Pharmacology Biochemistry and Behavior, Vol. 45, pp. 655-663, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Apomorphine Sensitization Effects: Evidence for Environmentally Contingent Behavioral Reorganization Processes

ERNEST N. DAMIANOPOULOS AND ROBERT J. CAREY[1]

Research and Development Service, VA Medical Center, and Department of Psychiatry, SUNY Health Sciences Center at Syracuse, Syracuse, NY 13210

Received 12 October 1992

DAMIANOPOULOS, E. N. AND R. J. CAREY. *Apomorphine sensitization effects: Evidence for environmentally contingent behavioral reorganization processes.* PHARMACOL BIOCHEM BEHAV **45**(3) 655-663, 1993.—Apomorphine-induced behavioral sensitization was investigated with a Pavlovian conditioning paradigm. Rats were administered apomorphine (2.0 mg/kg SC) daily for 7 days either paired or unpaired with a 10-min test environment placement. Initially, apomorphine induced hypolocomotion, but by treatment day 5, hyperlocomotion developed. Utilizing a videoimage analysis program which quantitated angular movement, it was determined that the increase in locomotion induced by repeated apomorphine treatment was due to an increase in rotational locomotion. Critically, rotation per se did not increase, but rather wide angle rotation toward the periphery of the test environment increased. Furthermore, a directional bias of rotation developed and stabilized which was unrelated to the animal's initial asymmetry bias. This emergence of a new locomotion pattern in conjunction with hyperlocomotion pointed to the need to reconceptualize behavioral sensitization phenomena into a new framework consistent with a progressive change in behavioral structure. Behavioral reorganization is presented as an alternative formulation to that of behavioral sensitization, as a drug-environment interactive process which is more compatible with the behavioral dynamics that emerge with repeated intermittent dopaminergic psychostimulant drug treatment.

Apomorphine Hyperlocomotion Behavioral reorganization Dopamine receptors Sensitization Drug state learning

REPEATED apomorphine stimulation in the intact rat can result in hypolocomotion or hyperlocomotion relative to nondrug levels (1,6). Low doses of apomorphine ($\leq$0.1 mg/kg SC), which primarily stimulate dopaminergic autoreceptors, induce hypolocomotion compared to nondrug locomotion levels, whereas repeated high doses of apomorphine ($\geq$0.5 mg/kg), which stimulate both pre- and postsynaptic dopamine receptors, induce hyperlocomotion (14,15,18). Interestingly, both low and high doses of apomorphine induce hypolocomotion initially but hyperlocomotion develops only with high doses and only after repeated treatments (14,15). The hypolocomotion effects induced by low and high doses of apomorphine, however, are not behaviorally equivalent. Low-dose apomorphine hypolocomotion is characterized by indices of sedation (e.g., yawning), while high-dose apomorphine hypolocomotion is characterized by intense sniffing, licking, and gnawing confined to a restricted spatial area (16). Thus the initial high-dose apomorphine hypolocomotion is actually hypermotoric behavior expressed in response forms incompatible with locomotion.

The emergence of hyperlocomotion with repeated high-dose apomorphine treatment from the initial hypolocomotion response has been characterized as a behavioral sensitization phenomenon (14,15). Alternatively, hyperlocomotion induced by repeated apomorphine administration may represent a change in the behavioral expression of the drug effect (7,8,26). That is, the drug-induced motoric stimulation which initially evokes repetitive movement in a confined space of a given test environment can, with repeated treatment, engage locomotor response mechanisms. Indeed, there is some evidence that repeated psychomotor drug administration produces qualitative structural changes in the locomotor pattern (7,8). In an attempt to address this issue, the present study assessed the effects of repeated apomorphine treatment in rats using a videoimage analysis system which permitted quantitation not only of locomotion but also of angular direction of movement. This videoimage system capability allowed detection of emerging new patterns of locomotion. Additionally, the repeated drug administration effects were evaluated in the context of the paired-unpaired drug treatment contrast of a

[1] Requests for reprints should be addressed to Robert J. Carey, Research and Development Service VA Medical Center, 800 Irving Avenue, Syracuse, NY 13210.

Pavlovian conditioning protocol. The use of this drug administration protocol allowed the differentiation between environmentally contingent drug administration effects from the numerous nonspecific biochemical effects associated with repeated drug treatment such as altered receptors, drug metabolism, and drug-induced stress effects.

METHOD

Animals

Adult 400–500 g Sprague-Dawley male rats were used in the present study. Upon arrival, the animals were weighed and housed in individual cages. The cages were in a climate (22 ± 1°C) and light-controlled room with 12L : 12D cycles. The animals were housed individually in 25 × 17 × 17 cm wire mesh cages with six cages in a row for a total of 30 cages. The animals were handled and weighed daily for 7 days subsequent to their arrival. All treatment protocols were approved by the Committee for the Humane Use of Animals of SUNY Health Science Center at Syracuse.

Drugs

Apomorphine HCl (Sigma) was dissolved in a vehicle solution containing 0.4 mg of ascorbic acid per ml of distilled water. Apomorphine was injected subcutaneously (SC) either in a 2.0 mg/kg or 0.5 mg/kg dosage. All injections were of equal volume and testing occurred during the light cycle.

Apparatus and Behavioral Measurement

Locomotion and rotation behavior were recorded automatically in a black 60 cm square enclosure with 40 cm side panels. This test chamber was in a light-controlled room. It was illuminated by four overhead dim red lights placed over the four corners of the test chamber. Ambient white noise (80 dB) was provided by a cassette tape player and was turned on immediately prior to placement of the animal in the test chamber and turned off upon removal from the test chamber. Spontaneous and drug-induced locomotion and rotation behavior were recorded with a Video Image Analyzing System (VIAS) (2). Online analog camera images of the freely moving animal were digitized and disc stored for later analysis. Computer evaluation of the stored data yielded summaries of locomotion in meters traversed during the test period in 5-min bins as well as frequency counts and direction of partial 1/4, 1/2, 3/4, and complete 1/1 rotations (based on system category determinations of 90°, 180°, 270° and 360° rotation) at each of four categories of diameter size: <20 cm, 20–<30 cm, 30–<55 cm, and ≥ 55 cm. In addition to the frequency counts within each diameter size category, the actual mean rotation diameters within each category were calculated so that the percentage of rotation locomotion relative to total locomotion could be determined.

