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Compulsive, Abnormal Walking Caused by Anticholinergics in Akinetic, 6-Hydroxydopamine-Treated Rats

Abstract. *In otherwise profoundly akinetic rats that had been severely depleted of brain catecholamines, anticholinergic drugs caused excessive walking. The effect did not appear until 10 days after surgery and then increased with time, suggesting that a phenomenon analogous to denervation supersensitivity may be involved. If the animals walked into corners, they were unable to turn around or back out. Their gait (extremely short steps) was reminiscent of that of patients with Parkinson's disease. The results are consistent with a mutually antagonistic interaction between cholinergic and dopaminergic brain systems and emphasize certain complexities in this interaction.*

Evidence suggests that destruction of ascending dopamine-containing pathways can produce deficits in locomotion. For instance, when 6-hydroxydopamine (6-OHDA) [a neurotoxin believed to destroy catecholamine systems relatively selectively (1)] is applied to the substantia nigra, to the nigrostriatal pathway in the hypothalamus (2), or intraventricularly (3), it can produce akinesia as well as many of the other symptoms of hypothalamic damage (4). Akinesia also can be produced by drugs, such as neuroleptics, which depress the action of catecholamine brain systems (5). Fur-

thermore, akinesia is a prominent symptom of parkinsonism, which has been related to the destruction of cells and depletion of catecholamines in the nigrostriatal system (6).

Anticholinergic drugs have long been valuable in the treatment of parkinsonism (7). However, some evidence, both clinical and in experimental animals, suggests that although anticholinergics are useful in alleviating rigidity, tremor, and catalepsy, they typically do not counteract akinesia (8).

Using rats depleted of brain catecholamines by intraventricular applica-

tion of 6-OHDA (9), we have found that short-step locomotion—a form of walking reminiscent of the gait of some patients with parkinsonism—can be temporarily induced by the anticholinergic atropine in otherwise profoundly akinetic rats.

Thirty-two male rats weighing 400 to 500 g (16 experimental and 16 control rats) were treated with pargyline (50 mg/kg) 30 minutes prior to surgery. In ten of the experimental rats, 200 μ g of 6-OHDA was injected into each lateral ventricle and 100 μ g of 6-OHDA into the third ventricle in a single operation (10). In the remaining six experimental rats, 200 μ g of 6-OHDA was injected first into one lateral ventricle and 48 hours later into the other. After the initial pargyline treatment, 12 control rats were given no further treatment. In the remaining four control rats, only the vehicle was injected into all three ventricles. Radioenzymatic assays for dopamine (DA) and noradrenaline (NA) confirmed the effectiveness of the 6-OHDA treatment in depleting catecholamines (11).

After the injections of 6-OHDA, the animals displayed various degrees of catalepsy and akinesia (12). In a test for cataleptic clinging (13), each rat was placed with its head and forequarters partway up onto the horizontal surface of an inverted, wire-mesh hanging cage. Its front paws grasped the upper horizontal mesh surface of the cage (that is, the cage bottom which, when inverted, was now on top), whereas its hindpaws

