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A Simple, Inexpensive Rotometer for Automatically Recording the Dynamics of Circling Behavior

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JERUSSI, T. P. *A simple, inexpensive rotometer for automatically recording the dynamics of circling behavior.* PHARMAC. BIOCHEM. BEHAV. **16**(2) 353–357, 1982.—An inexpensive, automated system for recording circling behavior in small animals is described. The apparatus provides information concerning: left and right full (360°) rotations, half (180°) turns, the time it takes for a rotation, the number of consecutive rotations in each direction, reversals (180° changes in direction), net rotations which are displayed directly without a source of electrical power, plus other parameters of circling behavior. The system is simple in principle and construction, and is virtually maintenance free. Depending on the options desired, a single rotometer can be built for approximately $350 or less than $70 by anyone able to use basic hand tools.

Rotometer Circling behavior Rotation Nocturnal rotation Amphetamine rotation

RATS or mice with unilateral nigrostriatal lesions circle or rotate predominantly in one direction when administered a variety of pharmacological agents [3]. The lesioned rodent model [12] has been used quite extensively to test the efficacy of various dopaminergic agonists and to screen drugs for their possible neuroleptic potency. More recently, normal, surgically unaltered rodents have been shown to rotate, although at lower rates than their lesioned counterparts, without drugs [2, 4, 8], or after the administration of pharmacological agenst that produce vigorous circling behavior in lesioned animals [3, 6, 7,].

The apparatus used to measure circling behavior (called a "rotometer") usually consists of a container that confines the animal and permits recording, by direct observation or automatically through an electromechanical arrangment, the direction and number of rotations. Counting left and right rotations directly [9, 10, 11] is tedious, time consuming, and subject to error because in many instances complete (360°) rotations cannot be determined unambiguously. Therefore it is desirable to count rotations "automatically."

One of the first automated rotometers [12] consisted of an electromechanical counter activated by a single microswitch. With these components, the direction and completeness of each rotation cannot be accurately determined unless an observer is present to note incomplete revolutions and changes in direction. This was also the case for another automated system [1]. Newer improved rotometers have been described [5, 13, 14] which sense direction, discriminate full rotations from incomplete oscillations, and automatically print-out the data. Although these apparatuses are reliable, the electronics is rather sophisticated and their cost ranges from several thousand to more than ten thousand dollars.

Similar to these more expensive rotometers, the automated apparatus described in this report also allows determination of left and right full rotations. But in addition, this inexpensive system provides information concerning: half (180°) turns, the time it takes for a rotation, the number of consecutive rotations in either direction, reversals (i.e., 180° changes in direction), plus other parameters of circling behavior (e.g., latency to the first rotation). Moreover unlike other rotometers, this simple apparatus records net rotations (i.e., rotations to the left minus rotations to the right) without any source of power.

METHOD

Apparatus

The rotometer consists of a clear acrylic flanged hemisphere, or bowl, 16 inches in diameter (AIN Plastics, Mt. Vernon, NY 10550), with a hole (1/2 in. dia.) drilled in the center at the bottom to allow the exit of animal droppings. A 1/2 inch brass grommet is epoxy cemented into the hole to prevent the animal from gnawing away the acrylic and enlarging the hole. The hemisphere, which is lightly sanded until it is translucent, is supported from its flange by two 12 inch wall brackets, and covered with a circular piece of 1/4 inch plywood approximately 18 inches in diameter (see Fig. 1). A stainless steel shaft (1/8 in.×2 in.), passing vertically through a hole (5/16 in. dia.) drilled in the center of the white plywood cover, is supported by a housing (AA2-1) containing stainless steel ball bearings (E2-1; all precision machined parts were obtained from PIC Design, Ridgefield, CT 06877). A spacer (B4-13) separates an aluminum gear blank (3/4 in. outside dia., BK2-6), fastened flush with the lower end of the shaft inside the bowl, from the bearing housing. A circular