Design and Procedure

The evaluation of repeated apomorphine administration on locomotion and rotation behavior was carried out using a Pavlovian drug conditioning protocol (25). Accordingly, the test chamber stimuli served as the Pavlovian conditioned stimulus (CS) and apomorphine stimulation through peripheral injection served as the Pavlovian unconditioned stimulus (US). Locomotion and pattern of rotation changes to the drug and conditioning procedure were measured as the unconditioned (UR) and conditioned (CR) responses. Statistically, the design was a 2 × 7 design in which the first dimension was a between-subject treatment dimension while the second dimension was a within-subject treatment dimension. This design is the standard Pavlovian conditioning design which includes repeated paired and unpaired treatment protocols (25). Eighteen animals each were randomly assigned to the paired and unpaired treatment groups. However, 2 of the 18 animals assigned to the unpaired group did not complete all treatments of the experiment and the data pertaining to these animals were not included. Locomotion and rotation pattern results were analyzed by means of ANOVAs and, following significant interaction effects, by specific tests for critical difference between two means at the $p = <0.05$ alpha level (30).

In the paired treatment protocol, the animals received an apomorphine injection (2.0 mg/kg SC) 10 min prior to a 10-min placement into the test environment while the animals in the unpaired treatment protocol received the apomorphine injection (2.0 mg/kg SC) 30 min after removal from the test environment. On completion of the 10-min placement into the test environment, the animals were placed back into their home cage. The paired treatment protocol was designed to induce conditioning of hyperlocomotion to test environment cues while the unpaired treatment protocol provided the appropriate control treatment baseline contrast; for example, an assessment of nonassociative drug-induced sensitization and test environment habituation effects. These treatments were administered for 7 consecutive days, one trial per session.

After a 7-day drug withdrawal period, the animals were given one nondrug test trial of 10-min duration in the test environment. One day following the nondrug test, paired and unpaired treatment animals in each drug test group were given three reacquisition apomorphine trials (days 1–3 treatment), prior to the drug tests. At this point, the animals in the paired and unpaired treatment groups were subdivided and matched separately in each group in terms of the total meters traversed on day 7 of acquisition and were then assigned in equal numbers to two drug test subgroups which differed only in sequence of drug testing: 0.5 mg/kg and then 2.0 mg/kg apomorphine, or, 2.0 mg/kg and then 0.5 mg/kg apomorphine. The two drug tests were carried out 7 days after completion of the reacquisition trials on separate days. All animals in these tests were given either 0.5 mg/kg or 2.0 mg/kg, depending on group assignment, 10 min prior to placement into the test chamber for a 10-min test trial. Thus in these final drug tests, all animals received the paired drug treatment.

Statistical Analyses

The results of days 1, 3, 5, and 7 were analyzed by a 2 × 4 repeated measures ANOVA. Separate ANOVAs were performed for the locomotion, rotation results, and, subsequently, for several derived locomotion and rotation measures. Additionally, 2 × 3 ANOVAs were performed on the results of the posttreatment nondrug and two apomorphine drug tests.

RESULTS

Locomotion

Figure 1 shows the locomotor behavior in terms of meters traversed for the paired and unpaired treatment groups during days 1, 3, 5, and 7 of the 7-day repeated apomorphine treatment. The locomotion results shown in Fig. 1 were analyzed with a single ANOVA. The F-tests showed no significant paired vs. unpaired treatment effects, $F(1, 32) = 3.444$, $p >$

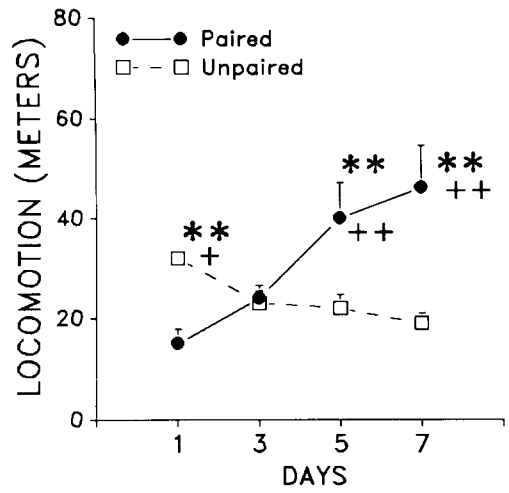

FIG. 1. Means and SEMs of locomotion measured in meters traversed for animals in the paired (n = 18) and unpaired (n = 16) treatment groups for days 1, 3, 5, and 7 of apomorphine treatment. Asterisks (**) indicate post hoc Newman-Keuls specific test comparisons following ANOVA for mean locomotion differences between paired and unpaired treatment groups on days 1, 3, 5, and 7 ($p < 0.01$). Crosses (+, ++) indicate within-treatment comparisons of mean locomotion differences for the paired ($p < 0.01$) and unpaired ($p < 0.05$) treatment groups, respectively.

0.05), a significant within treatment effect, $F(3, 96) = 12.009$, $p < 0.01$), and a significant interaction effect of treatment and days of treatment, $F(3, 96) = 31.424$, $p < 0.01$. Specific post hoc Newman-Keuls test comparisons between paired vs. unpaired treatment effects on days 1, 3, 5, and 7 showed, interestingly, that the initial response to apomorphine on day 1 in the paired group was that of locomotion suppression ($p < 0.01$). This initial hypolocomotion response, however, changed over into hyperlocomotion by day 5 ($p < 0.01$) and continued to increase up to day 7 ($p < 0.01$). Nonetheless, 5 of the 18 paired animals continued to respond throughout the seven sessions with the hypolocomotion response shown on day 1 (i.e., their locomotion did not increase above the paired group mean locomotion of day 1). The data of these animals is included in all the data analyses but is noted in order to indicate that not all animals develop hyperlocomotion with repeated apomorphine treatment.

