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Conditioned Apomorphine-Induced Turning in 6-OHDA–Lesioned Rats

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HUDSON, J. L., C.-S. FONG, S. J. BOYSON AND B. J. HOFFER. *Conditioned apomorphine-induced turning in 6-OHDA-lesioned rats.* PHARMACOL BIOCHEM BEHAV **49**(1) 147–154, 1994. – Apomorphine-induced turning has been used to evaluate the extent of unilateral nigrostriatal denervation after 6-hydroxydopamine (6-OHDA) lesions and subsequent functional striatal reinnervation by catecholaminergic grafts. It has been noted that the pregraft rotational pattern is usually double peaked and that fetal ventral mesencephalic grafts or dopaminergic drugs will alter the second peak but leave the first relatively unchanged. We hypothesized that the first peak may be the result of factors extrinsic to the nigrostriatal dopamine system, specifically a conditioned turning response, and would, therefore, be unperturbed by the above treatments which increase dopaminergic (DA) inputs. This was investigated by injecting 6-OHDA, unilaterally, into the nigrostriatal pathway of several groups of young Fisher 344 rats. One experimental group was repeatedly tested with 0.05 mg/kg apomorphine and the rotations quantified. A second group received similar injections of apomorphine but were prevented from rotating. Vehicle control animals were also studied for both of the above experimental groups. Subsequent to the above treatment, all animals were tested unrestrained repeatedly on apomorphine. Our results support the conditioned response hypothesis in that the first peak is not present with the initial unrestrained apomorphine behavioral trial but is present upon the second and subsequent unrestrained trials. Moreover, the restrained but apomorphine-injected rats, as well as the control animals, manifest no first peak upon their first freely moving apomorphine test; the second and subsequent unrestrained apomorphine trials, in these groups, do manifest a first peak. We conclude that the first peak represents respondently (Pavlovian) conditioned rotations and is, therefore, an indirect secondary behavioral result of unilateral nigrostriatal dopaminergic denervation and repeated apomorphine administration in an environment allowing unimpeded movements. These rotations are unlikely to be directly related to the cellular changes induced by dopaminergic manipulations in this system and, therefore, their presence in studies of striatal denervation and reinnervation using apomorphine-induced turning behavior should be interpreted accordingly.

Rotation Parkinson's disease Learning Classical conditioning Dopamine Substantia nigra Striatum

PARKINSON'S disease (PD) is an idiopathic neurodegenerative disorder of the basal ganglia that results in the loss of midbrain dopaminergic neurons projecting to the striatum. Clinical signs and symptoms of the disease, induced by dopamine (DA) deficiency, are progressively disabling; therefore, many laboratories are investigating the possible efficacy of catecholamine-containing grafts to the denervated striatum in an attempt to palliate or alleviate symptoms (1,6,7,11,14,20). To evaluate this approach, a rodent model of PD has been developed. Destruction of the nigrostriatal dopamine projection bilaterally in rats precipitates profound akinesia, adipsia, and aphagia (12,16). Although an accurate model, such animals are quite difficult to maintain.

Alternatively, stereotaxic unilateral destruction of the nigrostriatal dopamine system yields a rat that behaves relatively normally in the absence of external stressors (18). These chemolytic lesions, using the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA), allow the striatum ipsilateral to a nigrostriatal pathway injection to be selectively DA denervated while leaving the contralateral striatum and other pathways relatively intact. The denervated caudate/putamen becomes supersensitive to dopamine and dopamine receptor

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agonists (17); this aspect of the model may then be exploited as a behavioral test in that an animal will turn contralateral to the denervated side when given a direct DA receptor agonist, such as apomorphine, and the number of rotations correlates well with the extent of the lesion (5,10).

As shown previously by our laboratory, extensive depletions of dopaminergic terminals in one striatum will result in a behavioral response of greater than 300 turns per hour after a low dose of apomorphine (0.05 mg/kg SC); an animal manifesting such behavior has a >99% probability of possessing a 90% or greater striatal dopamine depletion (5,10). Using criteria similar to these, we and others have transplanted fetal ventral mesencephalic neuroblasts to test the hypothesis that reinnervation of the denervated striatum, and thereby normalization of rotational behavior, would occur (1,11). After transplantation, substantial overall decreases in the turning behavior in response to 0.05 mg/kg of apomorphine SC are seen.