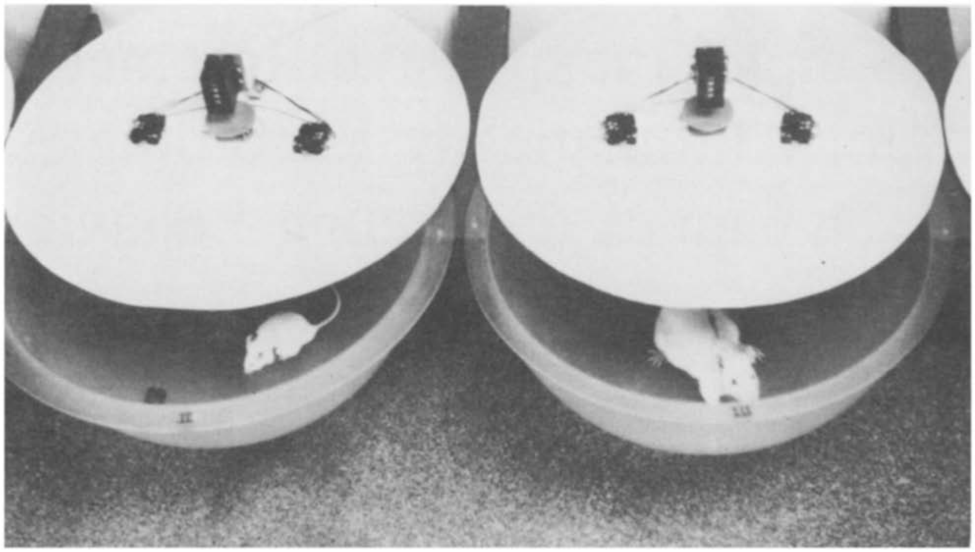

FIG. 1. Rotometers supported by wall brackets. Cover containing the direction sensing counters is slid away to show the mouse with harness unattached and rat in place.

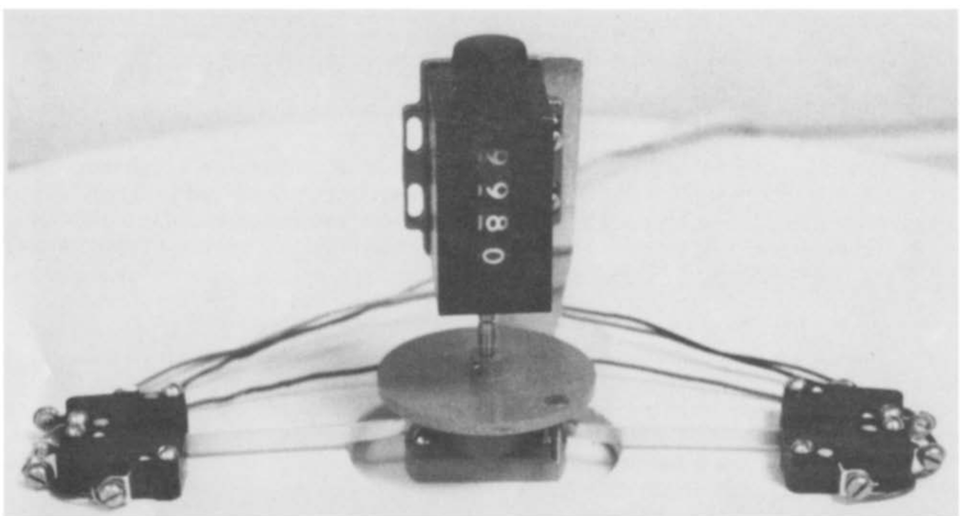

FIG. 2. Direction sensing counters: rotor, two pairs of microswitches, and a revolution counter indicating −20–20 net rotations to the right.

ferrous disc (3/4 in. dia.) is epoxy cemented to the gear blank which can now support a button alnico magnet (previously described [5]; 1/2 in. × 3/4 in., Ace Scientific, Linden, NJ 07036) attached to the animal harness.

The harness is a flexible soft steel wire (24 gauge) which passes through a short (1 in.) length of copper tubing (1/8 in. dia.). A loop formed by crimping the free end of the wire over the upper end of the tubing is slipped over the animal's head and snugged-up just behind the forelimbs. When the animal is in the harness, the button magnet is then attached to the gear blank.