Rotation Behavior

Rotation locomotion. Because locomotion in meters traversed includes both rotational and linear locomotion, the locomotion behavior was further analyzed in terms of percent of rotation locomotion relative to the total locomotion exhibited by each animal. Percent of rotation locomotion was defined as the ratio of total distance traversed in terms of rotation at all four rotation diameters relative to the total locomotion in meters traversed by each animal. The results are shown in Fig. 2. As can be seen from Fig. 2, percent of rotation locomotion increased from day 1 to day 7 differentially in the paired and unpaired treatment groups and these results were analyzed by an ANOVA. The F-tests showed a significant treatment effect, $F(1, 32) = 8.434$, $p < 0.01$, no significant days of treatment effect but a significant interaction of treatment × days of treatment, $F(3, 96) = 4.725$, $p < 0.01$. Specific post hoc Newman-Keuls tests showed that the paired treatment mean percent of rotation locomotion was higher than that of the unpaired treatment group on days 3 ($p < 0.05$), 5 ($p < 0.01$), and 7 ($p < 0.01$). These results indicate that the increased locomotion in the paired group as shown in Fig. 1, was increasingly expressed as rotation locomotion.

Rotation pattern analysis. The rotation results were further analyzed to determine the nature of rotation pattern change suggested by the results shown in Fig. 2. Figure 3 shows the rotation pattern change in terms of frequency of distribution according to diameter size level on days 1, 3, 5, and 7. The rotation responses are presented in combined form to include all rotations 1/4, 1/2, 3/4, and 1/1 as this parameter did not reflect differential drug effects. As can be seen from Fig. 3, the combined rotation frequencies of partial and full rotations ranging from the smallest to the largest diameter size of rotation: <20 cm, 20-<30 cm, 30-<55 cm, and ≥ 55 cm are approximately equally distributed across diameter size category on day 1. This rotation pattern, however, changed on days 5 and 7 selectively in the paired group. The F-tests of the ANOVAs of these results for days 1, 3, 5, and 7 showed that on day 1, apomorphine treatment in the paired group vs. the unpaired treatment group had an overall suppressive effect on rotation, as it did on locomotion. This apomorphine suppressive effect was manifested in reduced overall rotation frequency levels across all diameter-size categories, $F(1, 32) = 21.929$, $p < 0.01$). On day 3, however, this overall suppression effect was no longer present but there was a significant interaction of treatment with diameter size of rotation. Specific post hoc Newman-Keuls tests showed the interaction effect to be confined to a reduction of rotation in the smallest, <20 cm, diameter size in the paired vs. the unpaired group ($p < 0.05$). On days 5 and 7, the F-tests again showed no overall paired vs. unpaired treatment effect but a significant effect for diameter size and a significant interaction of treat-

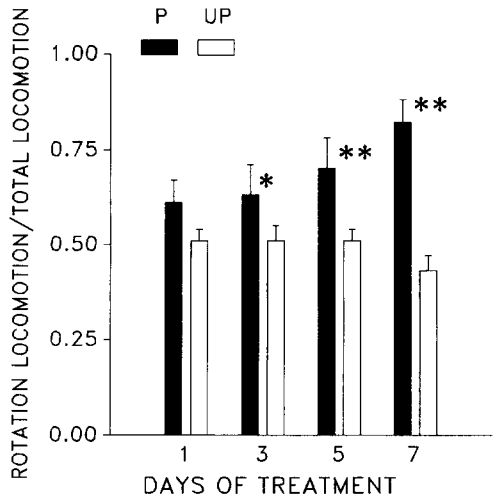

FIG. 2. Means and SEMs for percent of rotation locomotion on days 1-7 of apomorphine treatment. Rotation locomotion distance for each animal was calculated using the standard circumference formula for all partial and full rotations at each diameter size category. The summated rotation distance was divided by the total distance traversed to determine percent values for each animal. Asterisks denote $p < 0.05$ (*) and $p < 0.01$ (**) levels of statistical significance.

