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# Norepinephrine Stimulates Behavioral Activation in Rats Following Depletion of Nucleus Accumbens Dopamine

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SWERDLOW, N. R. AND G. F. KOOB. *Norepinephrine stimulates behavioral activation in rats following depletion of nucleus accumbens dopamine.* PHARMACOL BIOCHEM BEHAV **33**(3) 595–599, 1989. —Intraventricular (ICV) infusion of norepinephrine (NE) produces locomotor activation in rats that is greatly potentiated by prior depletion of whole brain catecholamines by ICV injection of 6-hydroxydopamine (6OHDA). In a series of experiments, the neural substrates of this potentiated locomotor response were examined. One group of animals received ICV infusion of 6OHDA to deplete whole brain catecholamines. Other rats were pretreated with desmethylimipramine (DMI) and then received 6OHDA infusions into the nucleus accumbens (NAC) to selectively deplete dopamine (DA) from this region. One week later, all animals were tested for their locomotor response to ICV infusion of NE. Both groups of rats exhibited a greatly potentiated locomotor response to ICV NE compared to corresponding sham-lesioned animals. Both ICV and NAC 6OHDA-injected animals also exhibited a supersensitive locomotor response to the DA receptor agonist apomorphine. These results suggest that NE-induced locomotor activation in ICV 6OHDA-treated rats results from the actions of NE on supersensitive NAC DA receptors.

Apomorphine Dopamine Locomotion Mesolimbic Norepinephrine Nucleus accumbens

A wealth of evidence now supports the notion that brain catecholamine-containing neural systems play a major role in mediating states of behavioral activation in the rat. Intraventricular application of either NE or DA increases locomotor activity in rats (11), while destruction of whole brain catecholamine systems with intraventricular 6OHDA causes marked impairment of activated behaviors (6). Drugs that block brain receptors for NE or DA diminish behavioral activation in the rat (2), and the effects of many psychostimulants, including amphetamine (19), cocaine, methylphenidate (15) and apomorphine (1) are prevented by pretreatments with DA receptor antagonists.

More refined experimental approaches have revealed that some brain catecholamine systems, in particular the mesolimbic dopamine system, appear to play a crucial role in some aspects of behavioral activation in the rat. Direct application of exogenous DA into DA terminal fields within the NAC stimulates locomotor activation (32). Selective destruction of brain DA within the NAC disrupts nocturnal locomotor patterns in familiar cages and exploratory behaviors within novel environments (16), and blocks amphetamine (14), cocaine- and methylphenidate-stimulated locomotion (15).

A less clear role in mediating behavioral activation is played by brain NE-containing systems. While NE appears to be a crucial substrate for spinal motor reflexes (5), its involvement in mediating behaviors of supratentorial origin has not been consistently demonstrated. Early studies implicated brain NE systems in the substrates underlying intracranial self-stimulation (ICSS) behaviors in rats, but findings from more sophisticated pharmacological (28) and lesion studies (17) have demonstrated a principal role for DA, and not NE, in mediating ICSS behaviors. Selective destruction of brain NE systems does not impair spontaneous locomotor activity (18) and does not disrupt the locomotor-activating properties of several psychostimulants (18) or neuropeptides (30).

One finding positively implicating brain NE systems as a crucial substrate underlying locomotor activation in the rat was reported by Segal *et al.* (26). In this report, animals previously treated with intraventricular 6OHDA demonstrated a "supersensitive" locomotor response to intraventricular (ICV) infusion of NE. The authors argued that this "supersensitive" response to NE resulted from the action of exogenous NE on brain NE receptors made "supersensitive" by prior denervation with 6OHDA. There have been many refinements in the techniques for 6OHDA-induced destruction of catecholamine terminals within the CNS, and it is now possible to selectively denervate small populations of catecholamine receptors. In the current set of experiments, this critical observation of a "supersensitive" locomotor response to NE in ICV 6OHDA-treated rats was replicated. The locomotor response to ICV infusion of NE was then studied in rats following selective 6OHDA-induced destruction of DA-containing terminals within the NAC. These findings suggest that the "supersensitive"