However, from the pre- and posttransplantation patterns of rotation, several questions have arisen as to determinants of this behavior other than DA denervation and reinnervation. First, after extensive reinnervation by DA-containing grafts, apomorphine-induced rotation is often markedly reduced but rarely completely disappears in spite of histochemical evidence that the striatum is filled by graft-derived dopaminergic neurites (15). Second, only the second of the typical two-peak pattern of rotation, after low dose apomorphine, appears to be altered by such transplants. That is, in DA-lesioned rats, within about 2 to 3 min of apomorphine injection the rat will exhibit a maximal rotation peak followed by a lower plateau phase. This is followed by a second peak of varying amplitude compared to the first, which subsequently falls to zero over the course of 60 min. The pattern changes with transplantation of dopaminergic neuroblasts, markedly reducing the second peak and plateau phase but only slightly affecting the first peak (19). Third, other manipulations to the DA-denervated striatum, such as the administration of DA-receptor blockers, appear to largely affect only the second peak and not the first.

On the basis of the above findings, the fact that preinjection rotations often occur in the rotometer bowls and other evidence from the literature, we postulated that some facet of the rotational response was independent of DA-receptor supersensitivity and perhaps was a conditioned or other secondary phenomenon (3,4,13). Though this phenomenon has been noted previously, its relevance to the transplantation literature and time course have not previously been addressed. To test these hypotheses, we performed unilateral stereotaxic 6-OHDA lesions on Fisher 344 (F344) rats. All rats were assigned randomly to one of five treatment groups. The groups were designed to separate the differential effects of time after the lesion, apomorphine administration, stress of restraint and/or rotations, and behavioral environment. This was accomplished by administering apomorphine or vehicle to rats that were either freely moving or physically restrained in a rotometer (phase I). Also, one group was not manipulated at all during phase I to control for time between the lesion and first apomorphine exposure. In phase II, all rats were given apomorphine freely moving. With phase III, all rats were given apomorphine but in a novel rotational environment.

METHOD

Lesioning

Sixty male F344 rats, 150–175 g, were used. Within a 36-h window, all rats were lesioned by the same investigator using the following procedure. Surgical anesthesia was produced by injection of 400 mg/kg of chloral hydrate IP. The animals were placed in a stereotaxic frame and the skin over the skull was incised and reflected. Bregma was used as the coordinate origin and a burr-hole was placed in the cranium over the injection site. After a small-needle puncture of the dura, 9 μg/4 μl/4 min of 6-OHDA (hydrochloride) was injected using a microliter syringe and a 26-gauge dome-tipped needle at AP −4.4 mm, ML 1.3 mm, DV −7.8 mm. The needle was removed after a 1-min delay and the wound closed. Convalescence occurred in home cages with food and water ad lib for the duration of the experiment.

Experimental Groups and the Triphasic Design

All sixty rats were randomly assigned to one of five experimental groups and all groups subsequently went through three sequential phases of behavioral testing. Automated rotometers were used throughout the experiments and consisted of 16-bowls and rotometer heads concurrently controlled by a PC (8). All data files acquired were analyzed via spreadsheet software and exported for display.

Phase I (Tests Performed at 3.5, 5, 7, 10, and 14 Weeks Postlesion)

Apomorphine unrestrained group. On each testing day, these 12 rats were placed into rotometer bowls and were allowed to acclimatize for 10 min. They then received a SC dose of apomorphine hydrochloride (0.05 mg/kg) in vehicle (normal saline with 0.02% ascorbate) and were allowed to move within the bowls for at least 60 min, or until they stopped turning. Following testing they were returned to their home cages until the next testing session.

Vehicle unrestrained group. These rats were treated in the same manner as the apomorphine unrestrained group except that the injection contained vehicle only (n = 12).

Apomorphine restrained group. For each testing session these 12 rats were kept from moving by placing them into standard clear colorless Plexiglas rat restrainers (Harvard Instruments); then the restrainer and rat were placed into the automated rotometer bowls. They were allowed to acclimatize for 10 min and subsequently received a SC dose of 0.05 mg/kg of apomorphine. The rats were left in their restrainers for at least 1 h postinjection and then returned to their home cages until the next testing session.

Vehicle restrained group. This group was treated identically to the apomorphine restrained group except that the test injection was of vehicle only (n = 12).

No test group. These lesioned rats, n = 12, remained in their home cages without experimental manipulation throughout the 14-week duration of phase I.

Phase II (Tests Performed at Weeks 16, 17.5, and 19.5 Postlesion)

This phase involved three identical behavioral tests of all five groups. All rats were placed, unrestrained and individually, into the rotometer bowls and were allowed to acclimatize for 10 min. Each received an injection of 0.05 mg/kg of apomorphine SC and was allowed to move unrestrained for at least 60 min.

Phase III (Week 21.5)

This phase involved a single test of all groups of rats, identically, in a novel turning environment. The new environment

was made as different from the previous one as possible. It was dark, flat, enclosed, and had cedar bedding, all of which were very different from the rotometer bowls. They received 0.05 mg/kg of apomorphine 10 min after introduction into the new environment. Their turning behavior was recorded for at least 60 min.

