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Automated Analysis of Stereotypic Behavior Induced by Psychomotor Stimulants

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BRANN, M. R., M. HACKER, M. FINNERTY, J. ELLIS, R. H. LENOX AND Y. H. EHRLICH. *Automated analysis of stereotypic behavior induced by psychomotor stimulants.* PHARMACOL BIOCHEM BEHAV 19(1) 57–62, 1983.—A newly developed rotation sensing device has been applied to the continuous monitoring of animal movement. Animals treated with morphine, amphetamine or apomorphine display different stereotypic movements which can be distinguished by the apparatus. Initial studies have indicated that the apparatus is able not only to identify but also to quantitate some measures of stereotypic behavior. For example, the number and direction of rotations (a measure of motor asymmetry), frequency of changes in movement direction (a measure of stereotypic movement) and periods of cessation of movement are affected differentially with acute morphine, apomorphine or amphetamine treatment. Moreover, using this apparatus, morphine was shown to increase the degree of rotational asymmetry of normal animals and of animals with unilateral lesions of the nigrostriatal pathway.

Automated monitoring stereotypic behavior Rotation 6-Hydroxydopamine d-Amphetamine Apomorphine Morphine

MOST automatic monitors of activity are incapable of distinguishing among stereotypic behaviors, qualitatively or quantitatively. Even the recently developed radar [7] and capacitance field [3] devices share these limitations. Stereotypic rotations have previously been counted automatically [5], but prior to the present report, the reliable quantitation of other stereotypic behaviors has required the use of a trained human observer.

The measurement of rotation was automated originally by Ungerstedt [10]. A rat was placed in a bowl and harnessed, so that every time the animal passed through one particular point in a circle it tripped a magnetic switching device. A refinement of this device by Greenstein and Glick [5] allowed the monitoring of the animal's entrance into each of four separate quadrants of a bowl by means of four photoelectric cells. The latter investigators defined a stereotypic rotation as a progression of an animal through four sequential quadrants of a circle [5], thus imposing qualitative criteria to be met by the measured behavior. Many investigators have suggested that laterality after drug and/or surgical manipulation is best characterized when both quality and quantity of rotations are considered [5,9].

The RoSe is a Rotation Sensing device which has the capability of providing a continuous record of an animal's precise angular position as a function of time, and thereby monitors the entrance of the animal into individual quadrants (or any other subdivision) of the bowl-shaped environment. This device has already been applied to the automated monitoring of rotational asymmetry induced by unilateral injection of antimicrotubular agents into the substantia nigra [2]. In the present paper, we describe the RoSe in detail and demonstrate that it can be used to distinguish among the behavioral effects of several drugs which alter dopamine-mediated neurotransmission. These behaviors are expressed as alterations in rotational asymmetry, general motor activity and the frequency of various stereotypic behaviors. For the purpose of this study we selected apomorphine, amphetamine, and low doses of morphine, all of which are known to increase asymmetry and motor activity as well as induce stereotypic behavior by "mimicking" dopaminergic neurotransmission. Apomorphine appears to act directly on post-synaptic dopamine receptors [9], while amphetamine and morphine act indirectly by promoting the release of dopamine from presynaptic terminals [4,9].

METHOD

Animals and Drugs

Male Sprague-Dawley rats (200–250 g) were housed in groups of six under a 12-hour light/dark cycle with food and water ad lib. Animals were transported from the colony room to the laboratory 1–3 hours before behavioral testing was conducted. Drugs dissolved in 0.9% NaCl were administered to the animals immediately before placing them in the behavioral chamber of the apparatus. Haloperidol (0.3 mg/kg) and amphetamine (5 mg/kg, 1.25 mg/kg, 0.31 mg/kg) were administered intraperitoneally (1 ml/kg). Apomorphine (2 mg/kg) and morphine (5 mg/kg) were administered sub-

cutaneously (1 ml/kg). Controls were given 0.9% NaCl (1 ml/kg) administered intraperitoneally.