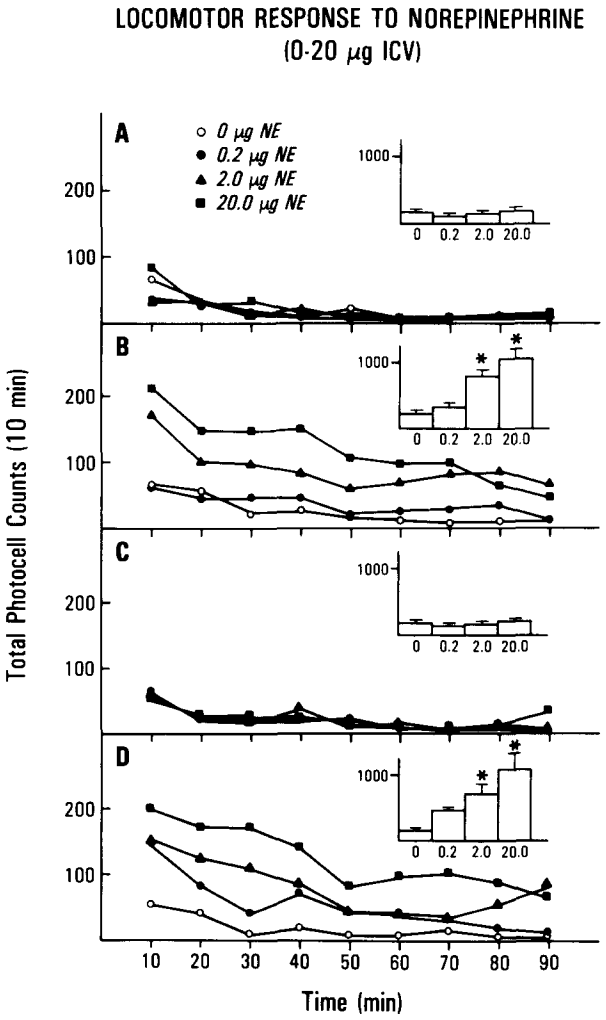


FIG. 1. Locomotor response to norepinephrine (0–20 μg ICV) in rats. Seven to thirteen days prior to testing, animals had received intraventricular infusion of vehicle (A) or 6OHDA (B), or intranucleus accumbens infusion of vehicle (C) or 6OHDA (D). Insert indicates total photocell counts over 90-minute test. *Indicates significant ($p<0.05$) difference from 0 μg dose by Newman-Keuls analysis following significant ANOVA.

locomotor response to ICV infusion of NE may result from the action of this exogenously applied transmitter on "supersensitive" DA receptors within the NAC.

## METHOD

Thirty-two 225–250 g albino Wistar rats (Charles River Laboratories) were handled individually for three minutes on the day of shipment arrival, housed in groups of three, maintained on a normal light-dark cycle and given food (Purina Rat Chow) and water ad lib. One week later, sixteen animals received ICV infusion of either 6OHDA (250 μg/25 μl, expressed as free base) dissolved in saline containing ascorbic acid (0.1 mg/ml) (n=9, 6OHDA ICV group) or of saline-ascorbic acid vehicle alone (n=7, VEH ICV group). Animals were anesthetized with pentobarbital (50 mg/kg IP) and secured in a Kopf stereotaxic instrument with the toothbar 5 mm above the interaural line. Intraventricular injection was made through a 30-gauge cannula at coordinates AP −0.6 (bregma), L 2.0, DV −4.2 (skull) at a rate of 2 μl/3 min. Cannulae remained in place for 1 min following infusion. A 7-mm 23-gauge stainless steel guide tube was then aimed one mm above the lateral ventricle at coordinates AP −0.6 (bregma), L 2.0, DV −3.2 (skull) and secured to the skull with two stainless steel screws and Silux dental cement. A 7-mm wire stylet filled the cannula.

