Short communication

Depletion of brain norepinephrine does not reduce spontaneous ambulatory activity of rats in the home cage

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Abstract

6-Hydroxydopamine (6-OHDA) lesions of brain noradrenergic neurons and terminals were made in rats to assess the importance of forebrain norepinephrine (NE) for mediating circadian patterns of spontaneous ambulatory activity that rats show in the home cage. 6-OHDA was injected intracranially into the fibers of the ascending noradrenergic dorsal and ventral bundle pathways or infused into the lateral ventricle or both. Rats living in a 12/12 h light/dark cycle exhibit a marked increase in ambulatory activity during the dark period in comparison to the light period and a ‘W-shaped’ pattern of activity during the 12 h of the dark phase. Results showed that near-total depletion of brain NE did not impair the capacity to generate normal patterns of spontaneous ambulatory activity that occur in the home cage. In the animals that sustained the most complete NE lesions, the amounts of activity generated at times of peak activity were exaggerated in comparison to the control animals, which is consistent with the possibility that NE in the brain exerts a moderating influence on behavior. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Neural basis of behavior

Topic: Monoamines and behavior

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Altered activity of noradrenergic neurons in the brain has long been thought to be critically involved in the pathophysiology of depression. The 'catecholamine hypothesis of depression,' first put forth over 30 years ago, proposed that depression arose from a deficiency of norepinephrine (NE) in the brain [4,22,23]. Although shortcomings of this formulation became evident soon after it was proposed, evidence nevertheless has continued to accumulate indicating that brain NE is important in depression (reviewed in Grant and Weiss [12]). The present study examined the effect of reducing brain NE on spontaneous motor activity of rats. Because altered motor activity is one of the most common changes seen in depression, the relationship between brain NE and motor activity has been of considerable interest since the catecholamine hypothesis of depression was proposed.

Despite the close connection between brain NE and motor activity suggested by the catecholamine hypothesis of depression, basic research has by no means provided unambiguous evidence showing that alteration of brain NE will affect motor activity. Experimental manipulation of brain NE, most commonly by making lesions of brain noradrenergic neurons, often has failed to produce a change in motor activity of animals [7,19,27]. A recent study using a genetic manipulation to investigate the role of NE (i.e., elimination of the gene for dopamine beta-hydroxylase) also reported no significant effect on spontaneous motor activity of mice unable to synthesize NE [28]. On the other hand, other studies have reported that NE depletion leads to a decrease in motor activity in a novel environment, suggesting a role for brain NE with respect to motor activity under these conditions [3,16,21]. In addi-
tion, early studies found that catecholamine-depleting drugs could reduce behavioral responses that are highly dependent on motor activity in rats (e.g., [17,18]). And, finally, a strong correlation between regional brain NE depletion and decreased motor activity has been shown in the context of a widely-studied animal model of depression [24,25,29,30].

In the present study, we investigated the influence of brain NE on diurnal changes in spontaneous ambulatory motor activity of rats. Freely-behaving rats, living in a 12 h on/12 h off light/dark cycle, exhibit a pattern of spontaneous motor activity such that the animals’ ambulatory activity is markedly elevated during the dark period of the day in contrast to the light period when animals are much less active. Moreover, animals show a characteristic pattern of activity during the ‘active’ dark phase. This pattern shows an upward spike in ambulatory activity at the beginning of the dark phase, after which activity then declines, only to increase somewhat around the middle of the dark period, after which it declines again, followed by marked increase leading up to the end of the dark period (i.e., a ‘W-shaped’ pattern). In this study, we examined whether destruction of NE neurons and terminals in the brain would affect these patterns of spontaneous ambulatory activity.