The upper portion of the shaft outside the bowl contains the "rotor"—a larger gear blank (2 in. outside dia., BL-6), drilled, tapped, and countersunk (3/16 in. from the outside) to take a #4-40 flathead machine screw. The shaft of a mechanical revolution counter (No. 4-X-7-1-R-CL-1:1, MacPherson Control Products, Westfield, NJ 07090), positioned over the rotor by a bracket loosely fastened to the plywood cover, is connected with polyethylene tubing to the upper end of the rotor's shaft (see Figs. 1 and 2).

A switch positioned to sense turns to the left (L_1) and one to indicate right turns (R_1) are mounted 180 degrees from another similar pair of microswitches (L_2 and R_2) (see Figs. 1 and 2). Switches in each pair (QAR Industrial Electronics, Mt. Vernon, NY 10550) are mounted "face-to-face" so that the actuating lever of one switch (No. E-22-85X) is also used to produce contact closure of the other (No. E-22-00AX). Each microswitch is wired to a separate channel of a 20

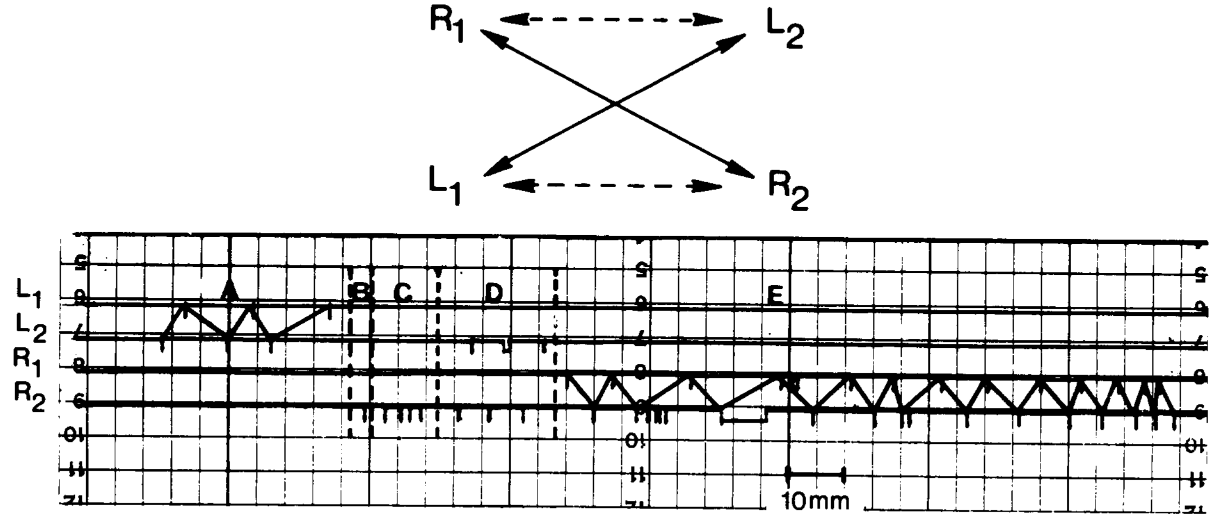

FIG. 3. Top: Arrangement of microswitch pairs (R_1–L_1, L_2–R_2). Solid arrows indicate sequence of switch closures for full (360°) rotations. Broken arrows indicate reversals—180° changes in direction. Bottom: Simulated chart recording illustrating −10 net rotations. A. Two and one-half rotations to the left (two full rotations plus an additional 180° turn). B. A reversal. C. Oscillations at switch R_2. D. Oscillations at switch pair L_2–R_2. E. A reversal followed by 12½ rotations to the right (12 full rotations plus an additional 180° turn).