TABLE 1

DOPAMINE AND NOREPINEPHRINE LEVELS (ng/mg PROTEIN, ±SEM) IN NUCLEUS ACCUMBENS, ANTERIOR STRIATUM AND HIPPOCAMPUS FOLLOWING INJECTION OF VEHICLE OR 6OHDA INTO THE LATERAL VENTRICLE OR NUCLEUS ACCUMBENS

| | Nucleus Accumbens | | Anterior Striatum | | Hippocampus | |
|---|---|---|---|---|---|---|
| | NE | DA | NE | DA | NE | DA |
| VEH ICV | 5.01 | 97.38 | 2.26 | 120.19 | 10.43 | 3.80 |
| (n=5) | (0.63) | (27.54) | (0.33) | (10.64) | (0.70) | (0.26) |
| 6OHDA ICV | 1.96* | 52.38 | 0.97* | 32.24* | 5.57* | 3.57 |
| (n=5) | (0.29) | (8.24) | (0.32) | (8.69) | (1.67) | (0.86) |
| VEH NAC | 4.16 | 93.00 | 2.38 | 116.01 | 9.60 | 4.25 |
| (n=5) | (0.52) | (8.71) | (0.78) | (11.69) | (1.67) | (0.48) |
| 6OHDA NAC | 3.77 | 6.34* | 2.24 | 32.06* | 9.29 | 3.76 |
| (n=5) | (0.67) | (4.05) | (0.37) | (6.28) | (1.05) | (0.67) |

*$p<0.05$, $t$-test comparison of 6OHDA vs. VEH animals.

A second group of sixteen animals received intra-NAC infusion of either 6OHDA (8 μg/2 μl, expressed as free base) dissolved in saline-ascorbic acid vehicle (n=9, 6OHDA NAC group) or saline-ascorbic acid vehicle alone (n=7, VEH NAC group). Each animal was injected with desmethylimipramine (DMI) (25 mg/kg IP, dissolved in distilled water). Thirty min later, animals were anesthetized and secured in a Kopf stereotaxic instrument as above. Bilateral injections of 6OHDA or vehicle were then made through 30-gauge cannulae at coordinates AP +3.2 (bregma), L ±1.7, DV −7.8 (skull) at a rate of 1 μl/3 min. Cannulae remained in place for 1 min following infusion. A 7-mm 23-gauge stainless steel guide tube was then aimed and attached one mm above the lateral ventricle as above.

Beginning one week after surgery, the locomotor responses of these animals were measured on four days, each separated by one nontest day, using sixteen wire cages. Each cage measured 20×25×36 cm with twin infrared photocell beams across the long axis 2 cm above the cage floor. Prior to the first test day, all animals were habituated to the photocell cages for 180 min. On each test day, animals were again habituated to the photocell cages for 90 min. They then received ICV infusion of one of four doses of norepinephrine HCl (NE) (0, 0.2, 2.0 or 20.0 μg in saline-ascorbic acid vehicle), in a randomized design used to control for potential order effects of repeated infusions. Infusion was accomplished by replacing the stylet wire with an 8-mm stainless steel 30-gauge injector attached to a 1-m length of PE 10 tubing filled with infusate. The tubing was then raised above the animal's head until flow began and 2 μl were infused over a 30–60-sec period. Following infusion, the stylet wire was replaced and the animal was placed immediately into the photocell cage, where activity was recorded for 90 min. Individual group comparisons of photocell counts were made using a two-way analysis of variance (ANOVA) with repeated measures on dose and time. Individual dose effects were analyzed using a Newman-Keuls comparison following significant main effects of dose by ANOVA. The level of significance was $p<0.05$.

