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BRIEF COMMUNICATION

Exposure to Amphetamine After Substantia Nigra Lesion Interferes With the Process of Behavioral Recovery

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MINTZ, M. AND R. TOMER. *Exposure to amphetamine after substantia nigra lesion interferes with the process of behavioral recovery.* PHARMACOL BIOCHEM BEHAV **25**(6) 1307–1311, 1986.—Recovery from unilateral substantia nigra lesion may be indicated by re-emergence of circling in the pre-lesion preferred direction. Following 6-OHDA-induced lesion of the dominant SN, we examined: (a) The effect of the delay from lesioning on amphetamine-induced rotation asymmetry, and (b) The effect of early post-lesion exposure to amphetamine on later rotation asymmetry. d-Amphetamine was initially injected either 7, 14, 21, or 30 days after lesioning. Transient circling in pre-lesion preferred direction (contralateral to lesioned side) was more frequently encountered on days 7 and 30 after lesioning, as compared to days 14 and 21. The contralateral rotation observed on day 7 is attributed to degeneration-induced DA release, whereas contralateral rotation noted on day 30 is believed to reflect the operation of post-lesion compensatory processes within the spared DA neurons. In response to subsequent amphetamine administration 30 days after lesioning, rats with previous exposure to the drug circled ipsilaterally, whereas most rats given amphetamine for the first time in that session rotated contralaterally to the lesion. These findings suggest that post-lesion administration of amphetamine interferes with the process of recovery.

Amphetamine 6-OHDA lesion Substantia nigra Recovery Rotation Dopamine Depletion

UNILATERAL lesions of the nigrostriatal (NS) pathway usually lead to ipsilateral amphetamine-induced rotation [7,15]. This behavior reflects the post-lesion dopaminergic (DA) dominance of the non-lesioned hemisphere. Yet, notwithstanding the significant lesion-induced DA depletion, transient periods of rotation directed contralaterally to the lesion were observed shortly after lesioning [15], or even weeks later [11,12]. An extreme example of such contralateral circling was recently reported by Robinson and Becker [14] who found that unilateral DA depletion as high as 95% was followed, in some rats, by contralateral, rather than ipsilateral rotation, 5 weeks after lesioning. This contralateral rotation may reflect the operation of compensatory processes within the lesioned system, including presynaptic increase of DA turnover in the spared cells [1,8] and development of supersensitivity of post-synaptic DA receptors [16]. If indeed contralateral rotation may be regarded as an index of behavioral recovery, it is surprising that findings of such contralateral rotation were reported in only a few studies. One possible explanation is related to the DA-depleting properties of amphetamine. Thus, in most studies, animals are repeatedly challenged with amphetamine during the immediate period after the lesion. In contrast, Robinson and Becker [14], who reported contralateral circling, refrained from doing so for five weeks after lesioning.

In the present study, we characterize the contralateral rotation as a function of the time interval between unilateral 6-OHDA-induced lesion of the substantia nigra pars compacta (SNc), and rotation assessment. We also examine the hypothesis that the effect of the lesion and the effect of early exposure to amphetamine after lesioning, on subsequent circling asymmetry, are additive.

METHOD

Subjects were 44 male Wistar rats, weighing 280–410 g at the beginning of the experiment. Animals were housed individually, under a reversed 24 hr light/dark cycle. Food and water were available ad lib throughout the experiment.

Amphetamine-induced circling behavior was measured in a rotometer following IP injection of 1.0 mg/kg d-amphetamine sulfate (Sigma). Rats were harnessed to a photocell based device and the number of full left and right turns was recorded during four consecutive 15 min periods, i.e., for a total of 60 min. This baseline session served to determine the dominant hemisphere (the hemisphere contralateral to the preferred direction of circling), for each rat. Twenty-four to forty-eight hours later the SNc of the dominant hemisphere was lesioned in all animals. They were