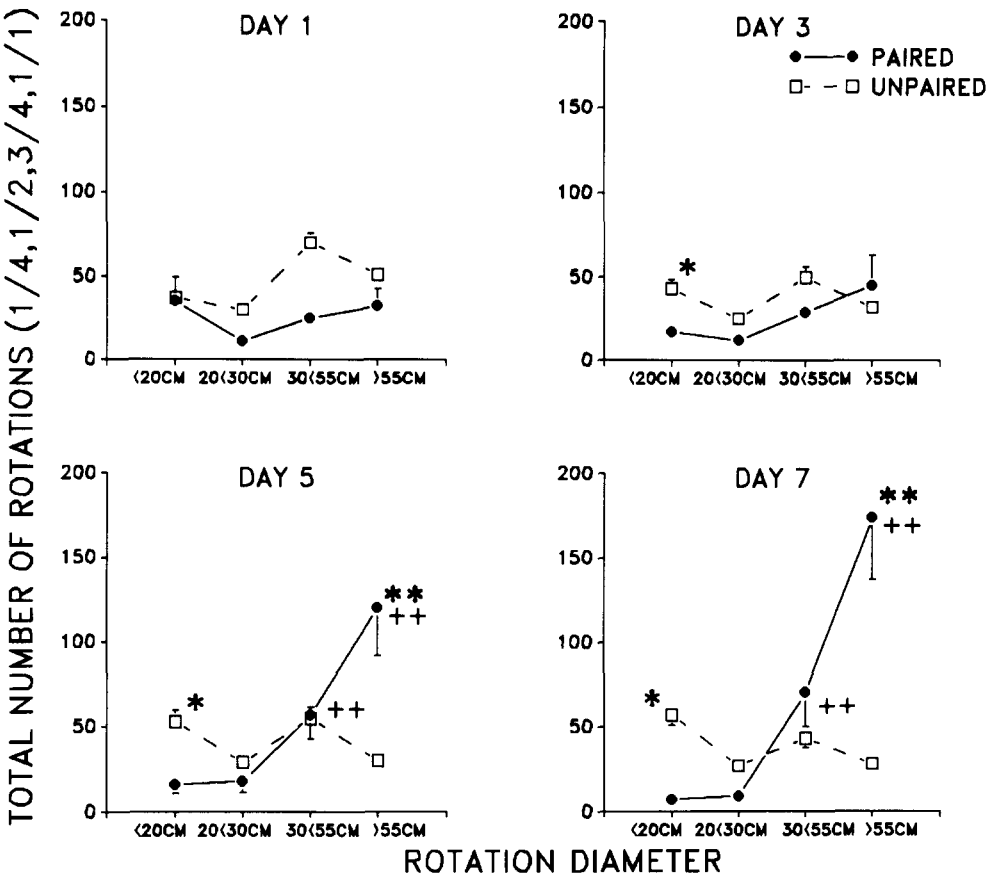

FIG. 3. Means and SEMs for total rotation frequency (i.e., sum of 1/4, 1/2, 3/4, and 1/1 rotations for each animal) as a function of size of rotation diameter ranging from <20 cm, 20-<30 cm, 30-<55 cm, to ≥55 cm. Results show total frequencies on days 1, 3, 5, and 7 of apomorphine treatment for the paired and unpaired treatment groups. Asterisks denote $p < 0.05$ (*) and $p < 0.01$ (**) levels of statistical significance for post hoc Newman-Keuls specific test comparisons following ANOVA between treatment means on days 1, 3, 5, and 7. Crosses denote $p < 0.01$ (++) specific within-treatment comparisons for the paired and unpaired treatment groups, respectively.

ment with rotation diameter size, suggesting that hyperlocomotion was being mediated by a redistribution of rotation frequencies toward large diameter rotation. Thus the repeated apomorphine treatment did not enhance rotational behavior per se but rather it modified the form of the rotational locomotion. Post hoc Newman-Keuls tests for specific effects showed that, for the paired group, the frequency of rotation in the highest category of rotation, ≥55 cm, was higher than all other diameter size levels within the same group ($p < 0.01$) and from those of the corresponding unpaired treatment group at this diameter size ($p < 0.01$). This same within-group pattern of results was observed for the specific treatment effect on the next highest category of rotation (i.e., 30-<55 cm) ($p < 0.01$). Additionally, there was a significant reduction in rotations at the smallest category size, <20 cm, ($p < 0.05$). Altogether, these results show a dynamic shift in rotation pattern from day 1 to day 7 selectively in the paired group. This rotation pattern change was expressed as a shift from an approximately equivalent frequency distribution to a highly skewed distribution of rotation frequencies weighted toward rotation at the largest diameter size category, ≥55 cm. Clearly, the shift to wide diameter rotations brought about the increase of rotational locomotion and of total locomotion as well.

Rotation direction bias analysis. Changes in rotation pattern were further assessed in terms of each animal's rotation bias as expressed by the direction of rotation. For example, if an animal had a counterclockwise directional bias of rotation on day 1 of testing, did this directional bias remain stable or even become enhanced with repeated apomorphine treatment as one might expect from a behavioral sensitization concept or from an inherent laterality bias? As is evident from Fig. 3, the major changes in rotation occurred between day 1 and days 5 and 7. Thus the directional bias of rotation was compared on days 1, 5, and 7. First, the number of clockwise and counterclockwise rotations were determined separately for days 1, 5, and 7 across all diameter size categories. Individual data were summed to form two frequency scores for each animal: a) total number of clockwise rotations; and b) total number counterclockwise rotations. A rotation direction bias ratio was then formed for each animal on days 1, 5, and 7 using the formula: p = no. of counterclockwise rotations/no. of counterclockwise + no. of clockwise rotations. A score of $p = 0.50$ would indicate no rotation directional bias, whereas

a score above 0.50 would indicate a counterclockwise rotation bias and, similarly, a score below 0.50 would indicate a clockwise rotation bias. Pearson's r correlations between rotation bias on day 1 with rotation bias on day 7 were low and did not differ statistically, 0.31 and 0.21 for the paired and unpaired groups, respectively. This absence of a statistical correlation is inconsistent with either a behavioral sensitization or with an inherent laterality bias prediction. Correlations between days 5 and 7, the days on which the new rotation patterns had emerged in the paired treatment group, revealed a striking difference. The correlations were: 0.83 and −0.09 for the paired and unpaired groups, respectively, and this difference was significant ($p < 0.01$).

The high correlation in directional bias in the paired group between day 5 and day 7 suggests that the paired animals shifted from bidirectional to stable unidirectional rotation locomotion which was independent of the initial idiosyncratic laterality bias (10). To determine whether rotation direction bias is an additional dimension of the behavioral reorganization of locomotion behavior, another analysis was performed. Specifically, the absolute or unsigned deviation of the rotation direction bias score for each animal as described above was determined separately for days 5 and 7 relative to day 1. In terms of this difference score, a 0 score would mean that the directional bias remained the same on day 5 relative to that of day 1 (or of day 7 relative to day 1). A score of 0.50 would mean a maximum degree of rotation bias change. Days 5 and 7 were selected as it was at this point that the pattern of frequency distribution across diameter size category emerged and stabilized as a distinctive and identifiable frequency pattern. This pattern, which was not present at the the beginning of drug treatment, qualifies as one instance of an emergent qualitative change resulting from the apomorphine treatment. A calculation of the absolute deviations of the rotation direction bias scores from those exhibited on day 1 for each animal would, therefore, present another parameter of the emergent qualitative change in locomotion behavior. The derived scores of each group were then analyzed by a single ANOVA. The F-test analysis showed a significant overall paired vs. unpaired treatment effect, $F(1, 28) = 6.471$, $p < 0.05$, with greater directional asymmetry in the paired relative to the unpaired treatment group means, 0.28 vs. 0.12, in a maximal range from 0 to 0.50. An overall within-treatment effect, $F(2, 56) = 3.187$, $p < 0.05$, was also found but no interaction of treatment × days of treatment, $F(2, 56) = 1.144$, $p > 0.05$. These results, indicate an overall increased asymmetry in the paired relative to the unpaired treatment group, especially in the absence of an interaction effect. Moreover, in 5 of the 18 animals of the paired group, there was a pronounced reversal in the directionality bias relative to day 1. One animal shifted from predominantly clockwise rotation, 67%, to predominantly counterclockwise rotation, 99%; the other four animals shifted in the opposite direction: 61%, 68%, 75%, and 99% counterclockwise rotation to 99%, 86%, 99%, and 99% clockwise rotation, respectively. None of the animals of the unpaired group exhibited such a degree of change.