To alter brain NE, lesions of the ascending noradrenergic pathways — the dorsal noradrenergic bundle (DNB) and ventral noradrenergic bundle (VNB) — were made using the neurotoxin 6-hydroxydopamine (6-OHDA). Subjects were male Sprague–Dawley rats weighing approximately 400 g at time of the study. Sixteen animals were assigned to one of three lesion groups (i.e., the axonal, ventricular, or compound lesion group) or the control group (n=4 per group). Animals were anesthetized with 1–3% halothane mixed with oxygen and placed into a stereotaxic instrument where surgery was performed. The ‘axonal’ lesion group received bilateral microinjections of 6-OHDA hydrochloride (Sigma) aimed at fibers of the DNB and VNB just anterior to the locus coeruleus (LC). Axons arising from NE-containing cells of the LC comprise the DNB, which provides over 70% of the NE in the rat brain including all the NE in the neocortex and hippocampus [9,13,15]. The VNB arises from noradrenergic neurons caudal to the LC and terminates in various regions of the diencephalon, providing particularly rich innervation to the hypothalamus [5,11]. After an incision was made in the skin covering the skull and the skin retracted, two small holes were drilled into the skull to introduce a 26-gauge cannula bilaterally into the brain. Stereotaxic coordinates were used for placement of the cannulae. For axonal lesions, the coordinates for cannula location were as follows: with nose piece –10.0; anterior/posterior (AP) –2.6 mm from lambda; medial/lateral (ML)±1.2 mm from midline; dorsal/ventral (DV) –7.0 mm and –8.0 mm from dura matter. 6-OHDA was freshly dissolved in artificial cerebrospinal fluid (aCSF) and delivered through the cannula into each side of the brain. A cannula was first lowered into one side of the brain to –8.0 mm, targeting axons of the VNB and 1 μl of 6-OHDA (4 μg/μl) was injected over a period of half a minute. After a wait of 1 min, the cannula was raised to –7.0 mm, targeting axons of the DNB and a second 1 μl injection of 6-OHDA was performed, followed by a wait of 1 min and then the cannula was removed. Thirty minutes after completion of the injection on one side of the brain, the procedure was repeated on the other side of the brain (the 30-min delay between injections was imposed to promote animal survival). The ‘ventricular’ lesion group received a microinfusion of 6-OHDA into the lateral ventricle. For this group, the surgical procedure was the same as described above except that a single hole was drilled in the skull for insertion of a single 26-gauge cannula. Stereotaxic coordinates used for this condition were as follows: with nose piece level with skull (−3.3°), AP –1.0 mm from bregma; ML±1.5 mm; DV –3.5 mm from skull (variable). After the cannula was lowered at this location, placement in the ventricle was confirmed by the observation of cerebrospinal fluid rising in a small length of silastic tubing attached to the cannula. 6-OHDA introduced into the ventricle normally would be taken up into dopamine (DA)-containing cells and terminals as well as NE-containing neurons and would also destroy DA cells and terminals. To prevent lesioning of DA terminals and cells, the DA uptake blocker GBR 12909 (Sigma) was infused through the ventricular cannula just prior to infusion of the 6-OHDA. GBR 12909 was dissolved in warm (37°C) aCSF (1 μg/μl) and 6 μl was infused at a rate of 2 μl/min. Following a wait of 15 min to allow time for the drug to bind to DA transporters, 4 μl of 6-OHDA (40 μg/μl) was then infused at a rate of 2 μl/min to complete the ventricular infusion procedure. The ‘compound’ lesion group underwent both the axonal lesion procedure and the ventricular lesion procedure. For this group, first the axonal lesion was carried out and then, after a recovery period of 2 weeks, the ventricular lesion procedure was conducted. All doses and surgical procedures used for the compound lesion group were the same as described above for the axonal and ventricular lesion groups. For the control group, these animals were anesthetized with halothane for the amount of time that surgery normally required but no surgery was performed (control animals were subjected to minimal manipulation so that their subsequent ambulatory behavior would not be affected by any experimental procedure and thus would constitute normal activity for a rat).

