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Abnormal gait sequence in locomotion after atropine treatment of catecholamine-deficient akinetic rats

(dopamine/methysergide/muscarinic pathways)

SERGIO M. PELLIS*, VIVIEN C. PELLIS*, REBECCA M. CHESIRE†, NEIL ROWLAND*, AND PHILIP TEITELBAUM*‡

*Department of Psychology, University of Florida, Gainesville, FL 32611; and †Department of Psychology, University of Hawaii at Manoa, 2430 Campus Road, Honolulu, HI 96822

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ABSTRACT **Excessive, abnormal locomotion occurs after a high dose (25–50 mg/kg) of atropine sulfate to rats already akinetic due to catecholamine deficiency from intraventricular administration of 6-hydroxydopamine. This abnormal locomotion involves an abnormal gait sequence [right (R) hindleg (H), left (L) foreleg (F), LH, RF] instead of the normal gait sequence (RH, RF, LH, LF). In such animals atropine progressively (*i*) decreases hindleg step size, (*ii*) decreases arching of the trunk, and (*iii*) increases foreleg step size. These factors combine to change the ratio of front/hind body support. If the body stretches too far and the hindleg step is too small, a given hindleg step supports insufficient weight to remove weight from the ipsilateral foreleg; consequently, the opposite foreleg must execute the next step, producing the abnormal gait sequence. Thus, atropine affects gait sequence indirectly; it acts on at least three variables that affect how body weight is distributed and shifted during locomotion. To maintain stability during such locomotion, gait sequence is appropriately altered.**

In animals made akinetic by selective depletion of catecholamine systems in the brain through intraventricular administration of 6-hydroxydopamine (6-OHDA), locomotion can be triggered by systemic injection of atropine sulfate, a cholinergic blocking agent (1, 2). Atropine has no such effect on normal animals. Furthermore, atropine methylnitrate, which does not pass the blood–brain barrier, has no effect on locomotion in 6-OHDA-treated rats (1, 2). Therefore, such locomotion appears to result from the blocking action of atropine on central brain pathways remaining intact in the catecholamine-deficient animal. The locomotion is excessive in amount and abnormal in character; the animals walk very slowly with short hindleg steps (1, 2). By contrast, locomotion stimulated by apomorphine, a dopaminergic agonist, although excessive in amount, does not have these abnormal characteristics (S.M.P., V.C.P., and P.T., unpublished observations).

Although anticholinergic drugs are used in conjunction with the L-isomer of 3,4-dihydroxyphenylalanine (L-DOPA), the precursor to dopamine, to treat rigidity and tremor in Parkinsonian patients, there is no evidence that anticholinergics are useful in promoting locomotion in such people (3). Clearly, however, in one animal model of Parkinsonism (the 6-OHDA-treated rat), locomotion is "released," or disinhibited (1, 4). Such locomotion may involve an abnormal gait sequence. We found that the described abnormal sequence was due to the action of atropine on three variables of body movement (hindleg step size, foreleg step size, and degree of body arching). In their interaction, these variables affect the animal's weight distribution, thus forcing a compensatory change in gait sequence.

MATERIALS AND METHODS

Eleven male Long–Evans hooded rats, weighing 400–500 g, were used. Unless otherwise specified, all drugs were obtained from Sigma. Surgery was done under Equithesin anesthesia (0.33 mg/100 g of body weight; chloral hydrate/sodium pentobarbital, 1:5) using standard stereotaxic procedures on six rats that had been pretreated with pargyline (50 mg/kg; i.p.) 30 min earlier. In a single operation, 200 μg of 6-OHDA hydrochloride (10 μg/μl) in a vehicle of 0.9% NaCl solution buffered by 0.1% ascorbic acid, was infused via a 28-gauge cannula into each lateral ventricle (1 mm posterior to bregma, 1.5 mm lateral to the midline, and 4.5 mm ventral to the skull surface). In addition, 100 μg was infused into the third ventricle (1.5 mm posterior to bregma; 9.0 mm ventral to the skull; and, at the level of the skull surface, 1.0 mm lateral, 10° laterally off perpendicular). For further details of the surgical procedure see Schallert *et al.* (1) and Whishaw *et al.* (5). An additional two rats were used as sham-operated controls. Three other intact rats were used to compare brain catecholamine content with 6-OHDA-treated animals.

