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Locomotor Activity and Stereotypy in Rats Following Repeated Apomorphine Treatments at 1-, 3-, or 7-Day Intervals

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MATTINGLY, B. A., J. E. GOTSICK AND C. MARIN. *Locomotor activity and stereotypy in rats following repeated apomorphine treatments at 1-, 3-, or 7-day intervals.* PHARMACOL BIOCHEM BEHAV 31(4) 871–875, 1988.—In two experiments, the effects of repeated intermittent administration of a relatively high dose of apomorphine (5 mg/kg) on locomotor activity and/or stereotypic behavior in rats was determined. In Experiment 1, male rats were given ten subcutaneous (SC) injections of apomorphine or vehicle and tested for locomotor activity and stereotypy. The first nine injection test sessions were given at 3-day intervals and the tenth injection test session was given 18 days following the ninth session. In Experiment 2, male rats were tested for locomotor activity following ten SC injections of apomorphine or vehicle with either a one- or seven-day interval between injections. Major findings were as follows: a) apomorphine produced progressively greater increases in locomotor activity with each succeeding injection (i.e., sensitization); b) sensitization to the locomotor activity stimulating effects of apomorphine developed with interinjection intervals of one, three, and seven days; c) the sensitization effect was maintained over the 18-day drug-free break; and d) the effect of apomorphine on stereotypic behavior did not significantly change with repeated injections. These findings indicate that even a single dose of apomorphine induces relatively long-lasting neurobiological changes. Moreover, these findings are consistent with the view that separate neural pathways mediate locomotor activity and stereotypy in rats.

Apomorphine Dopamine Locomotor activity Stereotypy Sensitization Rats

REPEATED treatments of rats with dopamine antagonists (e.g., haloperidol) produce a behavioral supersensitivity to dopamine agonists (e.g., apomorphine) (3). This increased sensitivity to dopamine agonists appears to be mediated, in part, by an increase in the number of dopamine receptors (3, 6, 12). Paradoxically, repeated treatments with dopamine agonists also result in a behavioral supersensitivity to dopamine agonists. This enhanced sensitivity to dopamine agonists with repeated exposure has been referred to as "reverse-tolerance," "up-regulation," and "sensitization," and has been demonstrated using several agonists (2, 5, 7, 16, 18, 21).

Research in our laboratory has also revealed a very strong sensitization effect in rats following repeated treatments with the dopamine agonist apomorphine. Indeed, the second administration of this drug in doses greater than 1.0 mg/kg often produces twice the effect on locomotor activity as does the first injection with a three-day interval between injections, and this progressive increase in activity continues to grow larger for up to 10–12 administrations of the drug (11). Unlike the chronic antagonist-induced behavioral supersensitivity, the neural mechanisms responsible for behavioral sensitization following repeated agonist treatments are unknown. Although an agonist-induced increase in receptor number or sensitivity would appear likely, available evidence indicates that striatal dopamine receptor sites either decrease in number or do not change following repeated agonist treatments (4, 12, 13, 18). Thus, receptor supersensitivity would not appear to account for the behavioral supersensitivity.

EXPERIMENT 1

Besides locomotor activity, acute injections of apomorphine also induce specific stereotypic behaviors in rats including repetitive sniffing, licking, and chewing responses (10). The main purpose of Experiment 1 was to determine whether apomorphine-induced stereotypy, like locomotor activity, would significantly change with repeated treatments with apomorphine. For this reason, two groups of rats were given nine injections of either apomorphine or vehicle with a 72-hr interval between injections. Following each injection, all rats were tested for stereotypy and locomotor activity. In addition, all rats were retested 18 days following the last regular test session to determine the relative permanence of the sensitization effect.

METHOD

Subjects

Eighteen male Wistar albino rats were experimentally naive and approximately 90 days old at the beginning of testing. All rats were housed individually and maintained on ad lib food and water. All behavioral testing was conducted during the light phase of the 12-hour light-dark cycle.

Apparatus

Activity measures were taken in two open-top BRS-Lehigh Valley cylindrical activity drums (Model 145-03). Each drum was 60 cm in diameter, 43 cm high, and was located in a separate experimental cubicle that was kept totally dark throughout testing. The interior of the drums was painted flat black and the floor was made of 4-cm diamond wire mesh. Each drum was equipped with two banks of three infrared photocells mounted on the outside of the drums. The photocells were approximately 12 cm apart and 2.5 cm above the drum floor. The photocell banks were connected to electromechanical counters in an adjacent control room by way of back-path eliminator diodes. Movement of the rat through a photocell beam sent a single pulse to the counters. Simultaneous pulses (i.e., pulses spaced less than 0.05 sec apart) such as might occur when two beams are broken near their intersection, were recorded as a single count by this method. Thus, activity was operationalized as the cumulative number of photobeam interruptions per unit time. Informal observations of apomorphine-treated rats in pilot studies indicate that nearly all photobeam interruptions are due to horizontal locomotor activity. Moreover, it is difficult to remove rats sensitized to apomorphine from the drums because of their extreme hyperactivity. It should be noted, however, that photobeam interruptions due to head bobbing and rearing cannot be distinguished from those resulting from horizontal activity using this apparatus.