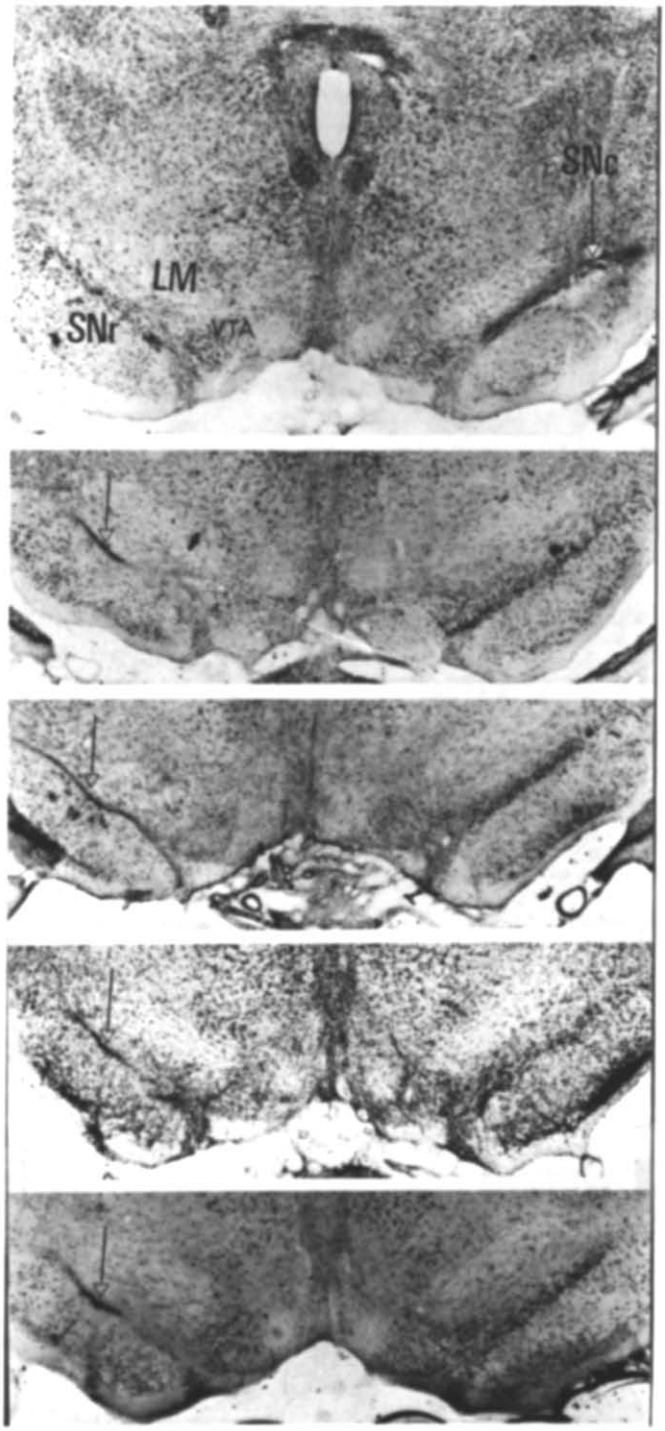

FIG. 1. Representative thionin stained coronal brain sections of rats sacrificed 33 days after a unilateral 6-OHDA (10 μg in 5 μl of solution) lesion of the substantia nigra pars compacta (SNc). Section thickness: 50–70 μm. Arrows indicate the degenerated layer of SNc. Note the similar extent of lesion in all samples. LM—medial lemniscus; SNr—substantia nigra pars reticulata; VTA—ventral tegmental area.

anaesthetized with chloral hydrate, and a relatively high dose of 6-OHDA (10 μg in 5 μl of cold saline with ascorbic acid, Sigma) was injected into the SNc during a 5-min period (coordinates: A 3.4; L 2.1; V 7.8; [13]). The cannula was left in position for another 5 min before withdrawal.

After lesioning, rats were divided into 4 independent groups, which differed only with respect to the timing of subsequent tests of amphetamine-induced rotation. Rotation was thus assessed on days 7, 8 and 30 after lesioning (group d-7); on days 14, 15 and 30 after lesioning (group d-14); on day 21 (in 3 animals, on day 17) (group d-21); and in the last group, on days 30 and 31 after lesioning (group d-30). In the latter group only, rotation following saline injection was measured on days 7 and 8 after lesioning, to equate the number of sessions preceding the testing on day 30, in all groups.