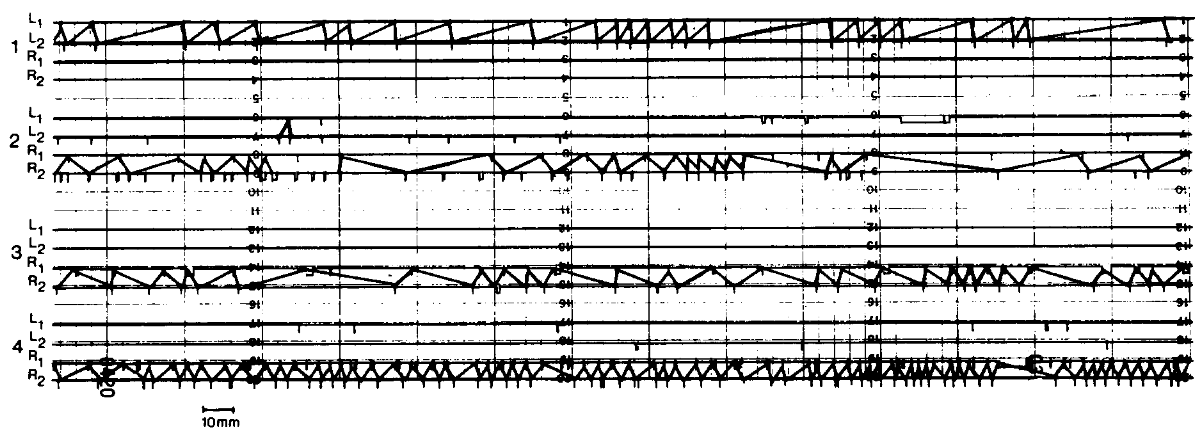

FIG. 4. Recordings from four rats injected with 1.0 mg/kg d-amphetamine sulphate; illustrating from top to bottom: +25, −22, −27, and −67 net rotations.

channel event recorder (Model A620X, Esterline Angus, Paramus, NJ 07652) so that switches sensing the same direction activate adjacent channels (see Figs. 3 and 4).

The animal is able to move freely within the confines of the hemisphere, and when circling occurs the rotor turns pushing in sequence the levers of the microswitches. Switch closures are automatically indicated as patterns of pen deflections on the chart of the event recorder. The solid arrows in Fig. 3, top, illustrate the sequence necessary for complete (360°) rotations, while reversals or 180 degree changes in direction are represented by broken arrows. A full rotation is counted when switches sensing the same direction are activated consecutively, e.g. L_2–L_1–L_2. R_1 occurring after L_2, in the previous example, constitutes a reversal, whereas closure of R_2 does not because the pattern L_2–R_2 indicates oscillation at the same switch pair and not a 180 degree change in direction (see Fig. 3, Bottom).

Rotations to the left and rotations to the right are determined by a graphic method illustrated in Fig. 3, Bottom and Fig. 4, whereby consecutive pen deflections of channels indicating the same direction are connected by adjoining diagonals, unless the rotation is interrupted by a reversal. A full rotation then appears diagrammatically on the chart paper as a triangle with an open base. After the entire recording session has been "diagrammed" in this way, the *vertex* of each open triangle is counted, beginning at either end of the chart paper, and the rotations in each direction are separately totalled. Net rotations are then calculated. In addition, the mechanical revolution counter always provides a direct continuous reading of net rotations, without any calculations, independent of the information obtained from the event recorder. Full rotations to the left are totalled, whereas rotations to the right are subtracted from the counter's numerical display as shown in Figs. 1 and 2. The net rotations obtained

TABLE 1

MEANS (±STANDARD DEVIATIONS) OF VARIOUS PARAMETERS OF CIRCLING BEHAVIOR

	Net Rotations	Rate of Rotation (min)	Number of Runs	Rotations per Run	Number of Reversals
Day 1	65.8 ± 58.1*	2.17 ± 2.69*	2.2 ± 1.7†	47.6 ± 59.9*	2.4 ± 3.3†
Day 8	49.3 ± 51.4*	2.56 ± 2.00*	1.2 ± 0.4†	47.0 ± 52.6*	0.4 ± 0.8†

Rats (N=10) were tested for one hour, a week apart, after d-amphetamine sulphate (1.0 mg/kg) administration. Values above (except for "reversals") were determined for the dominant direction of rotation.

*p<0.01–<0.001, linear regression analysis (r=0.80–0.89).