Design and Procedure

At the beginning of testing, the rats were randomly assigned in equal numbers, to either the apomorphine or vehicle-control condition. Apomorphine hydrochloride (5 mg/kg) was dissolved in 0.001 N HCl and injected subcutaneously (SC) 15 minutes prior to activity testing. Control rats were injected SC with an equivalent volume (0.5 ml/kg) of the 0.001 N HCl vehicle.

On each test day, the rats were weighed, injected, and then returned to their homecage. Stereotypy ratings were made 10 minutes following the injection during a 15-sec observation of each rat in its homecage. The stereotypy rating scale (15) was as follows: 0, normal activity, no repetitive movements; 1, predominant repetitive sniffing; 2, predominant repetitive licking; 3, predominant repetitive chewing. Using this system an animal exhibiting approximately equal amounts licking and chewing would be given a score of 2.5.

Locomotor activity measures began 15 minutes after the injection. The rats were placed individually into the activity drums and activity counts were recorded at ten-minute intervals for a total of 30 minutes. All rats were tested in this manner for nine sessions with a 72-hour interval between sessions. An identical tenth test session was conducted 18 days following the ninth session. During this 18-day rest period the animals remained undisturbed in their homecages.

RESULTS

Locomotor Activity

Figure 1 (left panel) presents the mean activity counts for the groups during the 10-test sessions. These data were analyzed using two separate mixed-factor analyses of variance (ANOVA). The first ANOVA compared the groups' activity across the first nine activity sessions and the second ANOVA compared the groups' activity before and after the 18-day break (i.e., session 9 vs. session 10). As may be seen

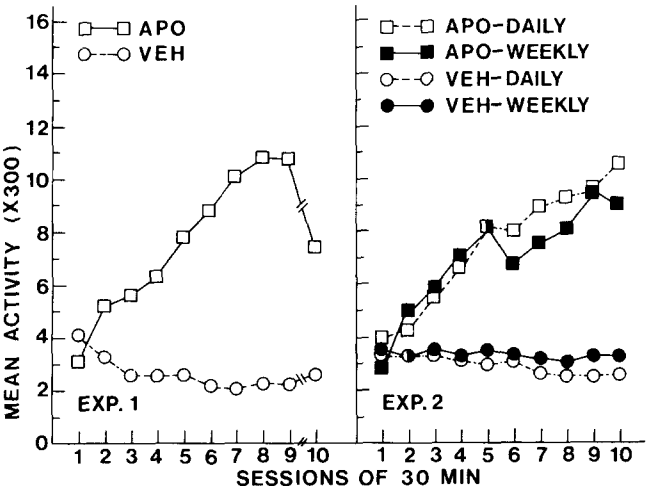

FIG. 1. (Left panel) Mean activity counts per 30-min session for the apomorphine (APO) and vehicle (VEH) groups. An 18-day drug-free rest interval was given between sessions 9 and 10. (Right panel) Mean activity counts per 30-min session for the APO and VEH groups tested at either daily or weekly intervals.

in Fig. 1 (left panel), the activity of the apomorphine and vehicle groups was comparable on the first session but significant group differences emerged with repeated testing. Indeed, the apomorphine group's activity progressively increased across the nine activity sessions, whereas the control group's activity slightly decreased over these sessions. As expected, the ANOVA performed on these data revealed a significant drug effect, $F(1,16)=14.25$, $p<0.01$; session effect, $F(8,128)=12.54$, $p<0.001$; and Drug × Session interaction, $F(8,128)=27.72$, $p<0.0001$.

Following the 18-day rest interval (session 10), the apomorphine-treated rats were still significantly more active than the vehicle-control rats, drug effect, $F(1,16)=24.52$, $p<0.001$. However, the effectiveness of apomorphine in stimulating locomotor activity did slightly decrease over the 18-day rest interval, session effect, $F(1,16)=15.78$, $p<0.01$; the Drug × Session interaction, $F(1,16)=22.54$, $p<0.001$.

In summary, apomorphine (5 mg/kg) produced a progressively greater effect on locomotor activity with each succeeding injection with a three-day interval between injections. More important, this behavioral sensitization to apomorphine was maintained for 18 days following the last injection.