Four weeks after surgery was initiated, animals were placed individually into standard polycarbonate laboratory cages (45×25×20 cm). Each of these cages was mounted within a photocell assembly (ANA 1219, Riverpoint Electronics) that permitted the continuous monitoring of the activity of the animal in the cage. The photocell assemblies were connected to a computer which recorded
and tabulated the number of interruptions of the photocells by each animal. Animals were housed directly on bedding and received ad libitum food and water. Five days was allowed for acclimation to individual housing, followed by 7 days of monitoring the animals’ nocturnal spontaneous motor activity. On each day, the recording apparatus was activated 2 h before onset of the dark phase and recording ended 5 h after lights on. Ambulatory motor activity was expressed as ambulation counts per hour, an ambulation count being defined as interruption of a photocell beam that had not been broken in the previous four beam breaks and thus represented horizontal locomotion by the animal.

At the conclusion of the study, the animals were sacrificed and brains removed. The following brain regions were dissected and retained for analysis: LC region of brain stem (LC), hypothalamus (HYP), hippocampus (HIPP), prefrontal cortex (PFC) and striatum/nucleus accumbens (STR). Brain samples were frozen at −85°C for analysis of catecholamine content using high pressure liquid chromatography (HPLC). Tissue samples were analyzed for NE content to assess the extent of the lesion; striatal tissue was principally analyzed for DA to assess whether DA-containing terminals had been protected from destruction by infusion of GBR 12909 prior to icv infusion of 6-OHDA. Tissues were homogenized in 0.1 M perchloric acid containing an internal standard. Catecholamines were separated using reverse-phase, ion-pair HPLC and were quantified by an electrochemical detector (ESA Coulochem II) attached to a computerized data acquisition system (Perkin Elmer Turbochrom 4). Protein content of each sample was measured by the Lowry technique so that catecholamine content could be expressed as pg NE (or DA) per mg tissue protein.

As expected, all lesion groups (axonal, ventricular, compound) showed marked depletion of NE in brain regions known to be major targets of the ascending noradrenergic pathways, i.e., cortex and hippocampus for the DNB and hypothalamus for the VNB. The results are shown in Table 1. 6-OHDA administered icv was found to be somewhat more effective for depleting NE in the terminal regions of the DNB than was 6-OHDA aimed at the axon bundles, although a difference between these techniques was not observed in the hypothalamus. Hypothalamic NE proved somewhat more resistant to neurotoxic depletion than did forebrain NE, consistent with previous reports [20]. The compound lesion group showed the largest reduction of NE, indicating that the ventricular and axonal lesion techniques had cumulative effects. In all animals of the compound lesion group, NE was undetectable in the hippocampus and cortical NE was almost equally reduced. The compound lesion technique also was able to deplete hypothalamic NE to an average of 10% of control, with two animals of the group found to have less than 10% of the control level of NE remaining. DA levels were not reduced by either the axonal or ventricular lesion techniques as estimated by DA content in striatum/nucleus accumbens, while the compound lesion technique produced a small reduction in DA (i.e., 30%) that is not considered to be functionally effective [6,14].

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>HIPP</th>
<th>PFC</th>
<th>HYP</th>
<th>LC</th>
<th>STR</th>
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<tr>
<td></td>
<td>pg/mg</td>
<td>% of</td>
<td>pg/mg</td>
<td>% of</td>
<td>pg/mg</td>
</tr>
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<td></td>
<td>protein</td>
<td>control</td>
<td>protein</td>
<td>control</td>
<td>protein</td>
</tr>
<tr>
<td>Control</td>
<td>1483±117</td>
<td>100%</td>
<td>1916±118</td>
<td>100%</td>
<td>6627±498</td>
</tr>
<tr>
<td>Ventricle</td>
<td>15±15</td>
<td>100%</td>
<td>203±89</td>
<td>100%</td>
<td>2230±220</td>
</tr>
<tr>
<td>Axonal</td>
<td>428±157</td>
<td>100%</td>
<td>432±177</td>
<td>100%</td>
<td>1808±442</td>
</tr>
<tr>
<td>Compound</td>
<td>0±0</td>
<td>100%</td>
<td>12±7</td>
<td>100%</td>
<td>692±165</td>
</tr>
</tbody>
</table>

The data are given as group means±S.E.M. The control group was non-lesioned. The ventricular group received 6-OHDA injected into the lateral ventricle; the axonal group received 6-OHDA injected into the fibers of the dorsal and ventral bundle ascending noradrenergic pathways; the compound group received both treatments. Sensitivity of the HPLC electrochemical detection was 2.0–3.0 pg/mg protein for hippocampal and cortical norepinephrine.