Postoperatively, the rats received a liquid diet by intragastric tube until they accepted palatable or standard diet orally (6). Three weeks postoperatively, three of the six 6-OHDA-treated rats were injected i.p. with atropine sulfate (50 mg/kg) (dissolved in 0.9% NaCl), followed three days later by 50 mg/kg i.p. of 1-methyl-*d*-lysergic acid butanolamide (methysergide) maleate (suspended in 0.9% NaCl; Sandoz Pharmaceutical, Hanover, NJ). In the remaining three rats the reverse sequence was used. Three days after the last injection of atropine or methysergide, the rats were injected i.p. with an equal volume of saline. The saline did not release locomotion in these akinetic animals. Atropine had the same effects on locomotion whether injected first or second, so the results are pooled for all six rats. The data for methysergide (which releases a different form of locomotion) will be presented elsewhere.

During 60 min following injection, the animals were placed on a 104 × 76 × 74-cm table top bounded by three walls, and 16-mm movie film was taken at 24 frames per sec, as the rats progressed from akinesia to forward walking. At first after injection, the animals were placed in the table center so that all movements preceding forward locomotion would occur without wall contact. Once they walked from center to wall, the animals were placed at one end of the enclosure so that long sequences of locomotion, again free of wall contact, could be filmed across the entire length of the table top. Over

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Abbreviations: 6-OHDA, 6-hydroxydopamine; R, right; L, left; H, hindleg; F, foreleg.

‡To whom reprint requests should be addressed.

the next hour, this procedure was repeated at 10- to 15-min intervals. By the end of the first hour the drug-released locomotion began to wane and filming ceased. Films were viewed frame-by-frame using an LW Motion Analyser (Lafayette Instruments, Lafayette, IN).

To facilitate analysis of the relationship between body parts during locomotion and to measure the size of each step topographically in relation to the body, Eshkol–Wachman movement notation (7) was used to describe simultaneous movements of each limb and body segment and position of each limb relative to the body. This is a globographic method in which the size, type, and direction of movements of all observable body segments are notated continuously and simultaneously for every frame of movie film. The use of the method has been detailed elsewhere (8–10), and only the results will be discussed. Measurements of step-cycle period and phase relationships were not attempted because the animals were recorded in an open field, and thus long sequences of uninterrupted straight-line walking did not occur frequently.

Subsequent to the locomotion experiments, the rats were decapitated and their brains were packed in dry ice. Tissues were stored at −70°C until determination of whole-brain concentrations of monoamines using HPLC with electrochemical detection (11). Each experimental animal had severe depletions of dopamine (mean depletion, 97%; range, 94–99%) relative to intact controls (mean, 1.61 μg/g of wet weight). Norepinephrine was also greatly depleted (mean depletion, 95%; range, 93–97%) compared with controls (mean, 0.71 μg/g of wet weight), and serotonin was slightly depleted (mean depletion, 31%) compared with controls (mean, 0.56 μg/g of wet weight).

RESULTS AND DISCUSSION

The gait sequence of atropinized 6-OHDA-treated rats was abnormal in comparison to undrugged or atropinized intact rats. Normal stepping sequence, typical of practically all tetrapods (12), is right (R) hindleg (H), R foreleg (F), left (L) H, LF, whereas, in the atropine-treated animals the gait was RH, LF, LH, RF. We first thought that this difference in stepping sequence might result from the action of atropine on some central program generator in the nervous system. However, the abnormal way in which the catecholamine-depleted atropinized rats shifted their body weight intrigued us, and by trying to understand this aspect of the locomotion, we were able to identify the body-movement variables affecting their gait sequence. Thus, the drug affects how the animal shifts its body weight, which, in turn, affects the sequence of stepping. Fig. 1 compares the sequence and the relative stance and swing time of an intact versus an atropinized rat while walking slowly. Note that the sequence of stepping, particularly of foot fall (moment foot makes contact with ground after stepping), is the reverse of that seen in normal animals.