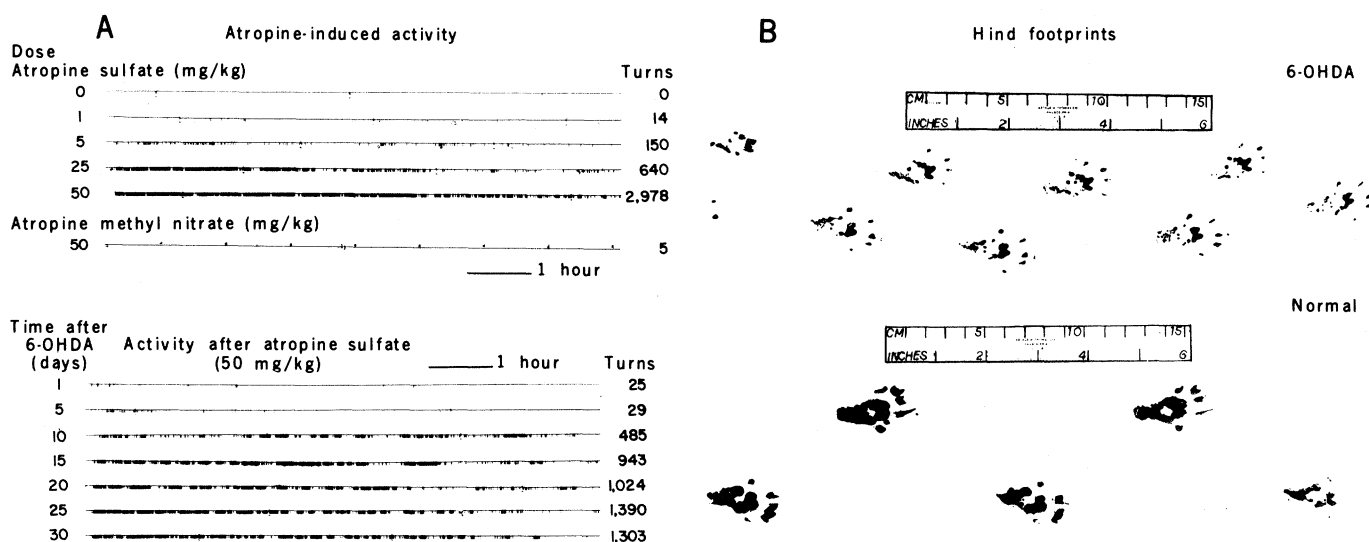


Fig. 1. (A) Top: A record of activity pattern in an otherwise akinetic 6-OHDA-treated rat injected at least 30 days after surgery with various doses of atropine sulfate or atropine methyl nitrate. Actual number of wheel turns are indicated on the right (turns were recorded by a counter on each wheel). Thin vertical slash marks reflect the pattern of activity (these do not always agree exactly with the number of turns because time markers also appear on the record). Bottom: A record of activity pattern after atropine sulfate (50 mg/kg) was injected on various days after surgery in another 6-OHDA-treated rat. Atropine has little or no effect until about 10 days after surgery but becomes increasingly effective in producing excessive walking thereafter. (B) Footprints from the ink-brushed hind feet of a 6-OHDA-treated rat (top) that was induced to walk by an injection of atropine sulfate (25 mg/kg). Rulers were placed next to each set of footprints before photographing and were later retouched for the figure.

grasped the vertical mesh wall. Eight control rats climbed up onto the horizontal surface virtually immediately (mean, 4 ± 1.4 seconds). In contrast, even when tested 30 days after surgery, ten 6-OHDA-treated animals clung much longer (70 ± 24 seconds). Five of them were so cataleptic that they clung for the duration of the test period (3 minutes). When activated by tail-pinch, however, they climbed up immediately. Otherwise, they eventually became fatigued while clinging and fell or slid down to the ground.

To measure the degree of akinesia, we placed all rats one at a time in the center of a circular open field (diameter, 80 cm) enclosed by a white wall 36 cm high. When tested 30 days after surgery, six of the 6-OHDA-treated rats failed to move more than a step or two for as long as 2 hours in the open field. In a 10-minute period, five others moved a few steps in one direction or another but did not progress far enough to reach the wall; the remaining five reached the wall in 3 to 8 minutes. In contrast, 16 control animals walked to the wall within 4 to 30 seconds.

We had previously observed that after receiving atropine sulfate injections, rats previously injected with 6-OHDA were no longer akinetic and immediately walked to the edge of the circular open field. Furthermore, they appeared to walk excessively relative to control animals also injected with atropine. Therefore, to quantify the excessive walking, ten 6-OHDA-treated animals and eight controls were placed individually in an activity wheel. On separate days, at least 30 days after surgery, they were each

Table 1. Effects of various doses of atropine sulfate on behavior in the activity wheel in 6-OHDA-treated and control rats, 30 days after surgery. Data are means $\pm$ standard error (S.E.).

