01$. The time × dose interaction is not significant.

In general, scopolamine-treated rats exhibited higher $\hat{\gamma}$ scores than saline controls, $F(2,23) = 7.82$, $p<0.01$; see Fig. 2. The main effect of time and the dose × time interaction are not significant. Raw data from a scopolamine-treated rat are shown in Fig. 3.

The effect of scopolamine on the proportion of trip types is shown in Fig. 4. In general scopolamine decreased trips to the center and increased trips of "4." This is likely the source of the dose × trip interaction, $F(6,73) = 5.98$, $p<0.01$. The main effect of trip type is also significant, but simply means that some trips are exhibited more frequently than others regardless of treatment. The

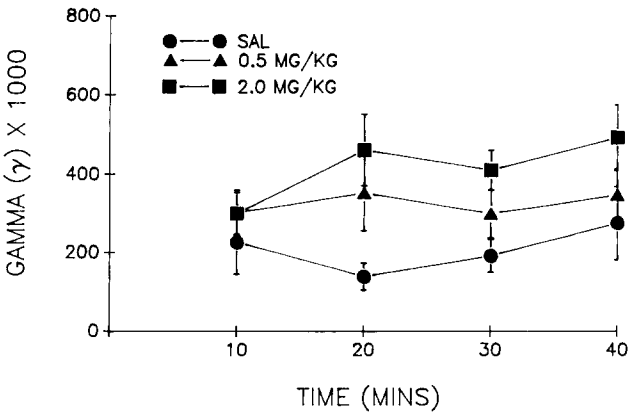

FIG. 2. Mean $\hat{\gamma}$ scores exhibited by saline- and scopolamine-treated rats. $\hat{\gamma}$ is an index of locomotor stereotypy; higher values indicate more stereotypic, or more repetitive patterns of locomotion in the open field.

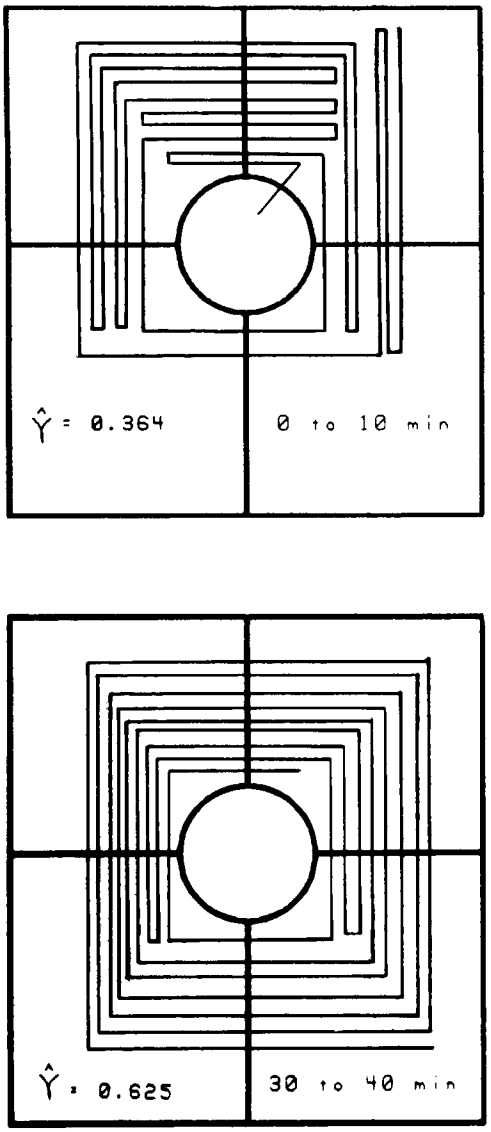

FIG. 3. Raw data from a rat injected with 2.0 mg/kg scopolamine. In general, rats locomote near the walls. The rat's path begins near the center and spirals outward simply to facilitate the data collection process. The rats do not simply circle the perimeter of the open field in one direction; changes in direction are common (this particular rat changes direction in the next trip).

main effect of time after injection and the three-way interaction are not statistically significant.

