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BRIEF COMMUNICATION

Mesolimbic Dopamine and Early Post-6-OHDA Lesion Enhanced Responses to d-Amphetamine

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LYNCH, M. R. AND R. J. CAREY. *Mesolimbic dopamine and early post-6-OHDA lesion enhanced responses to d-amphetamine.* PHARMACOL BIOCHEM BEHAV 32(2) 577–580, 1989.—Bilateral lesions of mesolimbic dopamine (DA) reliably produce an attenuated response to amphetamine's locomotor stimulatory effects when administered after two weeks of surgical recovery. Several studies have revealed enhanced amphetamine-induced hyperactivity during the first postlesion week, however. In the present study, animals with bilateral 6-OHDA lesions of nucleus accumbens and olfactory tubercle DA showed an exaggerated response to 1.0 mg/kg amphetamine during this early period but were hypoactive in the absence of drug treatment. Neurochemical assay at 5 days revealed increased DA metabolism in the tubercle. Shifting patterns of postlesion amphetamine response under conditions of reduced mesolimbic DA are suggestive of dynamic adaptations in nondopaminergic systems.

6-Hydroxydopamine Amphetamine Hyperactivity Nucleus accumbens Olfactory tubercle

EXTENSIVE experimental evidence points to the mesolimbic dopamine (DA) system as the neuroanatomical substrate for d-amphetamine's locomotor stimulatory effects. For example, DA infusion into the nucleus accumbens of the rat induces locomotor stimulation (6, 11, 18). Conversely, moderate to severe bilateral depletions of nucleus accumbens DA with 6-hydroxydopamine (6-OHDA) attenuate the locomotor hyperactivity produced by doses of 0.5–2.5 mg/kg amphetamine. In these lesion investigations, behavioral testing is typically conducted at 10 or more days postsurgery, with DA levels assayed at two to three months (3, 4, 10, 12, 14). If these animals are tested earlier after the neurotoxin infusion (e.g., on day three), however, an attenuation of amphetamine's locomotor stimulatory effects is not observed. That is, control-like levels of behavior are observed after amphetamine treatment at this time, even though the usual attenuated response is noted in the same animals two weeks later (12,13). Lesion animals may show an even greater response to amphetamine during the first week postsurgery (5,12). Early (1 to 36 hr) postlesion increases in neostriatal DA content [transmitter accumulation in degenerating nigrostriatal terminals (1)] have been linked to exaggerated amphetamine responses in the unilateral 6-OHDA model (19). It is possible that increased DA concentration in mesolimbic regions might also be responsible for the early postlesion enhanced amphetamine response after bilateral 6-OHDA infusion into the accumbens area, although the time course of neurochemical adaptations following neurotoxin treatment has not been characterized for this system. Thus, the following study was conducted to examine the role of dopaminergic dynamics in this early postlesion exaggerated response.

The first experiment was conducted to identify the period of enhanced response to the locomotor stimulatory effects of 1.0 mg/kg d-amphetamine. Sixteen male, Sprague-Dawley rats (Taconic Farms, Germantown, NY) served as subjects. The animals weighed 455–580 g at the time of surgery. Under deep Equithesin anesthesia (0.2 cc/100 g body weight), 10 animals were infused with freshly prepared 6-OHDA HBr (Sigma, St. Louis, MO, dissolved in 0.9% NaCl with 0.2% ascorbic acid) aimed at the nucleus accumbens and olfactory tubercle (AP +2.5 to +3.0, L ±2.5 and DV −8.0 mm from dura with the incisor bar set to +3.2 mm). A total of 2 μl 6-OHDA (3 μg/μl as the salt) was injected at 0.5 μl/min and the cannula was left in place for 5 min. Six vehicle rats were infused with the ascorbic acid. All animals were pretreated

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TABLE 1

MEAN μg/g DOPAMINE AND TURNOVER RATIOS

	Olfactory Tubercle		Nucleus Accumbens		Striatum	
	Veh	Les	Veh	Les	Veh	Les
Experiment One						
DA	8.01 ± 0.39	2.32 ± 0.35‡	6.29 ± 0.72	2.36 ± 0.54‡	13.25 ± 0.82	11.56 ± 0.69
Experiment Two						
DA	6.57 ± 0.72	2.81 ± 0.17‡	6.96 ± 0.66	3.82 ± 0.55†	10.10 ± 0.50	9.10 ± 0.55
HVA	0.46 ± 0.04	0.26 ± 0.02‡	0.51 ± 0.04	0.34 ± 0.05*	0.61 ± 0.08	0.50 ± 0.03
DOPAC	0.78 ± 0.10	0.42 ± 0.03†	0.96 ± 0.08	0.62 ± 0.09*	0.70 ± 0.06	0.61 ± 0.06
HVA/DA	0.07 ± 0.01	0.09 ± 0.01*	0.08 ± 0.01	0.10 ± 0.01	0.05 ± 0.01	0.06 ± 0.003
DOPAC/DA	0.12 ± 0.01	0.15 ± 0.01*	0.14 ± 0.01	0.17 ± 0.01	0.07 ± 0.01	0.07 ± 0.002