Postacquisition Conditioning and Drug Test Results

Locomotion behavior. The locomotion behavior results on the nondrug conditioning and 0.5 mg/kg and 2.0 mg/kg apomorphine tests were analyzed by a single ANOVA. The overall F-test analysis indicated a significant paired vs. unpaired treatment effect, $F(1, 32) = 14.545$, $p < 0.01$, a drug dose within-treatment effect, $F(2, 64) = 6.181$, $p < 0.01$, and a significant interaction effect of paired vs. unpaired treatment with drugs dose, $F(2, 64) = 5.47$, $p < 0.01$. Post hoc Newman-Keuls specific tests for mean locomotion of the paired (32.32 m) vs. unpaired (26.76) treatment groups indicated that the difference was not significant ($p > 0.05$) in the nondrug test for conditioning. Thus there was no evidence that the hyperlocomotion response observed on days 5 and 7 was conditioned to the exteroceptive test environment cues. However, in both the 0.5 mg/kg and the 2.0 mg/kg apomorphine tests, hyperlocomotion, as indicated by a comparison of the paired vs. unpaired groups, was observed ($p < 0.01$).

Rotation locomotion. Percent of rotation locomotion, as already described, was also analyzed on the nondrug and drug tests following the 7-day repeated apomorphine treatment. Figure 4 shows the percent of rotation locomotion on the nondrug test for conditioning and on the 0.5 mg/kg and 2.0 mg/kg apomorphine drug tests. An ANOVA was performed on these results. The F-tests showed a significant treatment effect, $F(1, 32) = 9.175$, $p < 0.01$, no within-treatment effect but a significant interaction effect, $F(2, 64) = 3.524$, $p < 0.05$. Specific post hoc Newman-Keuls test results showed that mean percent of rotation locomotion was not statistically different in the paired vs. unpaired treatment groups on the nondrug test but that it was higher in the paired group in both the 0.5 mg/kg ($p < 0.05$) and in the 2.0 mg/kg apomorphine tests ($p < 0.01$).

Rotation pattern analysis. Figure 5 shows the nondrug and 0.5 mg/kg and 2.0 mg/kg drug test results on rotation pattern following the 7-day drug withdrawal. In the nondrug conditioning test, the rotation patterns for the paired treatment group reverted back to those of day 1 and were similar to those of the unpaired group. However, the drug test results for the paired group, for both drug dose levels, showed a reinstatement of the distinctive rotation patterns observed on days 5 and 7 of acquisition. The F-tests of the ANOVAs of

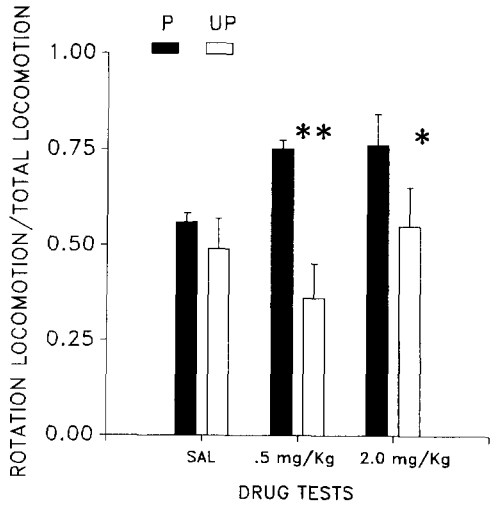

FIG. 4. Means and SEMs for percent of rotation locomotion for the 0.5 and 2.0 mg/kg apomorphine tests following days 1-7 apomorphine treatment. Rotation locomotion distance for each animal was calculated using the standard circumference formula for all partial and full rotations at each diameter size category. The summated rotation distance was divided by the total distance traversed to determine percent values for each animal. Asterisks denote $p < 0.05$ (*) and $p < 0.01$ (**) levels of statistical significance.

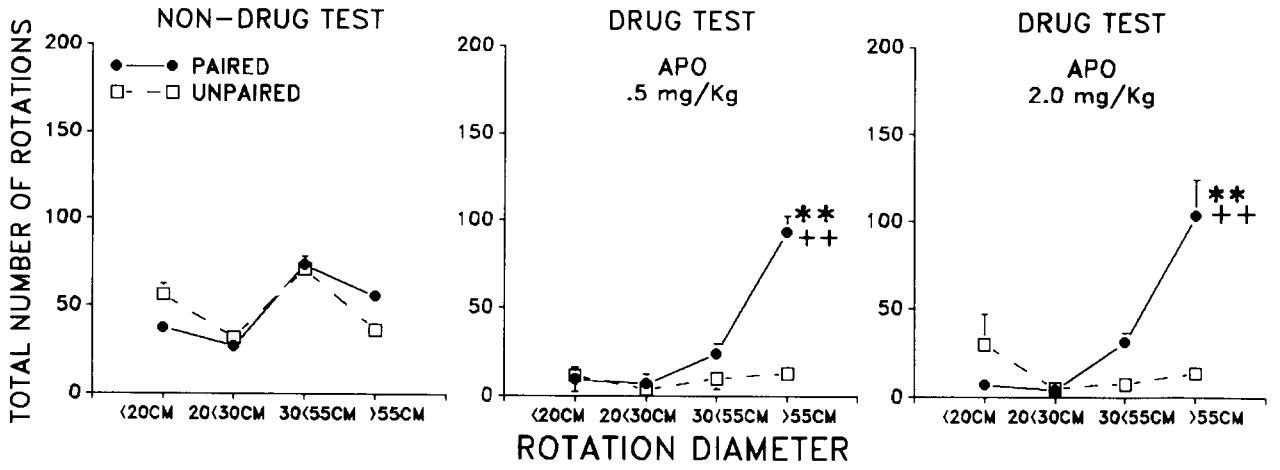

FIG. 5. Means and SEMs for total rotation frequency (i.e., sum of 1/4, 1/2, 3/4, and 1/1 rotations for each animal) as a function of size of rotation diameter ranging from <20 cm, 20-<30 cm, 30-<55 cm, to ≥55 cm. Results show total rotation frequencies on posttreatment nondrug and drug tests for animals in the paired and unpaired groups. Asterisks (**) indicate post hoc Newman-Keuls specific test comparisons following ANOVA for mean rotation rate differences between paired and unpaired treatment groups ($p < 0.01$). Crosses (++) indicate specific within-treatment comparisons of mean rotation rate differences for the paired group ($p < 0.01$).