Three days following the final NE infusion, twenty animals

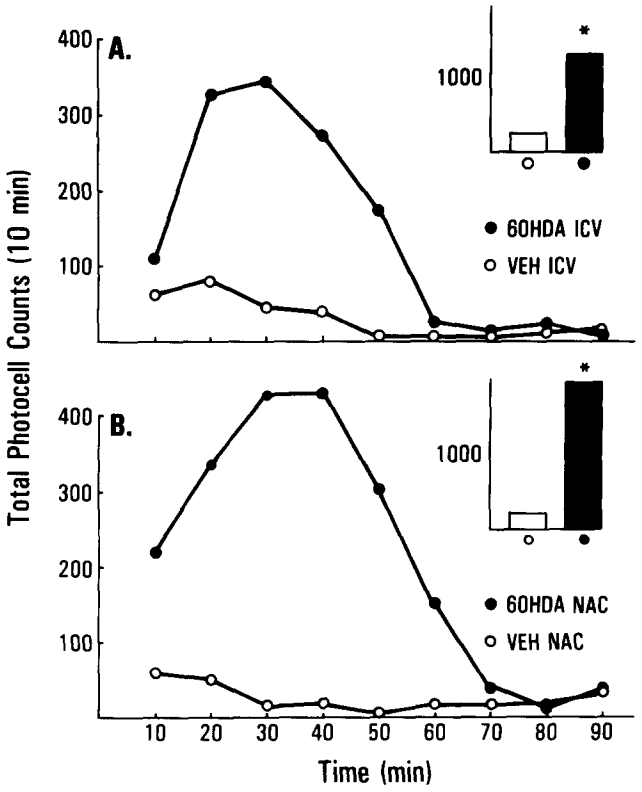


FIG. 2. Locomotor response to apomorphine (0.1 mg/kg SC) in rats following depletion of brain catecholamines caused by intraventricular infusion of 6OHDA (A) or intranucleus accumbens infusion of 6OHDA (B). Insert indicates total photocell counts over a 90-minute test period. *Indicates significant ($p<0.05$) main effect of lesion (6OHDA vs. vehicle) by ANOVA.

(n = 5, each group) were returned to the photocell cages for 90 min. They were then treated with apomorphine HCl (0.1 mg/kg in saline-ascorbic acid vehicle) given SC in a volume of 1 ml/kg; this dose of apomorphine is known to produce a robust locomotor activation in NAC 6OHDA-lesioned animals (33). These animals were then returned to the photocell cages, and locomotor activity was recorded for 90 min. Group comparisons were made using a two-way ANOVA with repeated measures on time, with significance level $p<0.05$. Following completion of behavioral testing, twenty animals (n = 5, each group) were decapitated and their brains were removed. Brain regions, including the NAC, anterior striatum, and hippocampus were removed from coronal slices and free-hand dissection as described previously (16) and stored at −80°C until assayed for DA and NE using electrochemical detection following separation by high-pressure liquid chromatography (HPLC) (8).

## RESULTS

Results of regional brain biochemical analysis are seen in Table 1. Analysis of 6OHDA NAC animals revealed significant depletion of DA in the NAC, $t(8)=9.02$, $p<0.05$, and anterior striatum, $t(8)=6.63$, $p<0.05$, but not in the hippocampus, $t(8)=0.75$, NS, compared to VEH NAC animals. There was no significant depletion of NE noted in any regions in 6OHDA NAC animals ($t<1$, all groups) compared to VEH NAC animals. Analysis of 6OHDA ICV animals revealed significant depletion of DA in the anterior striatum, $t(8)=6.4$, $p<0.05$, and moderate (46%) depletion of DA in the NAC, $t(8)=1.55$, NS. 6OHDA ICV animals did not register depletion of DA in the hippocampus compared to VEH ICV animals, $t(8)=0.26$, NS. Analysis of 6OHDA ICV animals also revealed a significant depletion of NE in the NAC, $t(8)=3.06$, $p<0.05$, anterior striatum, $t(8)=2.90$, $p<0.05$, and hippocampus, $t(8)=3.31$, $p<0.05$, compared to VEH ICV animals.

Locomotor activity exhibited a dose-dependent increase following ICV NE infusion in both ICV and NAC group animals (Fig. 1). This was reflected by a significant main effect of lesion [ICV: $F(1,15)=14.75$, $p<0.05$; NAC: $F(1,15)=37.44$, $p<0.05$], a main effect of norepinephrine dose [ICV: $F(3,560)=9.65$, $p<0.05$; NAC: $F(3,560)=16.86$, $p<0.05$] and a significant lesion × dose interaction [ICV: $F(3,560)=7.67$, $p<0.05$; NAC: $F(3,560)=15.15$, $p<0.05$]. A subsequent individual means comparison using a Newman-Keuls analysis revealed that this effect of NE was significant ($p<0.05$) for the 2.0 and 20.0 μg doses in both 6OHDA ICV and 6OHDA NAC group animals. Locomotor responses to ICV NE infusion in these two groups of animals were similar in amplitude, time course and dose-response properties (Fig. 1). In contrast, no significant effects of ICV NE infusion on locomotor activity were observed in either VEH ICV or VEH NAC group animals ($p>0.05$, all comparisons).