Stereotypy Ratings

Since stereotypy rating data did not meet ANOVA requirements, nonparametric analyses were used. Specifically, Mann-Whitney U-tests were used for between-groups comparisons and Wilcoxon Sign-Ranks tests were used for repeated measure comparisons. The median stereotypy rating scores for the two groups across the ten test days are presented in Table 1. As may be seen in this table, there was little change in stereotypy scores for the apomorphine-treated rats across the 10 test days. Of the nine apomorphine-treated rats, stereotypy scores did not change from session 1 to session 9 for four rats, the scores of three rats decreased by only 0.5, and the scores of the remaining

TABLE 1

MEDIAN STEREOTYPY RATING SCORES

Drug	Session 1	2	3	4	5	6	7	8	9	10*
APO†	1.5	1.0	1.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0
VEH	0	0	0	0	0	0	0	0	0	0

*An 18-day drug-free rest interval was given between session 9 and 10.

†The stereotypy rating scores of the apomorphine and vehicle groups differed significantly on each test session.

two rats decreased by 1.5. Mann-Whitney U-tests indicated that the two groups' stereotypy scores differed significantly on each of the ten test sessions, all Us=0, all ps<0.01. To determine whether the groups' stereotypy scores significantly changed with repeated testing, separate Wilcoxon tests were performed comparing each group's stereotypy scores on the first and ninth test sessions, and also comparing the groups' scores on the ninth and tenth session. None of these comparisons revealed a significant change in stereotypy scores (all ts>10, all ps>0.1). Thus, although apomorphine induced a significant degree of stereotypic behavior, unlike apomorphine-induced changes in locomotor activity, the level of stereotypy did not significantly increase or decrease with repeated apomorphine treatments. Moreover, changes in locomotor activity among the apomorphine-treated rats from session 1 to session 9 were not correlated with changes in stereotypy scores, Spearman Rho, r=.01, p>0.05.

EXPERIMENT 2

In Experiment 1 and in our previous studies (11), we have observed significant sensitization to apomorphine using interinjection intervals of 24 and 72 hours. Similarly, Castro *et al.* (5), using locomotor activity as a behavioral measure, reported sensitization to apomorphine in rats using a 48-hour interinjection interval. In contrast, sensitization was not found by these researchers when a 2-hour interinjection interval was used (5). These authors concluded that a drug-free period between successive injections was necessary for the induction of sensitization (5). Although their data indicate that some minimal time interval between injections is necessary for the induction of sensitization, whether sensitization to apomorphine will develop when apomorphine is administered at relatively long intervals is unknown. The results of Experiment 1 indicate that once the sensitization effect is established, it is relatively long-lasting. The purpose of Experiment 2, therefore, was to determine whether sensitization to apomorphine would develop with a 7-day interinjection interval. As in Experiment 1, two groups of rats were tested for locomotor activity following an injection of either apomorphine or vehicle. One-half of the rats in each drug condition were injected and tested daily and the remainder were tested at weekly intervals. Since stereotypy ratings did not significantly change with repeated testing in Experiment 1, only activity counts were recorded in Experiment 2.

METHOD

Subjects, Apparatus, and Procedure

Thirty male Wistar albino rats were experimentally naive and approximately 90 days old at the beginning of testing. Fifteen rats were randomly assigned to the apomorphine treatment condition and the remainder served as vehicle control rats. Eight of the 15 rats in each drug condition were injected (SC, 0 or 5.0 mg/kg apomorphine) and tested for locomotor activity daily for ten days and the remaining seven rats in each drug condition were injected and tested at weekly intervals for 10 weeks. In an attempt to minimize the confounding of test interval with age, one-half of the rats in the daily group were tested before the weekly group of rats and the remainder were tested following the completion of weekly group testing. Thus, in the daily test groups, eight rats (4 APO; 4 VEH) were between 160 and 170 days old at the beginning of testing. The apparatus and procedure was the same as in Experiment 1 except stereotypy ratings were not taken.

RESULTS

In order to determine whether the ten-week age difference affected the development of apomorphine sensitization, an ANOVA comparing the activity of the daily groups tested before and after the weekly groups was performed. Although the younger rats tested daily with apomorphine were more active overall than the older rats tested daily with apomorphine, this difference was not significant, F(1,6)=2.26, and comparable sensitization was observed in both groups. Consequently, the younger and older daily groups were combined for subsequent analyses.