Apomorphine

The lowest dose of apomorphine (0.5 mg/kg) produced hypoactivity; more than half of the rats failed to exhibit 4 or more trips during the 20-, 30-, and 40-min intervals. Therefore, data from this dose were not included in the statistical analyses. Data from the remaining doses of apomorphine are presented in 2 time intervals—0 to 10 min and 10 to 30 min after injection. Sufficient locomotion is present during the first 10 min to allow calculation of $\hat{\gamma}$ and proportions of trip types. The second and third 10-min intervals are combined to obtain sufficient locomotions for a reasonable estimate of $\hat{\gamma}$ and proportions of trip types. (Many rats

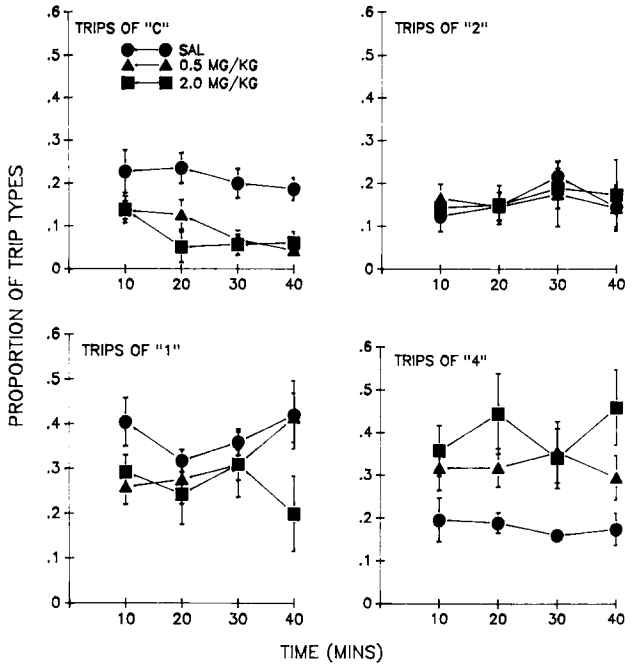

FIG. 4. The proportion of trip types exhibited by saline- and scopolamine-treated rats. "C" indicates a trip to the center; otherwise, trip types are defined as the number of lines crossed during a trip. Trips of "3" are the least frequent and are not shown. Since the proportions of trip types necessarily sum to 1.0, no information is lost by this procedure.

exhibited focused stereotypy and therefore exhibited little or no locomotion.) Data from the 30- to 40-min period are not presented because of the extreme lack of locomotion during that time period.

Lines crossed by apomorphine-treated rats are shown in Fig. 5. Data are presented as lines crossed per minute to facilitate comparison between the two time bins and to facilitate comparison with the scopolamine data. The main effect of dose is not significant ($p<0.07$). The dose × time interaction is significant, $F(3,68)=3.32$, $p<0.03$, and probably reflects the greater effect of

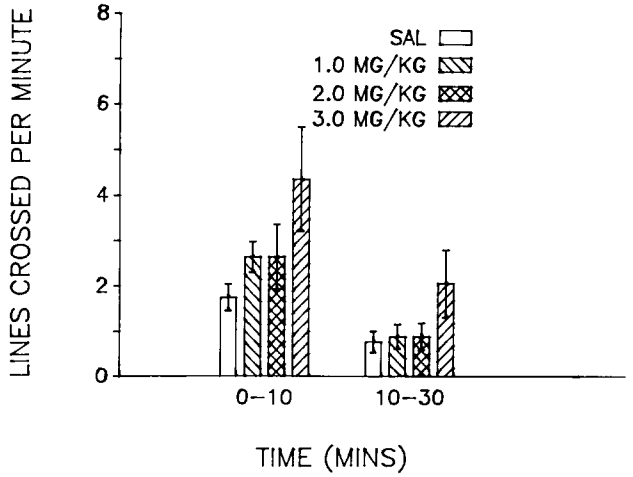

FIG. 5. Mean lines crossed by saline- and apomorphine-treated rats. (N = 18 per group.)

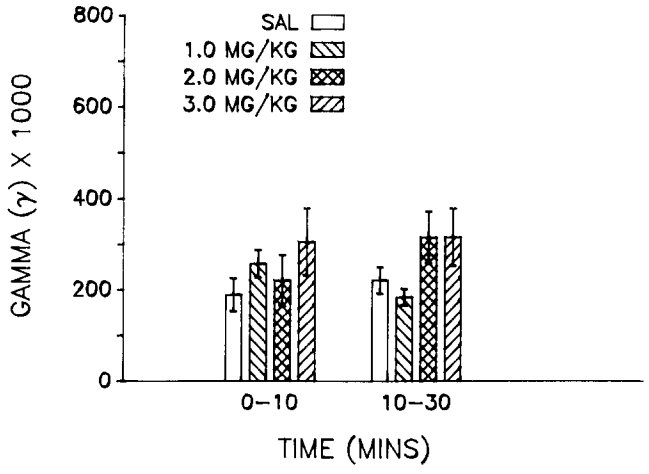

FIG. 6. Mean $\hat{\gamma}$ scores exhibited by saline- and apomorphine-treated rats.

apomorphine during the first 10-min interval. The main effect of time after injection is significant, but not relevant for the present.