The changes in rotation asymmetry were assessed with respect to two variables: (a) The effect of the time-interval between lesioning and the first injection of amphetamine was determined by comparing the rotation of these 4 groups on their first post-lesion amphetamine session (days 7, 14, 21 and 30, respectively), and (b) The effect of early post-lesion exposure to amphetamine on circling asymmetry at a later session was evaluated by comparing amphetamine-induced rotation on day 30, in three of the above four groups, previously exposed to amphetamine (groups d-7 and d-14) or saline (group d-30).

A net rotation score was calculated by subtracting the number of rotations toward the lesioned side, from the number of rotations directed contralaterally to the lesion. Positive and negative values therefore represent rotation contralateral and ipsilateral to the lesion, respectively. The net score was obtained for the entire 60-min session of rotation, as well as for each of the four successive 15-min periods comprising that session.

After completion of the behavioral assessment, rats were perfused with saline, followed by 10% formalin, and their brains removed. Fifty to seventy μm sections were cut on a freezing microtome and stained with thionin.

RESULTS

Histology

The effect of 6-OHDA injection was assessed in 4 to 11 coronal sections per animal. The final sample was composed of rats in which the examination of the sections under a microscope confirmed the existence of an extensive lesion of the SNc and relative sparing of the ventral tegmental area (VTA). Thus, in sections anterior to the VTA, only single cells could be seen along the medial border of the SNc. In contrast, all of the more posterior sections showed relative sparing of VTA cells medial to the SNc, together with an extensive lesion of the SNc cells. Microscopic examination revealed that both the anterio-posterior and the medio-lateral location, as well as the extent of the lesions, were very similar in all the animals. Figure 1 demonstrates several typical sections.

The Effect of the Time Interval Between Lesioning and First Post-Lesion Amphetamine Injection

Figure 2a summarizes the changes of net rotation from baseline to the first post-lesion amphetamine session in 4 independent groups, differing with regard to the time interval