*$p<0.05$, †$p<0.01$ and ‡$p<0.001$ compared to vehicle (independent t-test).

with 25 mg/kg IP DMI (1 ml/kg) to protect norepinephrine. Spontaneous locomotor activity was observed on days 1, 3 and 5 postsurgery. Crossing responses in an open field were recorded as previously described (16) by an observer blind to surgical and drug conditions. (Animals had been divided into matched lesion and vehicle-infused control groups on the basis of scores from the last of three presurgery observation sessions.) Each rat was also tested with 1.0 mg/kg IP d-amphetamine at 30-min postinjection on day 4 (n=5 lesion and 4 vehicle animals) or day 6 (n=5 and 2, respectively). Assignment to the amphetamine test day was randomized. [This dose of amphetamine reliably stimulates open field locomotor behavor in nonlesion rats (3,4).] At approximately six weeks postlesion, animals were decapitated under light ether anesthesia (loss of righting reflex) and sacrificed to determine the extent of DA depletion. Dopamine concentrations were determined from bilateral accumbens nuclei, olfactory tuberculi and striata, dissected as previously described (15). Samples were analyzed with high performance liquid chromatography coupled to electrochemical detection according to the procedure of Mayer and Shoupe (17) using a single glassy carbon working electrode and a LC-3 Bioanalytical Systems, Inc. detector. Concentrations were calculated as μg/g wet tissue by ratio to the internal standard dihydroxybenzylamine (8).

A second study was conducted to assess DA metabolism at a postlesion time point corresponding to the period of enhanced amphetamine response. Two additional groups of animals (matched on prelesion crossing scores) underwent surgical procedures as described above (n=9 lesion and 6 vehicle rats). Testing was begun on day 2, after the initial 24 hr period of nonspecific postsurgical behavioral suppression (observed in experiment one). Nondrug tests were conducted on days 2, 3 and 5. On day 4, animals were tested with 1.0 mg/kg d-amphetamine at 30 min postdrug. Immediately after the test for spontaneous activity on day 5 they were sacrificed and samples analyzed for DA and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) as described above.

In experiment one, 6-OHDA treatment produced significant, moderate DA depletions in both accumbens and the tubercle, while the striatum remained intact (Table 1). Analysis of spontaneous activity data (panel A, Fig. 1) revealed a significant suppression of crossing responses in vehicle animals on day 1, while the lesion group showed a significant reduction over all three days [repeated measures ANOVA: $F(3,42)=6.29$, $p<0.01$, for the interaction]. As responses to d-amphetamine were very similar for lesion animals tested on days 4 and 6 (means of 121.8 and 99.2, respectively) and for the two vehicle-infused groups (means of 52.5 on day 4 versus 47.5 for day 6), day 4 and 6 groups were combined. When amphetamine scores were converted to mean difference scores by subtracting the previous (nondrug) day's score (panel B, Fig. 1), it was determined that the lesion group showed a significantly enhanced locomotor response to drug, $t(14)=3.60$, $p<0.01$.

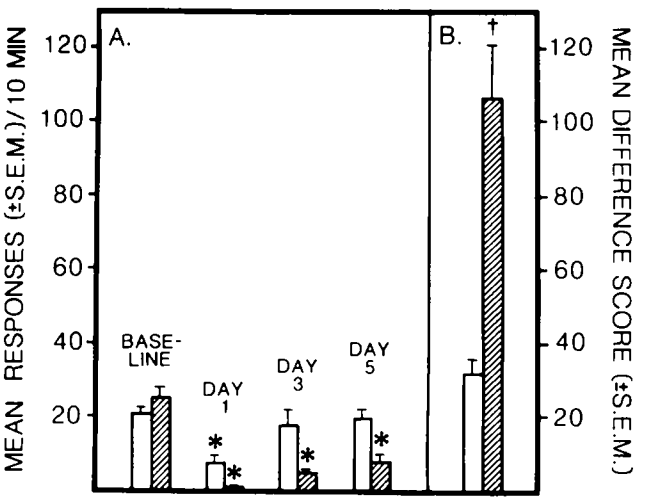

FIG. 1. Spontaneous locomotor activity (panel A) and response to d-amphetamine (Panel B) in Experiment One. Vehicle-infused group denoted with open bars and lesion with striped bars. *$p<0.001$ and †$p<0.01$ (Dunnett's post hoc tests in panel A and independent t-tests, panel B).