The $\hat{\gamma}$ scores exhibited by apomorphine-treated rats are shown in Fig. 6. None of the main effects nor interactions approach statistical significance. (The apparent increase in $\hat{\gamma}$ at 3 mg/kg is due largely to two extreme scores.)

The proportion of trip types exhibited by apomorphine-treated rats is shown in Fig. 7. Again there is a main effect of trip type (i.e., rats tend to exhibit some trips more than others regardless of drug treatment). The interaction between trip type and dose is significant, $F(9,200) = 2.27$, $p<0.05$, as is the three-way interaction, $F(9,200) = 2.13$, $p<0.05$. Apomorphine usually increased trips to the center and at some doses decreased trips of 4.

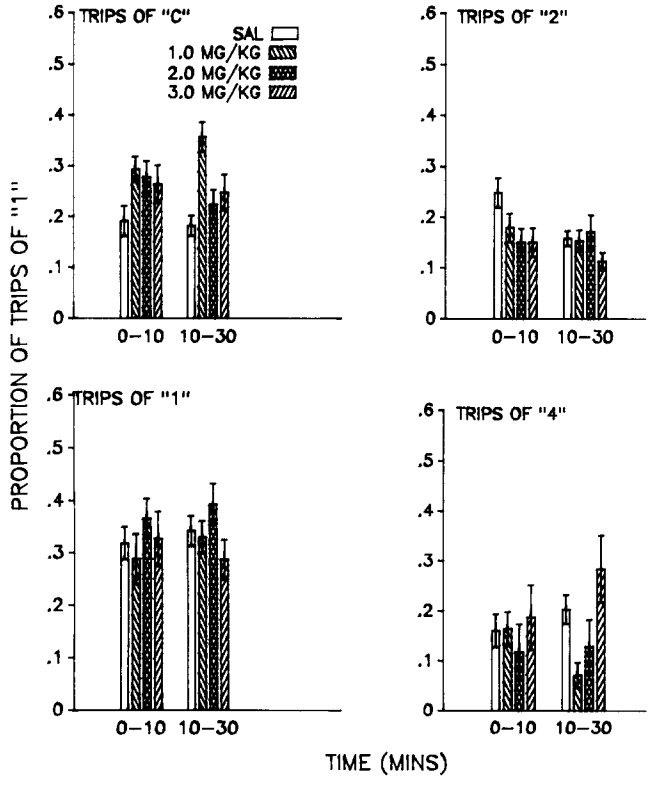

FIG. 7. Proportion of trip types exhibited by saline- and apomorphine-treated rats.

DISCUSSION

The purpose of the present research was to determine whether scopolamine and apomorphine produce locomotor stereotypy. The hypothesis was that apomorphine would produce dramatic locomotor stereotypy but that scopolamine would not. Contrary to expectations, scopolamine produced significant increases in locomotor stereotypy as measured by $\hat{\gamma}$. Apomorphine failed to produce locomotor stereotypy in the open field.

The failure of apomorphine to produce locomotor stereotypy is surprising for two reasons. First, the production of locomotor stereotypy by amphetamine suggests that dopamine is involved in locomotor stereotypy. Amphetamine-induced locomotor stereotypy is accompanied by increases in trips of "1" (trips from one end of the open field to another) and trips of "4" (trips around the perimeter of the open field) (11,12). In contrast, apomorphine increased trips to the center; a high proportion of trips to the center is usually accompanied by lower $\hat{\gamma}$ scores. Indeed, our subjective impression was that most apomorphine-treated rats wandered slowly and aimlessly throughout the open field. Thus, the effects of apomorphine on locomotion are very different from those of amphetamine.

Perhaps the doses of apomorphine used were inappropriate for producing locomotor stereotypy. However, the range of doses tested is consistent with the range of doses used in other laboratories [cf. (1)]. Apomorphine clearly stimulates postsynaptic dopamine receptors at these doses; if locomotor stereotypy is related to postsynaptic dopamine receptor stimulation it is not clear why locomotor stereotypy would not appear at these doses. Since we collected locomotor stereotypy data only from those rats that were locomoting, problems caused by rats exhibiting focused stereotypy were avoided. In short, the procedure maximized the likelihood of detecting apomorphine-induced locomotor stereotypy.

Second, the failure of apomorphine to produce locomotor stereotypy in an open field is surprising because apomorphine produced patterned locomotion in a smaller test chamber (4). However, the size of the test arena might be the crucial variable in this regard. A small test chamber might artificially restrict locomotion to produce the illusion of patterned locomotion.