Mean (±standard error) hourly ambulatory activity of the different groups during the 19 h of the day when activity was recorded is shown in Fig. 1, with the activity of each lesion group plotted against the control group. These data were analyzed by analysis of variance (two-way [group×h] with repeated measures across the hour factor) which was carried out for each of the three panels shown in the Fig. 1. No analysis generated an effect of group that approached significance, thereby indicating that depletion of brain NE by these lesion techniques did not cause a change in spontaneous motor activity in comparison to control animals. The total nocturnal ambulatory counts for each of the lesion groups, which was compared to the control group by t-test, showed no difference that approached significance. Moreover, inspection of Fig. 1 shows that even the ‘W-shaped’ pattern of dark phase activity that occurs in normal rats was intact in the lesioned animals. The only potential departure from this was seen in the ventricular lesion condition, where a somewhat reduced elevation in the first hour of activity after onset of the dark period was seen. However, the lack of an overall effect of group in the analysis of variance that compared this group with the control condition, which was
any overall difference in ambulatory activity from control rats but they also showed an unchanged pattern of nocturnal activity; i.e., like the control (normal) rats, lesioned rats showed a marked increase in activity at the onset of the dark period, followed by a decline and then another smaller peak in activity toward the middle of the dark period, followed finally by another large increase in activity leading up to onset of the light period. Finally, ambulatory activity during hours of light also was similarly low in all groups.

What is indicated by these data is that forebrain NE is not necessary for the rat to generate a normal pattern of ambulatory activity in a home cage situation during light and dark phases of the day. Lesions of NE neurons and terminals that virtually eliminated NE from the forebrain neither reduced total spontaneous motor activity nor impaired ability to generate increases in nocturnal ambulatory activity that characterizes normal rat behavior. But insofar as the technique used for affecting NE in the present study was to make a lesion that would reduce brain NE, an important issue to consider is whether compensatory increases in the function of noradrenergic systems in the brain (such as upregulation of postsynaptic adrenergic receptors) might have overcome effects of decreased transmission of brain NE. Thus it might be argued that, despite reduced NE levels in the brain, noradrenergic neurotransmission in the lesioned animals nevertheless was functioning well enough to mask motor deficits that will result from the depletion of NE. With regard to this issue, studies that have assessed compensation after experimentally-produced reduction of catecholamine content in brain agree that compensation indeed occurs but also indicate that compensation sufficient to return functional responses to normal generally requires 10% or more of the normal amount of NE or DA to be present [6,8,10,14]. In regard to the present experiment, the study of Curet and Montigny [8] is particularly relevant. These investigators examined electrophysiological responses of hippocampal neurons following electrical stimulation of the locus coeruleus (which will cause NE to be released in the hippocampus). In part of the study, responses were assessed in rats whose NE had been depleted by intraventricular infusion of 6-OHDA; electrophysiological measurement was made 14–21 days after the NE lesioning procedure had been carried out. In comparison to naturally-occurring physiological stimuli that activate LC neurons, electrical stimulation of LC is a very strong stimulus for NE release, so that this technique is likely to ‘test the limits’ by recruiting whatever NE is still available for release. In animals that had been infused with 6-OHDA, the normal influence of LC stimulation on hippocampal neurons was not diminished until NE depletion in the hippocampus was found to reach 90%, but with depletions above this level, the influence of stimulation began to decline and no influence at all of LC stimulation was seen (i.e., no evidence of any compensation being present) when NE depletion was measured as