these results which were carried out separately for each of the three postacquisition tests indicated that in the nondrug test, there was no significant paired vs. unpaired treatment effect, no diameter-size effect in terms of frequency of rotation, and no significant interaction of treatment with rotation diameter. These findings indicate the absence of conditioning of the drug-induced skewed rotation pattern to the test chamber cues on the nondrug conditioning test trial. In the apomorphine drug state condition (0.5 mg/kg and 2.0 mg/kg), however, the paired animals selectively exhibited the acquired rotation pattern of behavior shown on days 5 and 7. The *F*-test results for each drug test revealed a paired vs. unpaired treatment effect, $F(1, 32) = 3.097$, $p < 0.01$, and $F(1, 32) = 8.223$, $p < 0.01$, for the 0.5 and 2.0 mg/kg apomorphine tests, respectively. A within-treatment effect, expressed in diameter size differences in rotation frequency, was also observed, $F(3, 96) = 13.112, p < 0.01$, and $F(3, 96) = 9.200, p < 0.01$, for the 0.5 and 2.0 mg/kg apomorphine tests, respectively. An interaction of treatment and diameter size was observed as well, $F(3, 96) = 15.983$, $p < 0.01$, and $F(3, 96) = 7.964$, $p < 0.01$ for the 0.5 and 2.0 mg/kg apomorphine tests, respectively. Post hoc Newman-Keuls tests for specific effects showed that in each drug test the rotation frequencies at the largest size category of rotation, ≥55 cm, were higher in the paired group vs. the unpaired group ($p < 0.01$), and were also higher in the paired group within-treatment comparisons as well. ($p < 0.01$). These drug test results indicate that the skewed rotation pattern in the paired treatment group had been retained after the 7-day drug withdrawal period.

Session Duration Interval and the Emergence of Hyperlocomotion and Behavioral Reorganization of Locomotion

While the preceding analyses indicated a substantial change in the behavioral expression of apomorphine-induced effects over the course of repeated drug treatment, it is also possible that these changes could reflect a within-session shift in the drug effect. That is, with repeated treatment, the observed changes on days 5 and 7 might have been detected on day 1 with a longer session duration interval. An additional set of six animals were administered 2.0 mg/kg according to the paired treatment protocol as described earlier. These animals were observed for an extended session interval of 30 min. Mean locomotion (m) in the first, second, and third 10-min segment of the 30-min session showed a consistent decline in locomotion as the percent decrease in locomotion was 5% and 8% for the second and and third 10-min segments relative to the first 10 min segment. Percent of locomotion expressed as rotation locomotion also declined from the first 10 min to the last 10 min of the session. Similar analysis of frequency of rotation at each diameter size showed an overall decline in the second and third 10-min segments relative to the first 10-min segment but no redistribution of the frequency pattern for different diameter size categories. Thus the within-session changes were distinct on all measures from the between-session changes.

DISCUSSION

The emergence of hyperlocomotion in the animals that received apomorphine paired with test chamber placement is consistent with previous findings of apomorphine-induced behavioral sensitization effects on locomotion (14,15). Importantly, the present study showed, upon analysis of rotation patterns, that a new pattern of behavior developed concurrently with hyperlocomotion. Overall, there was an increase in percent of rotation locomotion relative to the total locomotion and a change in rotation pattern from an equal distribution of small, medium, and large diameter rotations to a predominance of large diameter rotations. Importantly, however, rotation per se did not increase; rather, the pattern changed. Because the shift toward large diameter rotations entails greater locomotion, this change in rotation behavior undoubtedly contributed to the development of hyperlocomotion. Not only did the increase in percent of rotation locomotion and the shift to large diameter rotations develop exclusively in the paired group of animals, but these changes were retained after

a 7-day drug withdrawal period. Additionally, the rotation directionality bias also changed with repeated treatment. Importantly, the directional bias was not significantly correlated with the initial rotation bias and in 5 of the 18 animals directional bias reversed. Furthermore, this acquired change in direction of rotation stabilized and was retained following drug withdrawal. Because drug-induced rotational direction bias in intact animals has been used to infer intrinsic hemispheric bias asymmetry in rodents (10), the observed shift and stabilization of rotation directionality with repeated drug treatment calls into question extant interpretations of laterality bias as being due to an intrinsic hemispheric dopaminergic imbalance. Instead, the present results point to the structural features of the test environment interacting with adventitious learning mechanisms during the drug state as determinants of locomotion directionality bias.