Saline Effects After Established Rotational Behavior

A separate group of 28 lesioned male F344 rats, in addition to the 60 animals described above, were tested unrestrained first on apomorphine and then subsequently a single time on saline in the rotometer. Four apomorphine behavioral tests were given separated by 2 weeks beginning 2 weeks postlesion. After repeated apomorphine trials, the first peak was established (see below). The rats were then placed into the rotometers, as before, only the drug injection was of vehicle alone. The animals were then allowed to move freely in the rotometers for 60 min.

Statistics

Means and standard errors were compared minute-by-minute for each group using an analysis of variance. The Instat® software package (Ver. 1.12a) was utilized to compute statistical parameters.

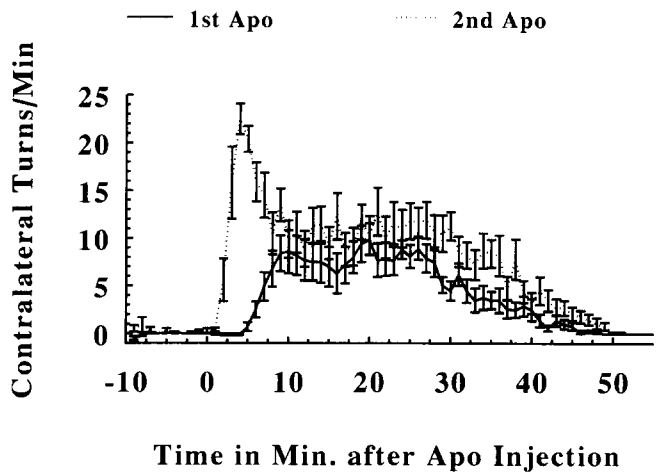


FIG. 1. Graph showing the means and standard errors of the apomorphine unrestrained groups' rotations for the first and second test (n = 11). Curves very similar to the second test resulted from all subsequent unrestrained apomorphine administrations. The initial rotational test shows no evidence of a first peak. The subsequent test curve reveals the pronounced first peak and some preinjection turns.

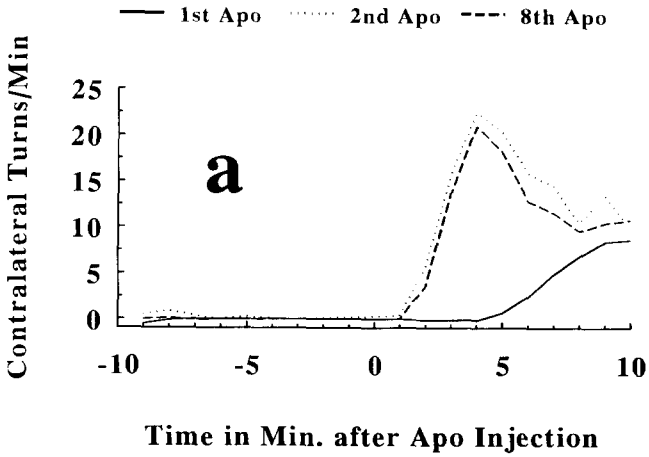
RESULTS

Because we were unable to determine which rats were >90% lesioned prior to phase I or II of the study, we selected rats for data analysis based upon their rotational performance in phase II. Previous work in our laboratory, based on an examination of 64 F344 rats, revealed that an animal rotating greater than 300 times in 1 h, in our rotometers in response to 0.05 mg/kg apomorphine SC, has a >99% probability of possessing a greater than 90% depletion of dopamine in the lesioned striatum (9). Thus, only rats meeting these strict criteria were included in the summary plots that follow. Although the possibility exists that experimental manipulations prior to phase II may have had some effect on the validity of this measure, we believe this potential effect to be minimal. Post hoc selection yielded similar numbers of rats in each of the experimental groups which originally contained 12 prior to subject elimination. The number of animals not rejected in each group after phase II were as follows: apomorphine unrestrained, n = 11; vehicle unrestrained, n = 10; apomorphine restrained, n = 9; vehicle restrained, n = 9; no test, n = 11. In addition, rats not rejected showed very similar means and variances between the experimental groups with regards to their total numbers of turns. Thus, the majority of the lesioned rats had extensive DA depletions.

Apomorphine unrestrained rats were tested in the rotometers freely moving in each of the three phases of the study. The first rotational test in phase I resulted in a response, obviously lacking a first peak, and beginning at 6 min postinjection (Fig. 1). The curve rises smoothly to a plateau and then trails off to baseline within 50 min. Each and all subsequent tests exhibited a marked initial rotational peak beginning at minute 1 and becoming maximal at minute 4, postinjection. This peak was followed by a decline in rotational rate that approached and met the plateau phase of the first rotational test about 8 min postinjection (Figs. 1 and 2). Tests 2 through 5 of phase I and the three tests in phase II were statistically different from the first test of unrestrained rotation for the first 8 min ($p < 0.01$) postapomorphine but not for the remaining minutes. Additionally, the first test revealed a tendency for the rats to rotate ipsilaterally during the preinjection period. In contrast, preinjection rotations in the contralateral direction were noted beginning with the second test and continuing until the sixth test, at which time they ceased.