noted above, means that the difference at this single time point is statistically attributable to chance; also, the failure to see a similar change in any other lesioned group indicates that this was in all likelihood a chance occurrence. In summary, not only did lesioned rats fail to show...
being total (i.e., 100%). Thus, Curet and Montigny reported that substantial depletions of NE (at least 90%) were needed to alter the influence of NE on hippocampal cells that was produced by electrically stimulating the LC, but even a strong stimulus for release such as this had no effect at all when NE depletion was judged to be complete. In the present study, NE lesions were quite extensive, with the compound lesion technique producing near-total depletion of NE in the forebrain. In this regard, Fig. 2 shows the individual activity data of the two animals in the compound lesion group that were found to have the most complete lesions in the study. For these two subjects (animals #7 and #9), no NE at all was detected in prefrontal cortex and hippocampus (assay sensitivity for these regions was less than 3.0 pg/ml protein, which was less than 0.2% of control levels) and the reduction in hypothalamic NE was 95% and 92% respectively. Examination of Fig. 2 reveals that these animals showed undiminished elevation of ambulatory activity in response to the onset of darkness and also showed a similar ‘W’ pattern of ambulatory activity during the dark period as was shown by control animals. These results in animals whose NE depletions were so large as to preclude the possibility of any significant compensation indicate, together with the other findings shown in Fig. 1, that forebrain NE is not necessary for the ambulatory behavior assessed in this study.

The results shown in Fig. 2 also may shed some light on the function of forebrain NE. Rather than showing a failure to respond, the two lesioned subjects whose data are shown in Fig. 2 can be seen to manifest exaggerated ambulatory responses at times when nocturnal ambulatory activity normally increases. In evaluating these results, it should be considered that the data shown for the two lesioned animals in Fig. 2 are means (±SEs) calculated from recordings made over 7 days, so that elevated activity at a particular hour was not an isolated occurrence on a single day but, instead, represents a consistent daily pattern. Therefore, animal #7 consistently showed (a) an exaggerated response to the onset of darkness, as well as (b) a large increase in activity near the end of the dark period which also occurred slightly earlier in time than did the usual pre-light increase in activity shown by normal animals. Animal #9 also showed a consistent exaggeration of ambulatory activity peaks in producing the ‘W-shaped’ pattern that characterizes dark-phase activity. Thus, eliminating forebrain NE in these animals seems to have removed a moderating influence on ambulatory activity; as a result, the animals responded in an exaggerated manner to the excitatory stimuli, both internal and external, that give rise to ambulatory activity at the time points where the exaggerated responses were seen. In a comprehensive review of basic research relating to the function of dorsal bundle noradrenergic pathway that originates in the LC, Robbins, Everitt and colleagues [19] concluded that the functioning of this system allows an animal to respond in a discriminating way to the environment (pg 146). While the theoretical formulation of Robbins and Everitt emphasizes how NE influences attention and stimulus processing, their formulation seems consistent with the possibility that forebrain NE serves to moderate behavioral responses in many situations.

When evaluating the results described here, these findings should not be interpreted as demonstrating that action of NE in the brain does not affect motor activity. First, the present study assessed spontaneous ambulatory activity in the home cage and did not examine motor responses that occur within brief periods of time and/or in reaction to challenging or novel events. Second and perhaps most important, the study described here utilized lesion techniques to assess the influence of forebrain NE on behavior. While this methodology has a long history with respect to the question of how NE is related to motor activity, it has distinct drawbacks. Long-term reduction or removal of brain NE, which is what the technique brings about, does not reproduce how a change in NE normally occurs to exert its influence; in physiological function, transient increases or decreases in synaptic NE concentration are what occur to affect function. As a consequence, such reduction or removal of a substance that acts acutely, particularly one that appears to exert a modulatory influence, ultimately may not prove to be a good method for assessing its effects. This may explain why techniques that acutely perturb NE neurotransmission (e.g., [1,2,26,31]) have observed effects on motor behavior with more regularity than studies in which brain NE has been removed by lesion techniques. Such observations argue strongly against overgeneralization of the present findings to conclude that brain NE does not affect motor activity.
But regardless of the possibility that making acute perturbations of NE action in the brain may reveal appropriate physiological functions of brain NE that are not seen when NE is chronically removed from the brain, the present results do indicate that the presence of NE in the forebrain is not required for the rat to generate normal diurnal patterns of ambulatory activity.

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References