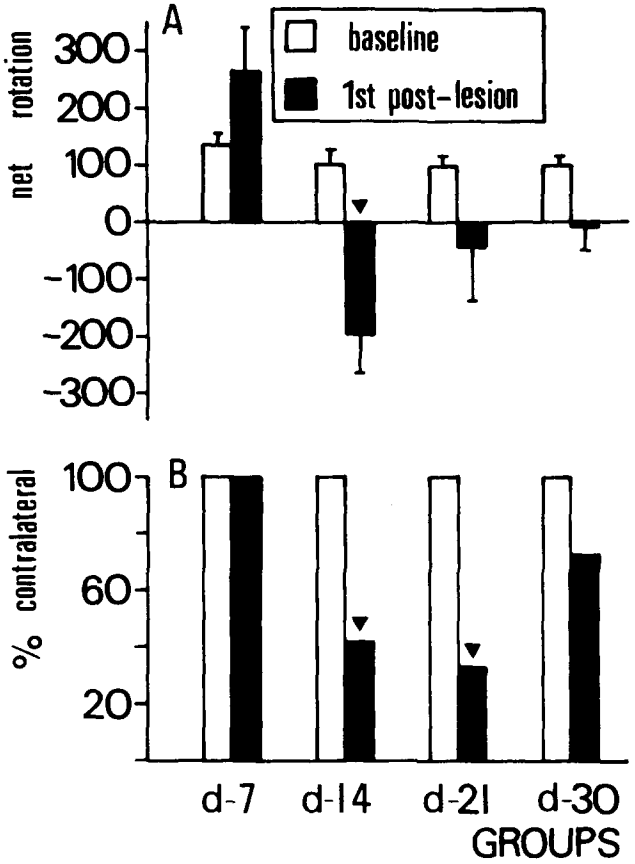

FIG. 2. The effect of time-interval from lesioning on rotation asymmetry was examined in 4 independent groups, to which amphetamine was first injected on days 7 (n=11), 14 (n=12), 21 (n=6) or 30 (n=15) after the lesion. Dominant SN (contralateral to preferred direction of circling during baseline session), was lesioned by injecting 6-OHDA. Rotation was assessed in response to 1 mg/kg d-amphetamine sulfate. A: Net rotation scores for 60 min, starting from amphetamine injection (mean+SEM) during baseline (open bars) and first post-lesion session (black bars). Two-way ANOVA revealed significant Group-by-Session interaction, $F(3,40)=4.21$, $p<0.02$. Black triangle indicates significant ($p<0.001$) change of net rotation score, compared to baseline. B: Percent of rats circling contralaterally to lesion (positive net scores) in the first 15-min following amphetamine injection during baseline (open bars) and the first post-lesion session. Triangles indicate significantly smaller proportion of rats rotating contralaterally to lesion on days 14 and 21 than on day 7 (test for the significance of the difference between proportions, $p<0.001$).

between lesioning and testing. The significant group-by-session interaction (two-way ANOVA, $F(3,40)=4.21$, $p<0.02$) indicates that the change in net rotation from pre- to post-lesion session was differentially affected by the various time-intervals between lesioning and testing. Thus, 7 days after dominant SN lesion, rats continued to rotate contralaterally to the lesion, whereas a longer interval (14 days) was associated with change to ipsilateral rotation. Post-hoc comparisons using the Scheffe test revealed that this reversal of asymmetry from baseline to the session on day 14 after lesioning was significant ($p<0.001$). Still longer intervals between lesioning and amphetamine administration, on the other hand, resulted in small asymmetry (21 days) or practically symmetrical mean net rotation (30 days), and the net scores did not differ significantly from baseline scores.

In order to further clarify these results, we looked at individual data and calculated the proportion of animals showing contralateral circling preference, in each group. Eighty-six percent of the rats tested after a 7-day interval circled contralaterally to the lesion, as compared with only 33% of the animals showing contralateral preference in any of the three other groups. These results, reflecting the entire 60-min session, indicate that the latter three groups do not differ from each other with respect to their rotation preference. However, a closer examination of the data suggested that the behavior of the animals is not uniform throughout the session, and these groups may differ from each other with respect to circling preference during part of the session only. Indeed, analysis of the rotation asymmetry in each of the 4 consecutive periods (15 min each) comprising the entire session, reveals some difference between these groups during the first 15-min period. Thus, net scores calculated for this initial period show that 29 rats of the entire sample (66%) began the first post-lesion session with contralateral rotation, and 12 of these changed into ipsilateral rotation towards the end of the same session. Figure 2b presents the percent of animals beginning the baseline and first post-lesion sessions by contralateral circling, in each group. It shows that contralateral rotation characterized all the rats tested for the first time 7 days after the lesion. Longer intervals (14 and 21 days) were associated with lower proportion of animals demonstrating contralateral circling (each of the latter was significantly different from the first group, $p<0.001$, test for the significance of difference between proportions). In contrast, after an even longer interval (30 days), a high proportion of rats beginning with contralateral circling is again revealed.

The Effect of Early Post-Lesion Amphetamine Injection on Rotation Recorded 30 Days After the Lesion

The rotation of 38 animals was tested on day 30 post-lesioning. Figure 3a compares this session's net rotation scores to baseline, in rats previously exposed to amphetamine either on days 7, 8 (d-7) or days 14, 15 (d-14), as well as in animals injected only with saline on days 7 and 8 (d-30). Two-way ANOVA revealed a significant group-by-session interaction, $F(2,38)=8.54$, $p<0.005$, indicating that the change in net rotation, from baseline to day 30 was differentially affected by previous exposure to amphetamine. The two groups injected with amphetamine on earlier post-lesion sessions demonstrated intense ipsilateral rotation on day 30; this was significantly different from their behavior on the baseline session (post-hoc analysis, $p<0.001$, for either group). In contrast, the group injected with amphetamine for the first time on day 30 showed symmetrical net rotation which did not differ significantly from its baseline behavior.