Both 6OHDA ICV and 6OHDA NAC group animals also exhibited a greatly potentiated locomotor response to apomorphine compared to their VEH group controls (Fig. 2). This was reflected by a significant group effect in ICV-infused animals [6OHDA ICV vs. VEH ICV: $F(1,9)=5.46$, $p<0.05$] and in NAC-infused animals [6OHDA NAC vs. VEH NAC: $F(1,9)=6.64$, $p<0.05$].

## DISCUSSION

Several studies have reported that NE-stimulated locomotor activity in rats is greatly enhanced by depletion of brain catecholamines with 6OHDA (10, 23, 26). In the current experiment, ICV infusion of NE resulted in a dose-dependent locomotor activation after depletion of whole brain catecholamines and after selective depletion of DA within the NAC and anterior striatum. In fact, NE-stimulated locomotion in NAC DA-depleted rats was indistinguishable in amplitude, time course and dose-response properties from NE-stimulated locomotion in whole-brain catecholamine-depleted rat. Both groups of animals also demonstrated a "supersensitive" locomotor response to the selective DA receptor agonist apomorphine. These results suggest that the locomotor-activating properties of NE in both NAC DA-depleted rats and whole brain catecholamine-depleted rats can be attributed to the action of exogenous NE on supersensitive DA receptors within the NAC and anterior striatum. The results have several important implications.

First, the finding that NE-stimulated locomotion was significantly enhanced in both groups of 6OHDA-treated animals confirms previous reports that denervation-induced changes in brain catecholamine systems interact with exogenously-applied NE to stimulate locomotion activation in the rat. However, these findings do not support the hypothesis that brain NE systems are normally an independent neural substrate for locomotor activity in the rat. While intraventricular injection of 6OHDA resulted in a significant loss of NE from the three brain regions assayed, the locomotor response to exogenous NE in these animals did not differ significantly from the locomotor response observed in animals that had sustained very selective depletion of ventral forebrain DA. Thus, the locomotor-stimulating properties of exogenous NE in catecholamine-depleted rats cannot be attributed simply to an effect of NE on supersensitive brain NE receptors.

Second, it is significant that a supersensitive behavioral response to NE in rats was observed after selective DA depletion from the NAC and anterior striatum. Previous studies demonstrated that infusion of 6OHDA into the NAC results in a significant increase in the number of NAC D2 receptors (27), and that stimulation of these receptors using the DA receptor agonist apomorphine results in a supersensitive locomotor activation (33). The present findings indicate that in a state of ventral forebrain DA receptor supersensitivity, NE can serve as a substrate for supersensitive behavioral changes.

In humans, schizophrenia is characterized by a significant excess of forebrain D2 dopamine receptors, identified by in vivo PET scan studies (34), post-mortem receptor-binding assays (4, 9, 21, 22) and in vivo neuroendocrine studies (36). Tardive dyskinesia (TD) in schizophrenics is also characterized by increased numbers of forebrain D2 dopamine receptors (4) as well as biochemical evidence of forebrain dopamine hyperactivity (9). Consequently, the behavioral abnormalities associated with schizophrenia and TD have been attributed to excessive forebrain DA activity (4,9). Several independent studies have also reported elevated levels of NE in the cerebrospinal fluid of schizophrenics (29) and of patients with tardive dyskinesia (13). Elevated levels of NE are also reported in the limbic forebrain of schizophrenic patients (7). Furthermore, symptoms of both psychosis (20,29) and TD (9,35) are exacerbated during states of stress or sympathetic arousal that are accompanied by increased brain NE activation. The current results suggest that elevated brain NE might contribute to behavioral pathology through the direct action of NE on forebrain DA receptors in states of DA receptor supersensitivity.

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