Spontaneous activity scores in Experiment Two also revealed significant ($p<0.01$, Dunnetts post hoc test) reductions from the last presurgery baseline for the lesion group on all three days of nondrug testing [repeated measures

ANOVA: $F(3,39)=5.35$, $p<0.01$, for the interaction]. Lesion animals also showed an exaggerated response to d-amphetamine treatment as mean difference scores were significantly greater than the vehicle group [means of 44.3 ± 6.3 versus 21.0 ± 3.8 for vehicle, $t(13)=2.78$, $p<0.05$]. Analysis of DA and metabolite levels from animals in this second study (Table 1) revealed significant, moderate DA depletions in the accumbens nuclei and tubercle, while striatal concentrations did not differ significantly from control. Both DOPAC/DA and HVA/DA ratios were found to be slightly, but significantly, elevated for lesion animals in the olfactory tubercle only.

Findings of the present investigation replicate the results from earlier studies reporting an enhanced amphetamine response in animals with bilateral mesolimbic DA loss during the first week postlesion. As early postlesion exaggerated responses have been demonstrated with photocell activity measures, the present findings extend these observations to open field activity. The later (after the first postsurgical week) attenuation of amphetamine locomotor activation, however, has been reliably demonstrated with both measures. Also, rats in the present experiments sustained a moderate DA depletion in both tubercle and accumbens regions, similar to that which has been reported for animals showing the later attenuated amphetamine response (i.e., ranging from 4 to 49% vehicle control levels). Therefore, it appears that these exaggerated responses represent the early portion in a postlesion continuum of changing amphetamine-induced locomotor stimulation.

Given that the early enhanced response to amphetamine is of a transitory nature, it may be linked to nonspecific 6-OHDA effects on efferent projections from the mesolimbic DA region. In fact, it has been proposed that accumbens efferents exert inhibitory modulation on an extra-accumbens locomotor-facilitory region (2,7). Disinhibition induced by nonspecific lesions of this mesolimbic DA region is manifest in spontaneous behavioral hyperactivity (2,20). By contrast, lesioned animals exhibiting hyperresponsivity to amphetamine in the present study were spontaneously hypoactive when tested without drug, arguing against transient nonspecific disinhibitory effects.

Concomitant measures of spontaneous (nondrug) behavioral hypoactivity and diminished mesolimbic DA function are compatible with the hypothesis that DA in this region facilites behavioral output. The observed reduction in mesolimbic DA concentration is not compatible, however, with an enhancement of behavioral response to amphetamine. On the other hand, the increased DA turnover suggests that amphetamine may be facilitating compensatory transmitter release from remaining (intact) terminals. Previous investigations have characterized neurochemical adapations following partial DA lesions in the nigrostriatal system, and found that surviving terminals show an increased synthesis and release following the lesion (9,21). While compensatory activity may account for the early amphetamine phenomena, however, the finding of spontaneous hypoactivity is problematic for interpreting the neurobehavioral significance of metabolic enhancement. Further, data from neostriatal systems indicates that compensatory activity is not a transient postlesion adaptation [at least in this system (21)], whereas the hyperreactivity to amphetamine's locomotor stimulatory effects appears characteristic of the early postlesion period (5,13).

Further studies aimed at identifying neurochemical adaptations after neurotoxin infusion into the mesolimbic DA region are necessary to characterize the mechanism for this transient increase in d-amphetamine response. Findings of a concomitant DA reduction cautions that the relationship between amphetamine-induced locomotor activity and the integrity of mesolimbic DA systems may not be as straightforward as previously presumed. That is, following bilateral 6-OHDA treatment to the mesolimbic DA terminal regions, severe and moderate DA loss has been observed in association with: 1) early enhancement of the amphetamine response, 2) attenuation at two to nine weeks postlesion (12–14), and 3) recovery to nonlesion rates of locomotor stimulation thereafter (4,13). The changing behavioral profile which has been observed suggests that there is a dynamic postlesion reorganization in the neurochemical substrate for this psychostimulant effect. Thus, alterations in nondopaminergic systems may be responsible for d-amphetamine's behavioral stimulatory effects under conditions of DA perturbation.

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