Dose (mg/kg)	Wheel turns per 12 hours	
	6-OHDA (N = 10)	Control (N = 8)
0	3 $\pm$ 3	13 $\pm$ 13*
1	60 $\pm$ 20	13 $\pm$ 6
5	133 $\pm$ 36	29 $\pm$ 21
25	371 $\pm$ 90	32 $\pm$ 16
50	818 $\pm$ 274	67 $\pm$ 20
<i>Atropine methyl nitrate</i>		
50	9.3 $\pm$ 3	26.9 $\pm$ 7

*In contrast to the open field test which was used to show that 6-OHDA-treated animals were clearly akinetic relative to control animals, the activity-wheel test was used to demonstrate primarily the hyperkinesia induced in 6-OHDA-treated rats by atropine. As a group, undrugged control animals showed little activity in the wheel for two main reasons: (i) they were run during the light portion of the light/dark cycle (8:00 a.m. to 8:00 p.m.; 23°C) and (ii) they were relatively mature; in our experience, younger rats show more running-wheel activity.

Table 2. Effect of atropine sulfate on clinging behavior in 6-OHDA-treated and control rats, 30 days after surgery. Data are means $\pm$ S.E.

Dose (mg/kg)	Clinging (seconds)	
	6-OHDA (N = 10)	Control (N = 8)
0	70 $\pm$ 24	4 $\pm$ 1.5
50	2 $\pm$ 0.16	1.5 $\pm$ 0.16

given injections of atropine sulfate (0, 1, 5, 25, and 50 mg/kg, intraperitoneally) in counterbalanced order (14). Table 1 shows that in the open field test, in contrast to controls, all 6-OHDA-treated rats given atropine showed a marked dose-dependent increase in walking (15). Atropine methyl nitrate (50 mg/kg), which crosses the blood-brain barrier less readily than atropine sulfate, had little or no effect. Therefore, the anticholinergic effects of atropine sulfate on locomotion appear to be centrally mediated.

The effects of atropine lasted about 6 to 8 hours. During this time catalepsy was also reversed: rats injected with 6-OHDA and then given atropine were able to climb the wire mesh as well as controls (Table 2).

Thus, our experiments demonstrate that atropine can temporarily reverse not only catalepsy, but also bradykinesia, and even an otherwise profound and long-lasting akinesia. Our findings are consistent with the view that there is a mutually antagonistic interaction between dopaminergic and cholinergic systems in the brain (7, 8). However, several complexities in this interaction remain to be clarified. First, atropine had little effect in reversing akinesia or in producing hyperkinesia for about 10 days or so after surgery and then became increasingly effective up to 30 days, after which the effect began to plateau (16) (Fig. 1A and Table 3). Atropine's effectiveness in inducing locomotion was greatest in those animals that were otherwise most akinetic and cataleptic (17). For instance, one very cataleptic rat with no detectable dopamine that had never been observed to walk spontaneously during the entire postoperative period produced nearly 3000 revolutions of its activity wheel after a single injection of atropine sulfate (50 mg/kg).

A phenomenon analogous to denervation-supersensitivity may mediate the effect of atropine. Somehow, as time passes after catecholamine depletion in the brain, the neural system responsible for the increased locomotion grows more sensitive to a blockade of cholinergic

neurons. To our knowledge, the mechanism for this action is still obscure (18). However, it is in accord with recent clinical evidence indicating that anticholinergics can lead to involuntary movements in some patients with parkinsonism (19).