Critically, the present results cannot be understood by invoking pharmacological mechanisms such as the mechanism of sensitization-tolerance. For example, if one conceptualizes the emergence of hyperlocomotion as being reflective of a shift to the left in the dose-response curve due to an increase in sensitivity to apomorphine, then, a higher dose than that used in the present study should induce hyperlocomotion on initial exposure to the drug. Mattingly et al. (14,15), however, observed that hypolocomotion occurs initially even at dose levels as high as 5 mg/kg. Another possibility is that the hyperlocomotion response might have occurred on day 1 but at a postinjection time later than the 10-min period used in the present study. Seemingly, sensitization to apomorphine might have advanced the drug response temporally so that this temporally delayed drug effect would become manifested only with repeated drug treatment. The present study results, however, showed a progressive hypolocomotion effect without a differential change in rotation pattern in the 30-min extended observation interval for one set of animals. Additionally, neither hyperlocomotion nor a shift in rotation pattern were observed in the posttreatment drug test in the animals of the unpaired group that had the identical drug treatment but unpaired to test chamber placement. Furthermore, except for the 10-min exposure to the test chamber, both paired and unpaired treatment animals experienced the apomorphine drug state in similar home cages. Thus if one were to argue that the home cage environment suppressed the occurrence of the temporally delayed apomorphine-induced hyperlocomotion effect in the unpaired treatment group, this response would have been equally suppressed in the paired group. Altogether, these considerations point to the necessity of developing a new approach toward understanding the behavioral sensitization effects of repeated psychostimulant drug treatment.

It is well established that the behavioral effects of high doses of apomorphine (≥ 0.5 mg/kg) are mediated by striatal and mesolimbic dopaminergic postsynaptic D1/D2 receptors (3,9,11-13,17,20-22,24,27,28). In evaluating high-dose dopaminergic drug effects, however, it is necessary to consider the sensory gating effects (23) that apomorphine imposes upon an animal's response to the test environment. For example, a large dose level of apomorphine, such as that used in the present study, initially induces an intense sniffing and licking behavior oriented exclusively to the stimuli with which the animal is in immediate contact. Consequently, there is reduced locomotion due to the animals being less attentive than nondrug animals to the distal cues of the test environment that elicit locomotor exploratory behavior (16). Thus for the animals of the paired treatment group, which are in the test environment during the apomorphine drug state, the proximal cues are prepotent and appear to occlude distal spatial aspects of the test environment. As a result, the apomorphine-paired animals achieve movement throughout the test environment through small shifts in spatial location guided by successive piecemeal sampling of circumscribed areas of proximal stimuli. At the end of this dynamic process, the test environment chamber walls provide both a limit of the locomotor response as well as a maximum level of proximal stimuli by providing simultaneous contact with horizontal and vertical surfaces. The convergence of surfaces for maximal proximal stimulus contact provides an explanation for the emergence of uniformity of locomotor pattern induced by apomorphine in the different animals following repeated treatment.

Importantly, interoceptive drug cues (4,5,19) are a constant and integral part of the complex stimulus context in which movement responses occur. These stimuli could account for the development of directionally stable locomotion patterns with repeated apomorphine treatment because the repeated association of drug stimuli with stereotypic locomotor patterns could lead to an S-R (stimulus-response) contiguity association and enable the drug stimuli to attain an increasingly determinant role in the occurrence of the behavioral expression of the drug effect (4,5). This S-R linkage through Pavlovian conditioning mechanisms would, in this manner, entail an association of a complex compound stimulus (S) which includes the interoceptive drug cue and proximal cues of the test environment with the animal's locomotor behavior (R).

Within the framework outlined above, the development of hyperlocomotion is interpreted as a behavioral reorganization process occurring in the drug state. However, the specific form of behavioral reorganization process depends upon behavioral contingencies tied to a specific environment. Thus, hyperlocomotion is not a necessary outcome in this schema. If the animal remains in place sniffing and licking, it may never come to chain locomotor movement to different sets of proximal stimuli and thereby establish contact with larger spatial areas of the test environment. This was confirmed by the fact that five animals of the paired group remained throughout the experiment at the initial hypolocomotion stage and did not show the behavioral reorganization expressed in changes of rotation pattern and acquired directionality of movement. Upon first exposure to the test environment during the drug state in the two postacquisition drug tests, the unpaired animals also showed hypolocomotion without behavioral restructuring similar to that of the animals of the paired group on day 1. At this point, the unpaired animals had already had 10 days of drug treatment and 10 days of nondrug exposure to the test environment, but they lacked the association of the drug state to the test environment stimuli. Similarly, rotation around the perimeter of the test chamber is not likely to develop if there is no vertical surface at the periphery (e.g., in an elevated flat surface without walls). At the same time, if the environment is a restricted spatial arena incompatible with locomotion, then other behaviors that maximize contact with proximal stimuli will increase with repeated treatment (e.g., rearing and climbing). Thus, the behavioral expression of the pharmacological effect of a psychostimulant drug entails a factor of plasticity that is contingent upon the environment and the animal's response to it.

Altogether, the present analysis and results call into question the use of the concept of behavioral sensitization to describe the enhanced locomotor effects of repeated psychostimulant drug treatment. While behavioral sensitization as a pharmacological concept has a precise operational meaning—

a shift to the left of the dose-response curve—it is nonetheless, too simplistic. Psychostimulant drugs engage attentional as well as conditioning and memory mechanisms. With repeated drug treatment, the drug effect becomes entwined in these complex integrative neural mechanisms to induce structural reorganization of behavior. Thus it becomes necessary to develop formulations of drug action that are commensurate with this complex interactive process. Rather than a shift in the dose-response function of a target response, repeated psychostimulant drug effects need to be reconceptualized into processes that entail a dynamic outcome of environmental contingencies with learning and behavioral plasticity mechanisms. Repeated psychostimulant drug treatments do not merely result in an increase of a specific target response, as suggested by the concept of behavioral sensitization; rather, they set the occasion for the initiation of a structural reorganization of behavior. The behavioral expression of the drug effect develops out of the drug impact upon sensorimotor integration processes in a specific test environment. In this context, learning and memory mechanisms interact with the drug state and the environmentally contingent behavioral patterns to consolidate and preserve such behavioral repertoires which, with repetition, can develop into stereotyped patterns. The concept of behavioral reorganization that is offered as an alternative descriptive concept presents a more comprehensive description of the behavioral dynamics initiated by repeated psychostimulant drug treatment compared to the extant widely espoused behavioral sensitization concept.

ACKNOWLEDGEMENTS

This research was supported by a Veterans Administration Merit Review Grant and by a National Institute of Drug Abuse Grant R01 DA05366-06.