Apomorphine restrained rats were placed in standard clear Plexiglas rat restrainers and then positioned in the rotometer chambers for all five tests in phase I. There they were given apomorphine subcutaneously and left in the restrainers until at least 60 min had passed. No phase I rotations could occur due to the restraint and, therefore, these trials are not plotted. Phase II tests, the sixth, seventh, and eighth apomorphine injections for this group, were carried out unrestrained in the rotometer chambers (Fig. 3). The first unrestrained test revealed a few preinjection ipsilateral rotations, postinjection contralateral turning beginning at about 5 min, and no first peak. Turns quickly rose to a plateau and subsequently declined, after 30 min, to baseline within 60 min. Apomorphine injections 7 and 8, phase II tests 2 and 3, respectively, show contralateral rotations beginning at about 1 min postinjection and peaking at 4 min. These then declined to a plateau and became indistinguishable from the first apomorphine unrestrained test at about 8 min postinjection. Also, preinjection contralateral turns were very obvious in phase II tests 2 and 3 but not test 1 (Fig. 3a).

Vehicle restrained rats were treated exactly as apomorphine restrained rats above except that they received vehicle rather than apomorphine injections all during phase I. No rotations were possible for these rats during this phase and they are, therefore, not presented graphically. In the apomorphine unrestrained test 1 of phase II, these rats exhibited a few preinjection ipsilateral rotations and no first peak, with contralateral rotations beginning at 6 min postinjection (Fig. 3b). These rose to a plateau and then declined slowly to baseline by 60 min. Tests 2 and 3 of phase II revealed postinjection rotations starting at 1 min and peaking at 4. These then decreased to approximate the plateau of test 1 at 8 min postinjection and continued to be indistinguishable from test 1 subsequently.



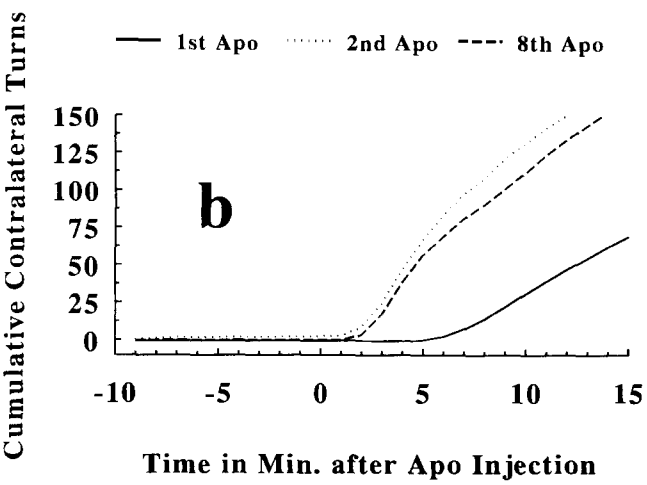
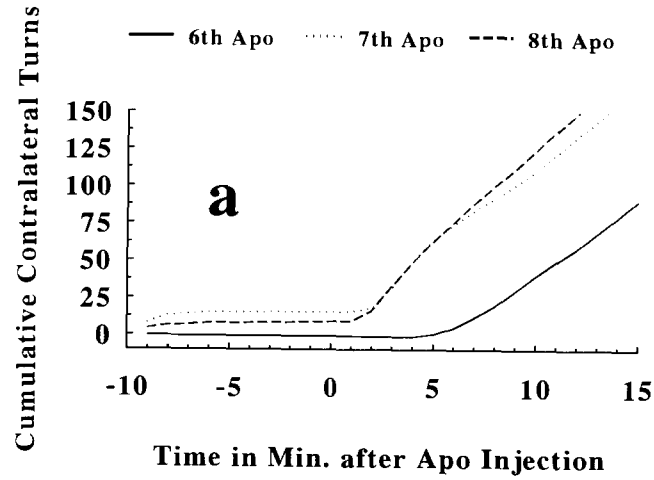


FIG. 2 Panel a is a graph showing an expanded view of the periinjection rotational time points in the apomorphine unrestrained group; the mean contralateral rotations per minute for the first, second, and eighth (last) apomorphine administrations in this group are shown (n = 11). The temporal characteristics of the first peak are more apparent in this magnified view. Panel b shows cumulative mean response plots for the first, second, and eighth apomorphine tests in the apomorphine unrestrained group. The first peak is indicated by the steeper slope in the second and eighth test records.

Tests 2 and 3 of phase II also had significant preinjection contralateral turning when animals were reintroduced to the testing environment.