Finally, the walking released by atropine in otherwise akinetic animals is not normal. We brushed the rats' hindfeet with black ink and photographed the tracks they made while walking (Fig. 1B). Relative to control rats injected with atropine, the 6-OHDA-treated rats injected with atropine took extremely short steps, both in the wheel and on a flat surface. To traverse a given distance, 6-OHDA-treated animals must therefore make many more steps than normal. For instance, to achieve its nearly 3000 turns in the activity wheel after an atropine injection, the otherwise completely akinetic animal described above walked more than 3.5 km by making more than 43,000 hindleg steps (estimate based on size of steps, circumference of activity wheel, and number of revolutions). In 6-OHDA-treated rats that had recovered a limited degree of spontaneous locomotion, short-step walking could be observed even without atropine. This gait is reminiscent of the short-step pattern seen in humans suffering from parkinsonism (20).

Also, when these animals walked into a 45° corner, they did not rear up in a vertical tactile scan and turn around as the normal animals did. Instead, they were trapped for indefinitely long periods. A somewhat similar phenomenon in undrugged anorectic rats with lesions of the lateral hypothalamus (stage II) has been described (21). It is clear that in the 6-OHDA-treated rat whose akinesia has been reversed by atropine, as in a rat recovering from lateral hypothalamic lesions, a locomotion system can be active with only partial recovery of some of the dimensions of movement involved in orienting and head-scanning that guide locomotion. Animals without brain dam-

Table 3. Effects of atropine sulfate (50 mg/kg) on behavior in the activity wheel of 6-OHDA-treated and control rats. The data indicate the development of hyperkinesia. Data are means $\pm$ S.E.

Days after surgery	Number of turns per 12 hours	
	6-OHDA (N = 4)	Control (N = 4)
1	12 $\pm$ 5	13 $\pm$ 12
5	15 $\pm$ 5	15 $\pm$ 12
10	282 $\pm$ 100	6 $\pm$ 3
20	797 $\pm$ 318	23 $\pm$ 3
30	1242 $\pm$ 631	14 $\pm$ 9

age that are injected with atropine also can be trapped in corners, but the deficits in movement systems are quite different (22). L-DOPA, a dopamine agonist, also can reverse akinesia in 6-OHDA-treated rats (23), but circling and other stereotypies such as gnawing, chewing, and grooming can quickly overwhelm forward locomotion in such animals. In combination with atropine, however, L-DOPA appears to produce a more normal form of walking.

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removed, and a sample of the caudate-putamen (average weight, $14.4 \pm .9$ mg) from each brain was assayed according to the method of L. L. Zschaeck and V. D. Ramirez, *J. Neural Transm.* 39, 291 (1976). With this technique we detected as little as 0.062 ng of transmitter per total tissue sample. Compared with controls, the 6-OHDA-treated rats showed little DA in the caudate-putamen (mean, $0.3 \pm .16$ ng/mg, which is 5.9 percent of control content; four of these animals showed no detectable DA). Noradrenaline was relatively unchanged (mean, $.22 \pm .03$ ng/mg, which is 89 percent of control content). Dissection was carried out macroscopically following landmarks that closely approximated the extent of the caudate-putamen. Thus, our samples may have included some tissue outside of this neural area.

12. All 16 rats given 6-OHDA also were initially aphagic, adipic, and showed severe sensory neglect as reported previously (2-4). Subsequently, 14 rats advanced to later stages of recovery typical of rats with lateral hypothalamic lesions. They began to eat palatable foods between 10 and 63 days but were still anorectic and required occasional supplementary intragastric feeding. Eventually, six of these rats began to eat dry food, drank water, and required no further supplementary feeding.
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14. Since the permeability of the rat brain to atropine is not great, large doses are often required to penetrate the blood-brain barrier and produce central effects. [I. Q. Wishaw, T. E. Robinson, T. Schallert, *Pharmacol. Biochem. Behav.* 5, 275 (1976); S. D. Harrison, Jr., T. R. Bosin, R. P. Maickel, *ibid.* 2, 843 (1974)]. It remains, of course, to be confirmed that such doses produce

their behavioral effects simply by anticholinergic action.