The above-mentioned difference between the groups was most noticeable at the beginning of the session, 30 days after lesioning. Figure 3b presents the percent of animals circling contralaterally in the first 15-min period of the baseline and day 30 sessions. As may be seen, the group which was spared early administration of amphetamine has the highest proportion of animals rotating contralaterally to the lesion. In each of the two groups that were subjected to amphetamine prior to day 30, significantly less animals began by rotating contralaterally (test for the difference between proportions, $p<0.01$). It therefore appears that both indices

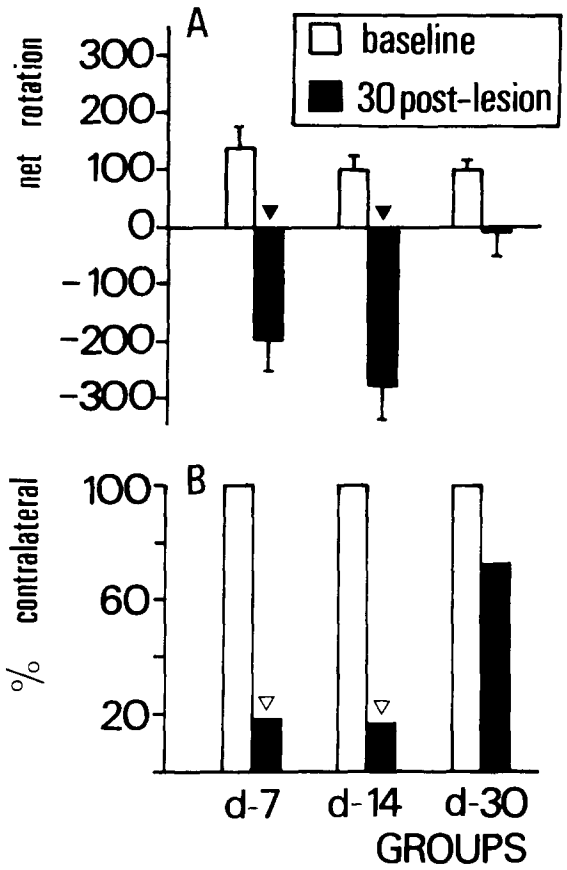

FIG. 3. The effect of early post-lesion amphetamine injection on rotation recorded 30 days after the lesion. Three independent groups of rats were previously exposed to amphetamine on days 7 and 8 (d-7, n=11) or days 14 and 15 (d-14, n=12) after dominant SN lesion, or were exposed only to saline on days 7 and 8 after the lesion (d-30, n=15). For details see Fig. 2. A: Net rotation scores for 60 min, beginning immediately after amphetamine injection (mean+SEM) during baseline (open bars) and on day 30 post-lesion (black bars). Two-way ANOVA shows significant Group-by-Session interaction, $F(2,38)=8.54$, $p<0.005$. Triangles indicate significant ($p<0.001$) changes from baseline values. B: Percent of rats circling contralaterally to lesion in the first 15 min following amphetamine injection, during baseline (open bars) and on day 30 (black bars). Triangles indicate significantly smaller proportion of rats circling contralaterally in groups previously exposed to amphetamine, compared to rats injected previously only with saline (test for the significance of difference between proportions, $p<0.01$).

(group mean net score, and the proportion of rats showing transient contralateral preference) reflect the fact that injecting amphetamine a short time after lesioning is associated with ipsilateral rotation 30 days after the lesion. It should be noted, however, that in group d-30, 6 (of 11) rats showing contralateral rotation at the beginning of the first post-lesion session, changed into ipsilateral circling within that same session.

DISCUSSION

Behavioral recovery following unilateral SN lesion may be reflected by return of rotational asymmetry to pre-lesion side preference [14]. In the present study, the lesion was limited to the dominant hemisphere, as determined by baseline assessment of side preference. Thus, failure of the lesion to change the baseline asymmetry, i.e., occurrence of post-lesion contralateral rotation, was interpreted as indicating the operation of compensatory processes. We have described the time course of behavioral recovery, and demonstrated the susceptibility of this recovery process to post-lesion amphetamine injections.