Vehicle unrestrained rats were placed in the rotometers freely moving throughout all three phases, as shown in Fig. 4a. During phase I, tests 1 through 5, they were given a SC injection of vehicle and left for more than 60 min in the rotometer chambers. Only a slight tendency was noted for the rats to rotate ipsilateral to their lesions. Test 6, the first apomorphine injection and the first test of phase II, showed no initial first peak with rotations beginning at 8 min postinjection and rising rapidly to a plateau that then declined to zero within 60 min. Tests 7 and 8, the second and third apomorphine injections and the second and third tests of phase II, respectively, exhibited a sharp increase in contralateral turning at minute 1 postinjection. This activity peaked at 4 min and became statistically indistinguishable from the first apomorphine test at 8 min postinjection. Additionally, preinjection contralateral rotations were very prominent immediately after introduction into the testing environment.

No test rats remained in their home cages, without manipulation, for the duration of phase I. The first phase II apomorphine test showed contralateral turns beginning at 5 min after the apomorphine injection (Fig. 4b). No first peak was evident. Turns rose to a plateau and then slowly declined to zero at 55 min. Tests 2 and 3 of phase II displayed substantial preinjection contralateral turning immediately after introduction into the testing chamber. Additionally, postinjection rotations began at 1 min and peaked at 4 min. Turning then declined to meet the plateau phase of the first phase II apomorphine test at 9 min after the injection, and approximated the previous test's pattern until its termination.



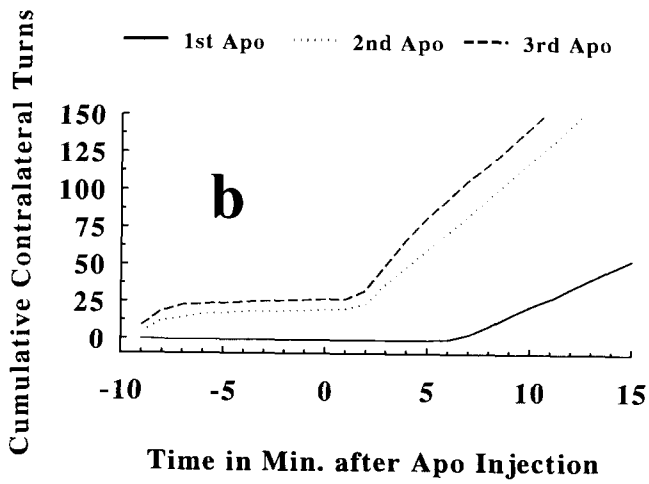


FIG. 3. Panel a shows expanded cumulative mean response curves corresponding to the sixth, seventh, and eighth apomorphine administrations (i.e., first, second, and third unrestrained apomorphine tests in phase II) in the apomorphine restrained group (n = 9). Preinjection rotations and numerous early postinjection turns are noted in the seventh and eighth tests. Panel b indicates expanded cumulative mean response curves for the first, second, and third unrestrained apomorphine trials (phase II) in the vehicle restrained group (n = 9). Shorter latency to turning and steeper curves are present in the second and third trials, as compared to the first trial.