REFERENCES

1. Bernard, M. M.; Scavone, C.; Frussa-Filho, R. Differential effects of single and long-term amphetamine and apomorphine administration on locomotor activity of rats. Gen. Pharmacol. 17: 465–468; 1986.
2. Bonatz, A.; Steiner, H.; Huston, J. P. Video Image analysis of behavior by microcomputer: Categorization of turning and locomotion after 6-OHDA injection into the substantia nigra. J. Neurosci. Methods 22:13–26; 1987.
3. Brundin, P.; Strecker, R. E.; Londos, E.; Bjorklund, A. Dopamine neurons grafted unilaterally to the nucleus accumbens affect drug induced circling and locomotion. Exp. Brain Res. 69:183–194; 1987.
4. Carey, R. J. Pavlovian conditioning between co-administered drugs: Elicitation of an apomorphine induced antiparkinsonian response by scopolamine. Psychopharmacology (Berlin) 104:463–469; 1991.
5. Carey, R. J. Stimulant drugs as conditioned and unconditioned stimuli in a classical conditioning paradigm. Drug Dev. Res. 16: 305–315; 1989.
6. 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 25:297–300; 1986.
7. Damianopoulos, E. N.; Carey, R. J. Effects of repeated cocaine treatment: Behavioral reorganization vs. behavioral sensitization. Soc. Neurosci. Abstr. 17:1428; 1991.
8. Damianopoulos, E. N.; Carey, R. J. Conditioning, habituation and behavioral reorganization factors in chronic cocaine effects. Behav. Brain Res. 49:149–157; 1992.
9. Gandolfi, C.; Dall'Olio, R.; Vaccheri, A.; Roncada, P.; Montanaro, N. Responses to D-1 and D-2 agonists after repeated treatment with selective D-1 and D-2 antagonists. Pharmacol. Biochem. Behav. 30:463–469; 1988.
10. Glick, S. D. Heritable differences in turning behavior in rats. Life Sci. 36:499–503; 1985.
11. Herrera-Marschitz, M. Neuropharmacology and functional anatomy of the basal ganglia. Stockholm: Karolinska Medical Institute; 1986.
12. Koob, G. F.; Stinus, L.; LeMoal, M. Hyperactivity and hypoactivity produced by lesions to the mesolimbic dopamine system. Behav. Brain Res. 3:35–41; 1981.
13. LeMoal, M.; Galey, D.; Cardo, B. Behavioral effects of local injections of 6-hydroxydopamine in the medial ventral tegmentum in the rat. Possible role of the mesolimbic dopaminergic system. Brain Res. 88:190–194; 1975.
14. Mattingly, B. A.; Gotsick, J. E.; Marin, C. Locomotor activity and stereotypy in rats following repeated apomorphine treatments at 1-, 3-, or 7-day intervals. Pharmacol. Biochem. Behav. 31:871–875; 1989.
15. Mattingly, B. A.; Gotsick, J. E.; Salamanca, K. Latent sensitization to apomorphine following repeated low doses. Behav. Neurosci. 102:553–558; 1988.
16. Moller, H. G.; Nowak, K.; Kuschinsky, K. Conditioning of pre- and post-synaptic behavioral responses to the dopamine receptor agonist apomorphine in rats. Psychopharmacology (Berlin) 91: 50–55; 1987.
17. Molloy, A. G.; O'Boyle, K. M.; Pugh, M. T.; Waddington, J. L. Locomotor behaviors in response to new selective D-1 and D-2 dopamine receptor agonists, and the influence of selective antagonists. Pharmacol. Biochem. Behav. 25:249–253; 1986.
18. Mueller, K.; Pell, J. L. Scopolamine produces locomotor stereotypy in an open field but apomorphine does not. Pharmacol. Biochem. Behav. 36:613–617; 1990.
19. Nielsen, E. B.; Scheel-Kruger, J. Cueing effects of amphetamine and LSD: Elicitation by direct microinjection of the drugs into the nucleus accumbens. Eur. J. Pharmacol. 125:85–92; 1986.
20. Pulvirenti, L.; Swerdlow, N. R.; Koob, G. F. Microinjection of a glutamate antagonist into the nucleus accumbens reduces psychostimulant locomotion in rats. Neurosci. Lett. 103:218–218; 1989.
21. Pycock, C. J. Turning behavior in animals: Commentary. Neuroscience 5:461–514; 1980.
22. Randrup, A.; Sorensen, G.; Kobayashi, M. Stereotyped behaviour in animals induced by stimulant drugs or by a restricted cage environment: Relation to disintegrated behavior, brain dopamine and psychiatric disease. Jpn. J. Psychopharmacol. 8:313–327; 1988.
23. Schneider, J. S.; Lidsky, T. I. (eds.) Basal ganglia and behavior: Sensory aspects of motor functioning. Toronto: Hans Huber Publishers; 1987.
24. Schwarting, R. K. W.; Steiner, H.; Huston, J. P. Asymmetries in thigmotactic scanning: Evidence for a role of dopaminergic mechanisms. Psychopharmacology (Berlin) 103:19–27; 1991.
25. Siegel, S. Pharmacological conditioning and drug effects. In: Goudie, A. J.; Emmett-Oglseby, M. W., eds. Psychoactive drugs. New York: The Human Press, Inc.; 1989.
26. Stahle, L.; Ungerstedt, U. Different behavioural patterns induced by the dopamine agonist apomorphine analyzed by multivariate statistics. Pharmacol. Biochem. Behav. 24:291–298; 1986.
27. Starr, B. S.; Starr, M. S. Differential effects of dopamine D1 and D2 agonists and antagonists on velocity of movement, rearing and grooming in the mouse. Implications for the roles of D1 and D2 receptors. Neuropharmacology 25:455–463; 1986.
28. Vaccheri, A.; Dall'Olio, R.; Gandolfi, D.; Montanaro, N.

Involvement of different dopamine receptors in rat diphasic motility response to apomorphine. Psychopharmacology (Berlin) 89: 265–268; 1986.
29. Wickens, J. Striatal dopamine in motor activation and reward-mediated learning: Steps toward a unifying model. J. Neural Transm. 80:9–31; 1990.
30. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill; 1962.