Apparatus

The behavioral chamber of the RoSe (Northeastern Neruosci. Prod., P.O. Box 371, Winooski, VT 05404) consists of two translucent 12 inch bowls which hinge together to form a spherical chamber. A small hole (1.5×0.5 inches) at the point in the sphere directly opposite the hinge is used as a viewing point. A linear potentiometer attached to the upper hemisphere senses the position of the animal. The freely turning shaft of the potentiometer runs through a small hole in the upper hemisphere to project 0.5 inches inside the chamber. The harness consists of a stiff wire, one end of which is fixed to a clip which in turn attaches to the shaft of the potentiometer. The other end of the wire, sheathed by plastic tubing, is looped snugly behind the forelegs of the rat. The electric leads from the RoSe are connected directly to the 7PI channel of a Grass polygraph.

Turning of the animal (and, thus, the potentiometer shaft) to the left and right result in opposing changes in the resistance of the potentiometer, and thereby generate opposing slopes on the Grass polygraph output. The design of the animal harness and the translucent hemispheres are similar to those included in a rotometer described by Greenstein and Glick [5].

Surgery

Animals were anesthetized with sodium pentobarbital (50 mg/kg IP) and placed in a stereotaxic apparatus. The tip of an 0.2 mm cannula was lowered to the substantia nigra pars compacta at coordinates LR 1.9, AP −2.6, and V 8.6, according to the Atlas of Pellegrino and Cushman [8]. To produce lesions in the nigrostriatal tract, 4 μl of a freshly prepared 0.9% NaCl solution containing 4 μg of ascorbic acid and 8 μg of 6-OH dopamine were injected at a rate of 1 μl/min. Sham lesioned animals were injected with 4 μl of 0.9% NaCl. In both groups the solutions were injected into the nigra on the side toward which the animals preferred to rotate before surgery.

Behavioral Assessment

In the initial study, each animal was monitored in a RoSe for two 60 minute intervals, separated by 48 hours. Animals were treated with morphine, amphetamine, apomorphine, or 0.9% NaCl immediately before placement in the apparatus. A given animal received the same drug on both days of testing, and each treatment group consisted of at least six animals. In the amphetamine dose response study, however, the animals were placed in the RoSe 20 minutes before drug treatment.

In the lesion study, animals were given morphine subcutaneously one and three days prior to surgery, and on days 2, 4, 6, 8, 14 and 16 following surgery. Immediately following injection, each animal was harnessed, placed in the center of the apparatus and monitored for one hour. On alternate days (3, 5, 7, 15 and 17) following surgery, the drug-free behavior of each animal was monitored for 20 minutes.

During the behavioral testing, animals were observed through the viewing port so that the ongoing behavior of the rat inside the apparatus could be correlated continuously with the output of the polygraph. Stereotypic behaviors such as rearing, grooming, posturing, sniffing, and licking were noted directly on the chart recordings during the testing session. Additionally, the trained observer wrote qualitative descriptions of general head and body movements.

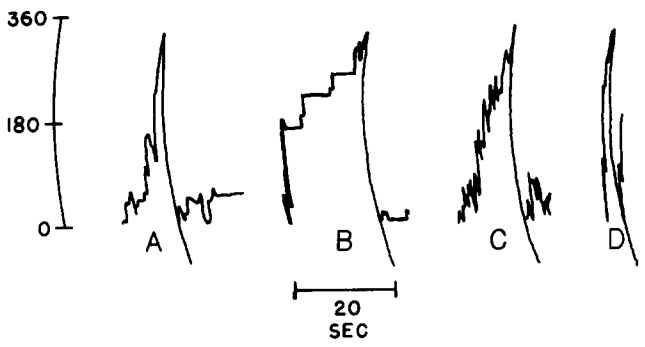

FIG. 1. Examples of 360° counter-clockwise rotations of animals treated with (A) saline, (B) morphine (5 mg/kg), (C) amphetamine (5 mg/kg), or (D) apomorphine (2 mg/kg). As the animal turns, the shaft of the potentiometer rotates, changing the resistance in the circuit and thereby causing the pen on the polygraph to deflect. The slope of the pen tracing indicates the direction of rotation, while the extent of the pen deflection corresponds to the magnitude of the turn. For example, the indicated rotations are full pen deflections with positive slopes which correspond to 360° counter-clockwise rotations.