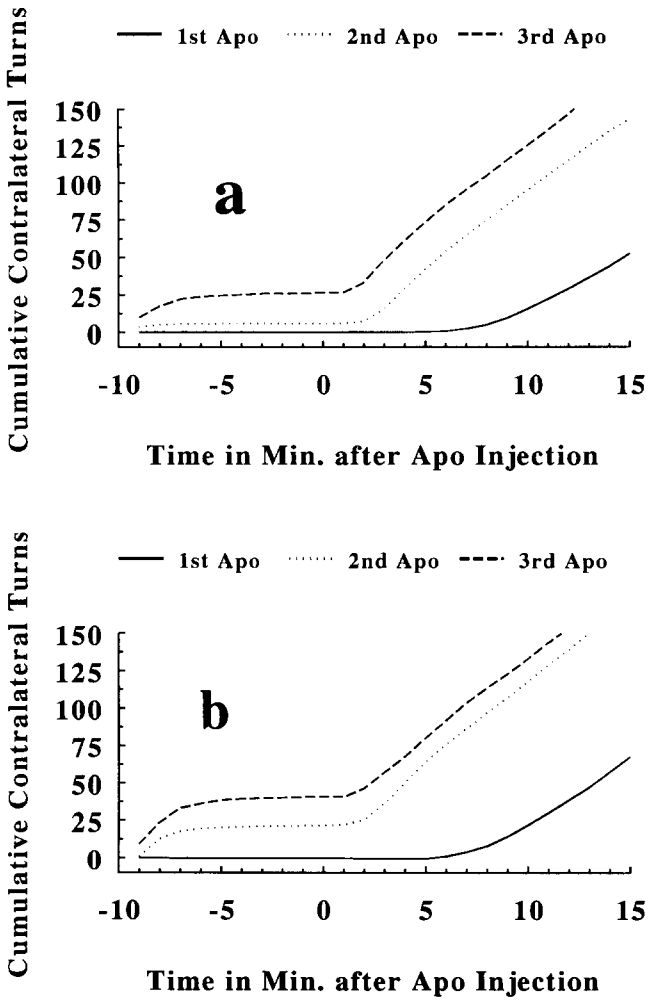


FIG. 4. Panel a indicates expanded cumulative mean response plots for the first, second, and third apomorphine tests (phase II) for the vehicle unrestrained group. The second and third tests show the steep response curve due to the first peak beginning at 2 min. The first peak is evident in the second and third, but not in the first unrestrained apomorphine tests. $n = 10$. Panel b shows expanded cumulative mean response plots for the first, second, and third unrestrained apomorphine tests (phase II) in the no treatment group. Pre- and postinjection slope changes are present in the second and third tests consistent with the presence of the first peak in these tests, but no such changes are seen in the first test ($n = 11$).

Statistical differences (ANOVA) were not seen between the five groups when comparing the first unrestrained apomorphine test in each group, regardless of time after lesions, to each other or when the second or subsequent apomorphine trials were compared to each other between groups. The differences were found to lie between the first and the second or subsequent unrestrained apomorphine tests. Figure 5a shows two cumulative response curves for all rats in all groups and Fig. 5b indicates the same data showing actual means and standard errors per minute, one curve each for the first and second unrestrained apomorphine trials irrespective of time after the lesion. Statistical differences between first and second unrestrained apomorphine tests for all rats ($n = 50$) were: 0–8 min $p < 0.0001$, 9–14 min $p < 0.05$. These data show the rapidity of onset of turning in the second test compared to the first and the steepness of the initial curve which is not present in the first trial, but remains in all subsequent trials (data not shown). It is clear that the second unrestrained apomorphine trial exhibits the early first peak, whereas the first does not; the curves become indistinguishable from each other following the first peak at minute 15.

Phase III results are shown in Fig. 6. All rats were tested in a completely novel environment and were grouped together for analysis. A few contralateral preinjection turns were recorded in the novel environment. The postapomorphine injection contralateral turns began at 2 and peaked at 3 min, although the amplitude of the first peak was less than test 3 in phase II. The plateau phase of the novel environment test was somewhat decreased but the duration of turning was similar to the third test of phase II, about 55 min. The method used to count rotations in phase III was the same as the other phases; however, although the thoracic harness and cable kept the rats from entering corners of the new chamber, they also

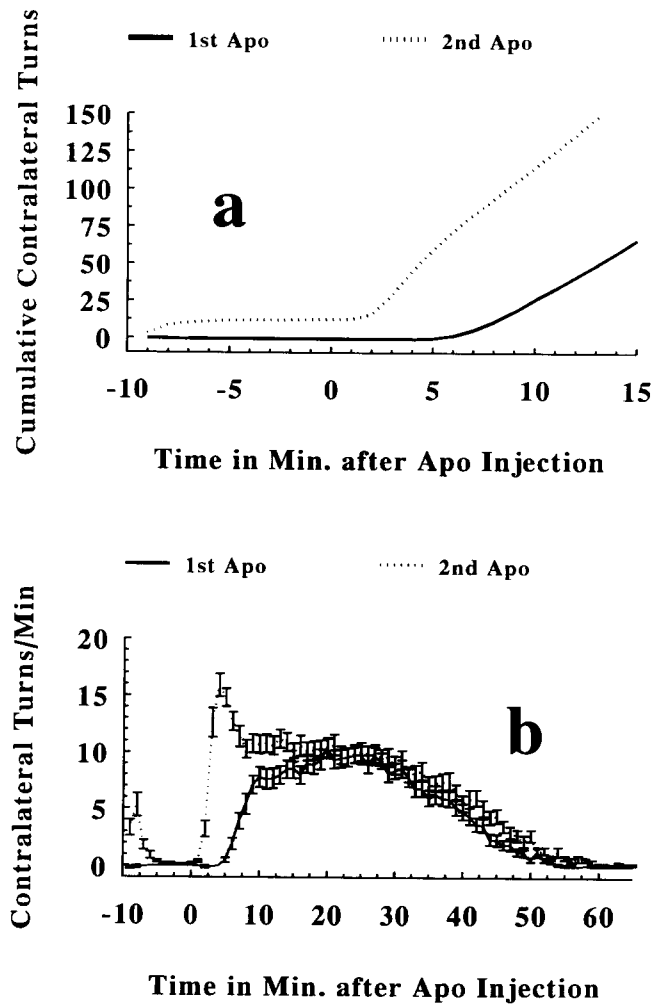


FIG. 5. Panel a represents cumulative mean response curves for the first and second unrestrained apomorphine tests regardless of the time administered relative to the lesion; all five groups were consolidated to generate these curves ($n = 50$). Graph (panel b) showing the means and standard errors, by minute, for the first and second unrestrained apomorphine tests for all groups. The pre- and early postinjection peaks are obvious for the second test and the remainder of the curves overlap.