†p<0.05–<0.01, paired t-tests.

from the revolution counter can save some time in determining left and right rotations from the chart record. First diagram the channels for the direction with the *least* number of rotations. Now simply add this number to the absolute value of the net rotations to get the total number of rotations in the other (i.e., dominant) direction.

Application

Six surgically unaltered female Sprague-Dawley rats (200–250 g; Sunrise Laboratory Animals, Whitehouse Station, NJ 08889) were tested for rotation without any drug from 1700 to 0900 hours and were retested under the same conditions one week later. Net rotations during the dark phase (1830 to 0630 hours) of the diurnal cycle were determined. The chart speed of the event recorder was 20 cm·hr^{-1}. Another group of normal female rats (N=10) was tested twice for rotation, one hour a week apart, after the intraperitoneal injection of d-amphetamine sulphate (1.0 mg/kg). Under these conditions the optimal chart speed was found to be 75 cm·hr^{-1}, whereas when mice were tested, better resolution of rotations could be obtained by increasing the speed to 150 cm·hr^{-1}. In addition to net rotations, several other parameters of circling behavior were determined: rate of rotation (obtained by measuring the open base of each triangle, show in Fig. 3, Bottom and Fig. 4), runs (i.e., sequences of consecutive rotations uninterrupted by reversals), rotations per run, and reversals.

RESULTS AND DISCUSSION

The mean (±SD) net rotations in the dominant direction for the nocturnal sessions were not significantly different from week to week (27.2±38.4 vs 19.0±22.9, p>0.1, paired t-tests) and were significantly correlated (p<0.05, r=0.8779, linear regression analysis) as previously reported [2,4]. The chart records indicated that some rats took well over an hour to complete a single rotation, and upon observation it appeared that animals would begin circling, sleep, then awaken to complete the initial rotation. This pattern of circling behavior is not evident from rotometers equipped with automatic digital counters which print-out at specified predetermined time intervals [5,13], because if the logic circuit is still storing portions of an uncompleted rotation and then a print-out occurs, subsequent completion of that rotation will not be recorded as a *full* rotation. Therefore, rotations completed after the print-outs are not counted and the continuous nature of circling behavior is masked by digital outputs.

Rats tested with amphetamine rotated in the same direction each week, and mean net rotations were significantly correlated and not significantly different for the two test sessions (see Table 1) as previously reported [6,7]. There was excellent agreement (a mean difference of ±0.6) between net rotations diagrammed from the chart records and those read directly from the revolution counters. The mean rate of rotation and the mean rotations per run were also significantly correlated between test sessions as indicated in Table 1. On the other hand, the mean number of runs and the mean number of reversals decreased significantly with subsequent testing. In other words, rats tested a second time tended to have similar number of net rotations with fewer runs and reversals. Therefore with the apparatus described in this report it is now possible to measure other parameters of circling behavior which may be differentially affected by the action of various drugs.

The present rotometer is simple in principle and construction. Therefore it is easy to build and there is virtually no "down-time." The event recorder is essentially unaffected by line transients or power surges which can seriously disrupt some logic circuits of more sophisticated electronic systems. Moreover, even in the event of a total power outage, net rotations still can be retrieved from the visual display of the mechanical revolution counter. There are some disadvantages with this system, however. Left, right, and total rotations are not immediately available, and the chart records must be "diagrammed" in order to obtain the information. Generally this procedure is not time consuming, but it can be if an animal makes many reversals or oscillations between switches. Usually this is not a problem with unilaterally lesioned rats or mice.

The primary advantage of this apparatus is its cost. The entire system, complete with five rotometers, can be built for approximately $1800 ($350 per rotometer). This is less expensive than just the electronics (Coulbourn Instruments, Inc., Leigh Valley, PA 10081, personal communication) for a more sophisticated system, similar to that previously described [5], using photocells and a logic circuit to sense direction and count full rotations. Moreover if only net rotations are desired, as is the case in many lesion studies, the revolution counter alone maybe sufficient. Then an entirely portable rotometer, requiring no power supply, can be built for less than $70.

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