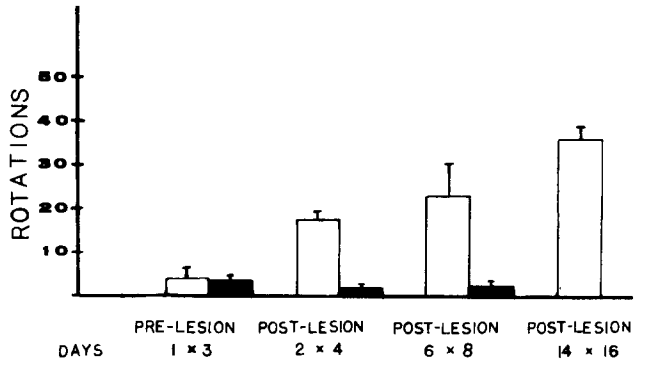

FIG. 2. Rotational asymmetries of animals which were challenged with systemic morphine (5 mg/kg) on different days after receiving a unilateral injection of 6-OH dopamine (□) or saline (■) into the substantia nigra pars compacta. The vertical axis indicates the number of net ipsiversive rotations (rotations toward minus rotations away from the lesioned side) executed in the hour following morphine challenge. Each bar represents the average of the two treatment days indicated on the horizontal axis.

RESULTS

To quantitate rotation, we followed Greenstein and Glick's definition of stereotypic net rotations [5]; that is, to execute a rotation, the animal must progress sequentially through four quandrants of a circle. The number of rotations in one direction are then subtracted from the number of rotations in the other direction to give the number of net rotations. Figure 1 shows the configurations of full rotations for animals under the influences of various drugs, as monitored by the RoSe. Note that although each animal rotated in the same direction, it was accomplished in a qualitatively differ-