High rates of contralateral rotation were evident for two time intervals: soon after the lesion (day 7), and, again, when rats were first tested 30 days after the lesion. Intermediate intervals resulted in the expected ipsilateral rotation. The contralateral rotation on day 7 cannot be considered to reflect behavioral recovery, since its occurrence (also reported by [11, 12, 15]), most likely reflects the degeneration-induced DA release. Although such release has been reported to be complete within 60 hr after the lesion [15], it should be noted that Ungerstedt's rats underwent repeated injections of amphetamine, a procedure which may accelerate the process of DA depletion. The degeneration-induced depletion may, therefore, last longer in untreated animals. Faull and Laverty [4] report a 50% depletion of DA, 3 days after SN lesion. Their Fig. 2 (p. 335) clearly shows that the depletion process did not reach an asymptote within the first week after the lesion, and that 10 days after the lesion the DA content in the denervated striatum was still approximately 20% of the value measured on the other side. Also, the findings of Anden *et al.* [2] show that even a complete hemisection of the forebrain results in sparing of 8–9% of DA concentration, 7 days after the lesion (our estimate, based on Fig. 1 of Anden *et al.* [2]). Eventually, when the degeneration process is completed, direction of rotation is expected to be ipsilateral to the lesion. This was indeed seen in the present study, 14 days after the lesion. Yet, long delays from lesioning were also associated with increased DA turnover in the spared NS fibers [1, 8, 12]. We suggest that this compensatory process may underly the contralateral rotation observed in a large proportion of rats 30 days after the lesion. Kozlowsky and Marshall [10] also consider increased turnover and release by the remaining DA terminals among cellular processes which may contribute to the compensation for the partial loss of DA cells. Such compensatory processes may be sufficient to overcome even substantial lesions, as indicated by the fact that even a 95% depletion may result in contralateral rotation 5 weeks after the lesion [14].

Repeated measures of amphetamine-induced behavior is an accepted method of assessing post-lesion rate of recovery [3]. However, the present results point out a serious flaw in this methodology. Thus, following injection of amphetamine on days 7–8 or 14–15 after the lesion, rats demonstrated intense ipsilateral rotation on day 30. In contrast, animals injected with saline on days 7–8 have shown substantial recovery, i.e., circling in pre-lesion preferred direction on day 30. Mintz *et al.* [11] suggested that amphetamine had an irreversible depleting effect on the post-lesion DA pool. The present findings confirm the above suggestion, showing that early post-lesion amphetamine injections interfere with the recovery process. Furthermore, the present results indicate that this deleterious effect of amphetamine occurs even after relatively long time intervals between lesioning and amphetamine administration. Thus, 54% of the rats that began by rotating contralaterally (in group d-30), changed to ipsilateral circling within that same session.

Contrary to our results, Feeney and colleagues [5,9]

showed a beneficial effect of amphetamine on lesion-induced behavioral deficits. However, their lesions were confined to the cortex, and the authors relate the facilitated recovery to amphetamine's capacity to antagonise the "catecholamine diaschisis," i.e., to activate the functionally depressed catecholaminergic structures remote from the site of the lesion [9]. In contrast, in our study the DA pathway was lesioned directly, and we relate our results to the ability of amphetamine to deplete the presumably spared cells within the lesioned structure. Depletion of striatal DA was reported after a single amphetamine dose in nonlesioned rats pretreated with iprindole, which prolongs the half-life of amphetamine [6]. The possibility of significant reduction of DA concentration within the lesioned pathway following even a single injection of amphetamine was demonstrated by Oberlander *et al.* [12]. In view of such susceptibility of the DA pool to amphetamine after a direct lesion of the DA system, it is suggested that better monitoring of recovery may be achieved by examination of spontaneous behaviors, rather than those requiring the administration of DA-releasing agents.

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