Under the influence of atropine, the animal's body becomes excessively elongated, lacking the convex arching seen in normal animals (Fig. 2); the hindleg steps become abnormally short and the foreleg steps become abnormally long (see ranges superimposed on the tracings in Fig. 2). It must be noted that the ranges for step sizes shown in Fig. 2 represent the largest individual rat steps seen relative to the body, not to the ground. All six 6-OHDA-treated rats began to walk forward within the first 10 min after atropine injection. Three of the 6-OHDA-treated rats walked with the abnormal gait sequence immediately upon walking forward. In contrast, during the onset of drug action three of the otherwise akinetic animals walked with the normal gait sequence, but eventually, at a critical point in the interaction of the changing amplitude of the three body-movement variables, all of them shifted to the abnormal gait sequence. At this point, after a given hindleg step, the foreleg on the other side stepped, allowing the animal to shift his weight foward without falling over.

The abnormal weight distribution producing this shift in gait sequence is illustrated by computer tracing and analysis (Apple IIe graphics tablet) of the proportion of body in front and behind the hindfoot as it contacted the ground after stepping (Fig. 3). When the hindleg steps became too short, the foreleg steps became too long, and the degree of body arching became too little, the hindleg step did not land far enough forward to support enough of the body weight behind it to allow the ipsilateral foreleg to stop bearing weight and step. If the animal had done so, it would have fallen over to that side. In the example illustrated, with ≈20% more of total body area in front of the point of support than behind it, the gait was still normal. With ≈60% more of the total body area in front, the gait was abnormal.

Insufficient transitions in gait for individual rats were available in the current study to specify with certainty the threshold proportion for gait change, but it appeared to involve an increased front-to-back proportion of ≥30%. Six tracings each for two intact rats were taken at the moment the hindleg landed, giving a mean hind-to-front ratio of 1.2 (SE = 0.1). Because atropine can sometimes release normal gait sequence locomotion early in its action on 6-OHDA-treated animals, it ought to be possible to identify a dose range for a more normal therapeutic action of atropine.

Our results show that this change from normal to abnormal gait was determined by movement variables affecting how the animal shifts its body weight as it walks forward. The relative

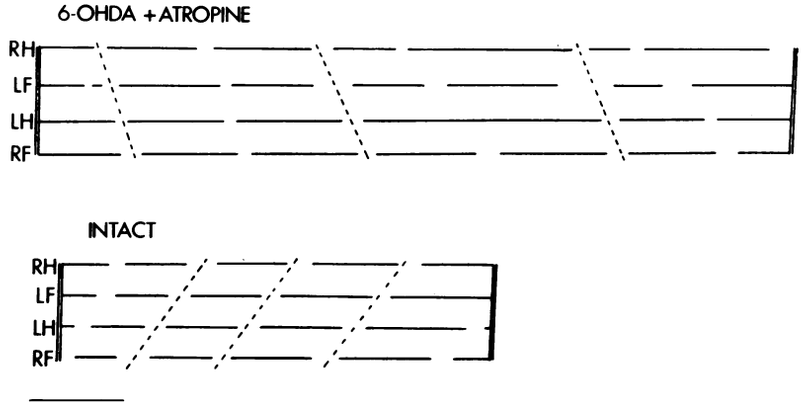

FIG. 1. Stance time (solid horizontal lines) and swing time (open spaces between horizontal lines) are shown for the steps of a 6-OHDA atropinized rat and for an intact rat. Oblique dotted lines highlight the reverse pattern of stepping for the two animals. A line showing 2 sec is presented in the lower left corner. Both sequences were filmed at 24 frames per sec.

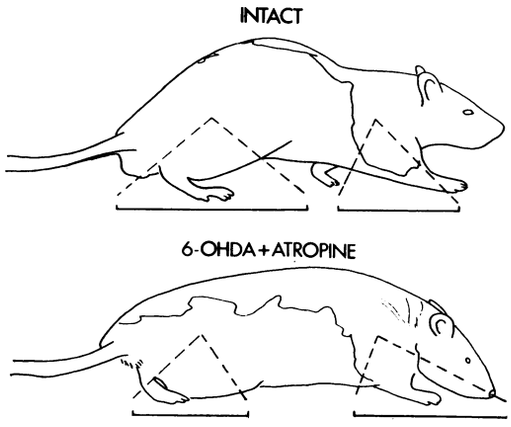

FIG. 2. Postures of an intact rat and a 6-OHDA atropinized rat are compared immediately preceding the landing of the left hindfoot in these tracings. In contrast to the intact rat, the 6-OHDA atropinized rat has an elongated torso, smaller hindleg step, and larger foreleg step (ranges shown by broken lines).