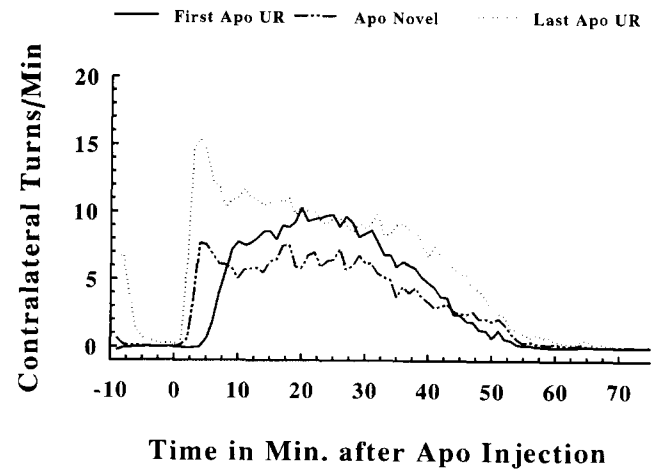


FIG. 6. Summary plots of the means of the first and last unrestrained apomorphine-induced rotational test compared with the rotational response in a novel test environment for all rats in phase III (n = 50). The first rotational peak is evident in the last but not first unrestrained test of phase II. The novel test environment elicited a few preinjection contralateral turns and an attenuated first peak.

somewhat hindered the rats' ability to turn. This makes the interpretation of the data presented in Fig. 6 difficult.

Twenty-eight additional male F344 rats were lesioned and tested on apomorphine and found to be well DA depleted. After their fourth unrestrained apomorphine trial in the rotometers, the fifth trial was carried out with vehicle injections alone (Fig. 7). This resulted in no rotations.

DISCUSSION

We have found that the initial rotational peak that is present in unilaterally 6-OHDA lesioned rats is never observed the first time an animal receives apomorphine freely moving in a testing environment; however, it is always present with every subsequent apomorphine administration. Our results indicate that the peak's emergence depends upon pairing between apomorphine administration and subsequent induced rotational behavior in well-lesioned rats. It is not dependant upon the time after the lesion, the stress of the rotational tests, the stress of the restraint device, or the repeated administration of apomorphine in the absence of rotational behavior. We postulate, therefore, that the first peak is a respondently conditioned rotational behavior that occurs only after a well-lesioned rat has experienced apomorphine-induced turning. Here, apomorphine is both the unconditioned and conditioned stimulus and the conditioned response is rotational behavior that occurs prior to unconditioned response (rotational behavior).

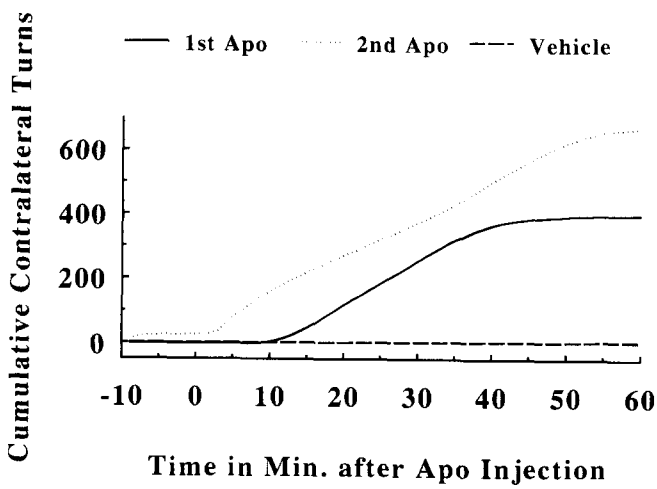


FIG. 7. Graph indicating cumulative response curves for the first and second rotational tests in a group of 28 lesioned F344 rats and their response to a subsequent saline injection in the same environment. The first peak is not present for the first apomorphine rotation but is manifested on the second and subsequent (data not shown) trials. Vehicle administration elicits no rotational response.

In the past, the literature has used a typical two-peaked pattern of rotation to be indicative of an animal that is well-lesioned in terms of unilateral DA depletion in the striatum. It is suggested from the present work that the two-peak pattern is a consequence of the interaction of apomorphine with supersensitive DA-receptors on the lesioned striatum, and the superimposed respondently conditioned rotations occurring early in this behavioral test.