Figure 1 (right panel) presents the mean activity counts of the four groups during the 10 activity sessions. As may be seen in this figure, the activity of the rats injected with apomorphine increased progressively across the 10 test sessions, whereas the activity of the vehicle control groups did not significantly change across sessions, drug effect, F(1,27)=49.82, p<0.0001; session effect, F(9,233)=28.46, p<0.001; Drug × Session interaction, F(9,233)=41.61, p<0.0001. More important, repeated injections of apomorphine produced progressively greater activity increases in both the daily- and weekly-injected groups, interval effect, F(1,27)=0.04, and Drug × Interval interaction, F(1,27)=0.60.

GENERAL DISCUSSSION

Consistent with previous studies [e.g., (5,11)], the present results indicate that repeated injections of apomorphine (5 mg/kg) produce behavioral sensitization. This sensitization effect, however, is clearly behavior-dependent. That is, the effect of apomorphine on locomotor activity significantly increases with repeated treatments, but the effect of apomorphine on stereotypic behaviors does not significantly change with repeated treatments. Of course, it may be argued that we did not see any significant changes in stereotypy because of the relative insensitivity of our stereotypy measure. For example, the rating scale we used primarily detects qualitative changes in behavior (e.g., sniffing vs. licking) rather than quantitative changes in the intensity or duration of stereotypic behaviors. Consistent with our results, however, other researchers using more extensive rating scales sensitive to both qualitative and quantitative changes in stereotypy have not found any significant changes in stereotypy following repeated treatments of apomorphine in doses ranging from 1.0 to

6.0 mg/kg (5,10). Thus, the absence of significant changes in stereotypy in the present study is probably not due to the insensitivity of the rating scale we used.

In contrast to the lack of change in the stereotypy in rats following repeated high doses of apomorphine, repeated low doses (<0.10 mg/kg) have been reported to produce significant sensitization of stereotypy (10). This low dose sensitization effect has been attributed to the preferential effect of low doses of apomorphine on striatal dopamine autoreceptors (10). Stimulation of dopamine autoreceptors has been reported to produce a decrease in dopamine synthesis and release. Consequently, Kinion, Merson and Kane (10) have suggested that the effect of repeated low doses of apomorphine may be functionally equivalent to that of repeated treatments with a dopamine antagonist. If so, then low doses of apomorphine may induce behavioral sensitization as a result of a "disuse supersensitivity" of dopamine postsynaptic receptors. Repeated treatments of apomorphine in doses high enough to stimulate dopamine postsynaptic receptors would not be expected to produce either behavioral or receptor supersensitivity. Although this explanation may account for the ineffectiveness of repeated high doses of apomorphine in producing sensitization of stereotypy, it cannot explain the development of sensitization observed using locomotor activity as a behavioral measure.

The differential effect of repeated high doses of apomorphine on locomotor activity and stereotypy in the present study supports the view that separate neural mechanisms mediate locomotor activity and stereotypic behaviors in rats (8, 9, 19, 20). Specifically, it has been suggested that agonist-induced stereotypic behaviors result from striatal dopaminergic receptor stimulation and that agonist-induced changes in locomotor activity are mediated by the mesolimbic dopamine pathway [see (1,8) for reviews]. If this view is accurate, then the present results suggest that striatal and mesolimbic dopaminergic pathways are differentially affected by repeated treatments with high doses of apomorphine. As mentioned previously, available evidence indicates that striatal dopamine receptor binding is either decreased or unaffected by repeated apomorphine treatments (13,17). This finding is consistent with the absence of sensitization to apomorphine found in the present study using stereotypy as the behavior measure. Unfortunately, the effect of repeated apomorphine treatments on dopamine receptor binding in mesolimbic regions has apparently not been determined.

The results of Experiment 1 also indicate that, once developed, sensitization to apomorphine is maintained for at least 18 days following the last apomorphine injection. Similarly, the results of Experiment 2 indicate that sensitization to apomorphine develops with intervals as long as seven days between injections. Together these findings indicate that the neurobiological effect of a single dose of apomorphine is maintained for at least one week and that the cumulative effect of repeated intermittent doses of apomorphine may be relatively permanent. Recent studies of behavioral sensitization using the indirect dopamine agonists, cocaine and amphetamine, have also observed relatively long lasting neural and behavioral effects [e.g., (14)]. In contrast to apomorphine, however, sensitization to the stereotypic effects of these drugs is observed (18).

In summary, the present results indicate that the sensitization to high doses of apomorphine develops with respect to locomotor activity, but not stereotypy. Moreover, the results indicate that sensitization to apomorphine is relatively long lasting and develops with intervals of up to seven days between treatments. Previous work in this laboratory (11) indicated that the sensitization effect is not simply the result of conditioned hyperactivity. Consequently, the present findings suggest that apomorphine treatments produce enduring neurobiological changes. At present the specific nature of these changes is unknown.

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