degree of control of peripheral influences over central programming of gait sequence in intact and unrestrained animals remains unclear (13). In the atropine-release of locomotion described here, however, the varying gait sequence patterns seen appeared to be completely predictable in terms of the body weight distribution existing at the instant preceding each step. Thus, gait sequence here appears to be dominated by peripheral influences. Our results in these brain-damaged catecholamine-deficient animals therefore support Bässler's view (14) that a chain-reflex approach to motor control may, in this instance, be more appropriate than thinking primarily in terms of a central pattern generator network.

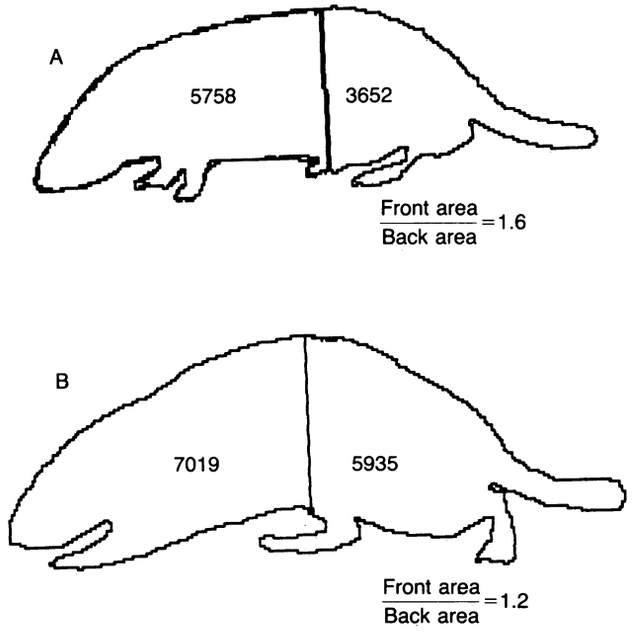

FIG. 3. Computer-generated outlines (Apple IIe graphics tablet) of two 6-OHDA atropinized rats at the moment the hindfoot contacted the ground after stepping. A vertical line from the midpoint of that foot reveals the proportion of body area in front and behind that point of support. The greater proportion of body area anterior in *A* led to abnormal gait sequences, whereas in *B*, the gait sequence remained normal even though the step was relatively small. However, in *B* the torso was more arched; hence, the hindfoot bore more weight when it landed, thus removing weight from the ipsilateral foreleg.

It should be noted as an additional possibility that the action of atropine on body elongation and stepping may be indirect. Atropine may act on the movement subsystem controlling head scanning, but may not act directly on the subsystem involved in locomotion (15, 16). Before walking occurs, atropine releases tactile-controlled lateral and forward snout scanning in 6-OHDA-treated akinetic rats. Perhaps, as the head scans and stretches forward, it secondarily recruits forward stepping by the forelegs and then by the hindlegs as an allied reflex associated with the animal's shift of body weight during scanning. But the hindlegs lag markedly in their recruitment, while the forelegs make longer steps, thus stretching the body further. This, combined with the short hindleg catch-up steps (perhaps also a symptom of insufficient recruitment of hindleg stepping), changes the body-weight distribution, thus possibly yielding the observed short-step locomotion with abnormal (reverse—i.e., LF, LH, RF, RH) gait.

To understand how step size affects weight shift during locomotion, it is important to measure step size relative to the body, not as an absolute quantity relative to the ground as is commonly done (17, 18). A step may be large or small in absolute space; what matters, however, is whether the step is large or small relative to the animal's body-weight distribution. For example, a small hindleg step need not produce the abnormal gait sequence if the small step occurs when the body is strongly arched, thus enabling the step to support enough of the body behind it to allow the foreleg on the same side to unburden. Although our bodywise analysis clarifies why gait sequence changes from normal to abnormal, such analysis does not address questions of degree of coordination between front and hind legs. For such an analysis of coordination, absolute, as well as bodywise, step size of fore- and hindlegs should be compared.

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