The curves shown here do not appear obviously two peaked. The reason for this is that we included all rats that were greater than 90% striatally DA depleted. Previous studies have indicated that rats rotating more than 300 times in 1 h, after 0.05 mg/kg apomorphine, had a greater than 99% chance of having such a lesion; therefore, all rats exceeding these criteria were included in this study (9). Other reports have used only rats with total rotations higher than 500 turns per hour and the two-peaked pattern. The second peak becomes more obvious if the rats turn at a higher total number. The second peak is the primary source of larger rotational totals due to its duration; thus, with larger totals, the second peak rises out of the plateau to a much greater extent than is shown in Figs. 1 and 5b. Due to this, the coexistence of rotations >500 per hour and the two-peaked pattern of rotation occurs.

Previously, apomorphine has been noted to elicit an environmentally paired turning response in unilaterally 6-OHDA treated rats (13). Although observed to be temporally related to introduction into the rotational environment and to drug injection (2), the parameters of this behavior have not previously been described in a temporal or mechanistic manner. The restraint paradigm allowed us to separate the pharmacological aspects of apomorphine-induced turning from the conditioning aspects.

The early postinjection rotations found in the second and subsequent unrestrained apomorphine tests are best characterized as Pavlovian or respondently conditioned circling. In classical terms, with respect to the first unrestrained apomorphine-induced rotations, the unconditioned stimulus is the injection of apomorphine. The measured unconditioned response is the turning elicited by this drug beginning at 8 min postinjection. The conditioned stimulus, albeit incidental, is probably multifactorial and may include: visual cues related to the rotometers and/or the investigator, olfactory stimuli, and tactile stimuli (especially the SC injection of apomorphine), amongst others. This conjecture is supported by the fact that simply placing the previously apomorphine-rotated rats into the rotometer bowls elicits spontaneous contralateral turning, a behavior that a lesioned animal does not manifest in the absence of pharmacological interventions. Also, the injection of apomorphine probably plays a significant stimu-

lus role in that it is closely temporally paired with the conditioned peak and also because an injection of vehicle will not elicit rotations.

Further evidence of the respondently conditioned status of the preinjection turning lies in the fact that these will extinguish over the course of five unrestrained apomorphine trials. As noted previously in our laboratory, the preinjection turns will spontaneously cease after about five repeated apomorphine tests. These turns are not reinforced, in the classical conditioning sense, by the presence of apomorphine and so extinguish over time. The early postinjection rotations, however, remain with repetitive tests.

The appearance of the first rotational peak is not associated with time after lesioning, stress, or apomorphine administration in the absence of turning, as is evidenced by comparison of all experimental groups. Because of this result, it seemed reasonable to treat each group similarly after phase II. Thus, in phase III, the novel environment elicited a turning response intermediate between the first unrestrained apomorphine trial and the subsequent ones. Preinjection rotations were few but contralateral. This is contrary to the ipsiversive turning seen prior to the first unrestrained apomorphine test in the rotometer bowls. The postinjection circling began at 2 min and peaked, although at a lower amplitude than the normal rotometer environment, at 4 min. The plateau phase was also somewhat attenuated compared to the plateau in the rotometer bowls but the time course was very similar. Because the novel environment mildly impeded the rotations of the animals, as noted above, it is premature to draw conclusions regarding generalizability of the influence of the rotation environment. However, it is clear that the rats rotated early and with a distinct peak postinjection, and this suggests that the respondently conditioned first peak is not easily separable by the environmental changes made. It also indicates the primary role of the apomorphine injection. Other manipulations are needed to delineate the cues used to elicit the first-peak in unilaterally lesioned rats.

An intriguing aspect to Pavlovian conditioning in hemi-Parkinsonian rats deserves mention. We have shown that the conditioned contralateral turning is only evident when experienced by the rat through sensory pathways involving proprioceptive, tactile, auditory, visual, and vestibular mechanisms. This is indirect evidence that the basal ganglia are not conditioned by this behavioral test and that their effect on motor systems may require concurrent corroborative sensory input for conditioning to occur. The potential, therefore, may exist to exploit this in a clinical setting. Parkinsonian patients are often unable to initiate movements similar to hemi-parkinsonian rats being unable to spontaneously rotate contralateral to their lesions, prior to apomorphine. Perhaps if increased DA input to the striatum or DA agonist treatment could be paired with increased repetitive appropriate sensory input in Parkinsonian patients, one could increase a patient's ability to override motoric deficits.

In summary, we have shown that the early peak in rotations after apomorphine administration in unilaterally DA-lesioned rats is a Pavlovian conditioned response; we suggest that these rotations should be treated as such in analyses regarding the extent of DA denervation and/or transplant-induced reinnervation. This will help remove some experimental bias in that a secondary manifestation of the lesion will be identified in the testing paradigm, thus rendering it more accurate as a reflection of drug or transplantation interventions in this model of Parkinson's disease.

ACKNOWLEDGEMENTS

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