Apomorphine did not cause as much hyperlocomotion as scopolamine in our hands. Others report a failure of apomorphine to produce any hyperlocomotion (9,10) or a disturbing lack of a dose-response relationship in hyperlocomotion (8,9). This is somewhat problematic since the ability of scopolamine to produce locomotor stereotypy might be related to the greater hyperlocomotion produced by scopolamine. The conclusion that apomorphine fails to produce locomotor stereotypy in an open field would be more strongly supported had apomorphine produced as much hyperlocomotion as scopolamine.

Even though apomorphine failed to produce locomotor stereotypy under these circumstances, scopolamine consistently produced locomotor stereotypy; this has implications for the neurochemical mechanisms underlying locomotor stereotypy in an open field. According to conventional wisdom, scopolamine indirectly increases dopamine release. However, recent direct tests of this hypothesis seriously question this conclusion (2,3). In addition, a case can also be made that caffeine modulates dopamine activity (16) but caffeine fails to produce locomotor stereotypy (11). Therefore, the hypothesis that dopamine is intimately involved in locomotor stereotypy should be viewed with caution for the moment.

ACKNOWLEDGEMENTS

Portions of this research were performed by J.L.P. in partial fulfillment of the requirements for the Master of Arts degree. This research was supported in part by TCURF5-23715 and by grant 1RO1DA05817.

REFERENCES

1. Costall, B.; Naylor, R. J.; Neumeyer, J. L. Differences in the nature of the stereotyped behaviour induced by apomorphine derivatives in the rat and in their actions in extrapyramidal and mesolimbic brain areas. Eur. J. Pharmacol. 31:1–16; 1975.
2. Damsma, G.; Westernik, B. H. C.; De Vries, J. B.; Horn, A. S. The effect of systemically applied cholinergic drugs on the striatal release of dopamine and its metabolites as determined by automated brain dialysis in conscious rats. Neurosci. Lett. 89:349–354; 1988.
3. de Belleroche, J. S.; Gardiner, I. M. Cholinergic action in the nucleus accumbens: Modulation of dopamine and acetylcholine release. Br. J. Pharmacol. 75:359–365; 1982.
4. Geyer, M. A.; Russo, P. V.; Segal, D. S.; Kuczenski, R. Effects of apomorphine and amphetamine on patterns of locomotor and investigatory behavior in rats. Pharmacol. Biochem. Behav. 28:393–399; 1987.
5. Joyce, E. M.; Koob, G. F. Amphetamine-, scopolamine- and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. Psychopharmacology (Berlin) 73:311–313; 1981.
6. Kelly, P. H.; Seviour, P. W.; Iversen, S. D. Amphetamine and apomorphine responses in the rat following 6-hydroxydopamine lesions of the nucleus accumbens septi and corpus striatum. Brain Res. 94:507–522; 1975.
7. Kirk, R. E. Experimental design: Procedures for behavioral sciences. Belmont: Brooks/Cole; 1982.
8. Maj, J.; Grabowska, M.; Gajda, L. Effect of apomorphine on motility in rats. Eur. J. Pharmacol. 17:208–214; 1972.
9. Mazurski, E. J.; Beninger, R. J. Stimulant effects of apomorphine (+)-amphetamine in rats with varied habituation to test environment. Pharmacol. Biochem. Behav. 29:249–256; 1988.
10. Meller, E.; Bordi, F.; Bohmaker, K. Enhancement by the D1 dopamine agonist SKF38393 of specific components of stereotypy elicited by the D2 agonists LY171555 and RU24213. Life Sci. 42:2561–2569; 1988.
11. Mueller, K.; Hollingsworth, E. M.; Cross, D. R. Another look at amphetamine-induced stereotyped locomotor activity in rats using a new statistic to measure locomotor stereotypy. Psychopharmacology (Berlin) 97:74–79; 1989.
12. Mueller, K.; Kunko, P. M.; Whiteside, D.; Haskett, C. Time-course of amphetamine-induced locomotor stereotypy in an open field. Psychopharmacology (Berlin) 99:501–507; 1989.
13. Mueller, K.; Peel, J. L.; Rewey, K. L. Effects of caerulein + haloperidol on amphetamine-induced locomotor stereotypy in rats. Life Sci. 44:717–724; 1989.
14. Sanberg, P.; Henault, M. A.; Hagenmeyer-Houser, S. H.; Russell, K. H. Topography of amphetamine and scopolamine induced hyperactivity: Toward an activity print. Behav. Neurosci. 101:131–133; 1987.
15. Sharp, T.; Zetterstrom, 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. 401:322–330; 1987.
16. Ungerstedt, U.; Herrera-Marschitz, M.; Brugue, M. C. Are apomorphine, bromocriptine, and the methylxanthines agonists at the same dopamine receptor? In: Gessa, G. L.; Corsini, G. U., eds. Apomorphine and other dopaminomimetics. vol. 1. Basic pharmacology. New York: Raven; 1981.