15. Running behavior was never observed. The anticholinergic scopolamine (up to 5 mg/kg) also induced comparable excessive walking in additional akinetic, 6-OHDA-treated rats ($N = 4$).
16. In rats made akinetic and cataleptic by daily injections of reserpine (2 mg/kg), atropine sulfate induced excessive short-step walking beginning at about 8 days after the first reserpine injection.
17. For example, a correlation coefficient of .79 between clinging duration and post-atropine activity in 6-OHDA-treated rats (measures taken at least 30 days after surgery) was significant ($P < .01$).
18. A DA receptor blocking agent (haloperidol, 2 mg/kg intraperitoneally) failed to prevent the increase in locomotion brought about by atropine (50 mg/kg) in 6-OHDA-treated rats ($N = 3$).
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9. We realize that there are many forms of Parkinsonism. At present, there is no animal model that mimics all of the symptoms [R. C. Duvoisin, in *The Basal Ganglia*, M. D. Yahr, Ed. (Raven, New York, 1976), pp. 293-303; C. D. Marsden, R. C. Duvoisin, P. Jenner, J. D. Parkes, C. Pycock, D. Tarsy, *Adv. Neurol.* 9, 165 (1975); U. Ungerstedt, A. Avemo, E. Avemo, T. Ljungberg, C. Ranje, *ibid.* 3, 257 (1973)].
10. The 6-OHDA was injected as a solution of 6-OHDA hydrochloride ($10 \mu\text{g}/\mu\text{l}$) in a vehicle of 0.9 percent NaCl solution buffered with 0.1 percent ascorbic acid [see I. Q. Wishaw, T. E. Robinson, T. Schallert, *Pharmacol. Biochem. Behav.* 5, 275 (1976)].
11. Halfway through the present experiments, six experimental and eight control animals were killed to obtain an estimate of the effectiveness of the 6-OHDA treatment in depleting brain catecholamines. (The remaining animals are still being tested behaviorally.) The animals were killed by decapitation, the brains were quickly

Intraspecific Defense: Advantage of Social Cooperation Among Paper Wasp Foundresses

Abstract. *Foundress associations and high frequencies of conspecific nest usurpation are most common where densities of Polistes metricus are high. Here nest usurpation occurs primarily in single-foundress colonies resulting in multiple-foundress colonies having significantly greater productivities than single-foundress colonies. This is not true at low densities. Conspecific pressures and not predation or parasitism provide an advantage to cooperating wasp foundresses in P. metricus.*

The ecological factors conducive to the evolution of insect sociality are largely undocumented. Some authors have suggested that various selective pressures have conferred an advantage to a social organization (1), while others argue that two major selective forces, predation (or parasitism) and the benefits of group foraging, are responsible for the evolution of group living (2). I now report that in certain habitats, conspecific pressures provide a pronounced selective advantage to individuals of cooperative foundress associations in the paper wasp, *Polistes metricus*.

Many species of *Polistes* are facultatively social before the emergence of the first workers, with varying percentages of the foundresses (gynes) being joined by other overwintered foundresses that, for some reason, have not started nests of their own. Such joining foundresses are commonly dominated by the queen (dominant foundress) and essentially behave as workers. These subordinate foundresses are thought to contribute relatively little to the production

of colony reproductives (3). After the emergence of the first workers, colonies are eusocial, with a reproductive division of labor between the queen and her workers, who assist the queen in the production of other workers, males, and potential foundresses (3). Comparative studies of conspecific multiple- and single-foundress colonies in the same habitat should provide evidence of the advantages, if any, accruing to members of a social organization relative to a solitary existence.

Field studies were conducted from 7 April to 1 September 1977 on 74 naturally nesting *P. metricus* colonies located in five sites near Lawrence, Kansas. Site 1 contained 40 wooden boxes (10 by 20 by 30 cm, the open side covered with "chicken wire" mesh) fastened to 1.5-m high poles arranged uniformly in a 5- by 8-m grid. Site 2 contained 20 similar-sized metal boxes dispersed over a 50- by 80-m trailer park. Fourteen of the wooden boxes and eight of the metal boxes were occupied by nesting *P. metricus*, which indicates that nest sites were not