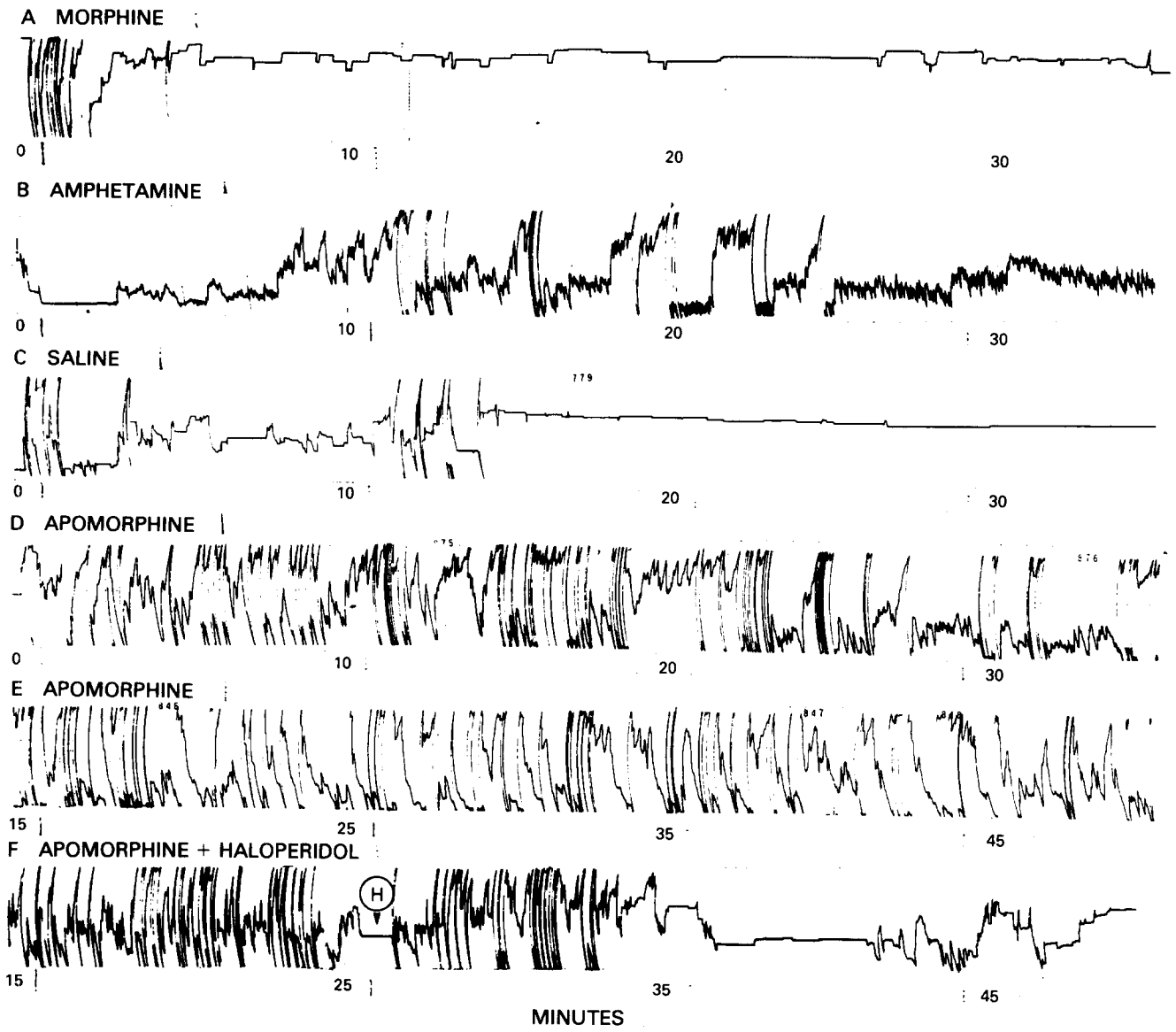

FIG. 3. Sample polygraph recordings of animal behavior monitored by the RoSe. The horizontal axis indicates time after injection and placement in the apparatus. The vertical axis indicates animal position (see text). (A) Morphine (5 mg/kg) (note the blocked pattern). (B) Amphetamine (5 mg/kg) (note the rapid "up-and-down" pen deflections of small amplitude). (C) Saline (complex and varied pattern). (D) Apomorphine (2 mg/kg) (note large number of long, smooth pen deflections). (E) Apomorphine at later times. (F) Apomorphine followed by haloperidol (0.2 mg/kg) (point marked H) at 27 min (compare with E).

ent manner. Saline-treated animals execute varied and complex sequences of movement (series of complex and varied pen deflections) (Fig. 1A). Morphine-treated animals (Fig. 1B) exhibit pauses (horizontal pen tracings) during their rotations. Amphetamine-treated animals (Fig. 1C) execute rotations marked by rapid side-to-side movements of the head and shoulders, which produce rapid deflections of the pen. Finally, apomorphine-treated animals (Fig. 1D) turn rapidly, employing long, extended movements (long smooth pen deflections).

The influence of unilateral 6-OHDA lesions of the substantia nigra on rotation, in animals challenged with morphine, is illustrated in Fig. 2. Rotational asymmetry increased markedly in a time-dependent manner after the lesion in animals receiving 6-OHDA, but not in sham-lesioned animals. By observing the behavior of each animal following the injection of saline or morphine on alternate days of testing, we found that the administration of morphine accentuated the rotational asymmetries of the lesioned animals.

In order to assess the consistency of the rotational preferences of normal (non-lesioned) animals, we developed a paradigm consisting of two days of testing. On each day we defined the preferred side of a given animal to be the side toward which the most stereotypic turns were executed. For morphine-treated animals, the same side was preferred on the first and second day of testing ($N=18$, $p<0.05$; rank test).

TABLE 1

DOSE RESPONSE RELATIONSHIP AND INHIBITION BY HALOPERIDOL OF AMPHETAMINE INDUCED INCREASE IN NUMBER OF STEREOTYPIC MOVEMENTS

Time after Injection (min)	0–10	10–12	20–30	30–40	40–50	50–60	Total 10–60
Saline	15.2 ±3.5	6.3 ±3.3	13.5 ±3.2	6.3 ± 3.7	8.2 ±4.2	0.0 ± 0.0	33 ± 9.3
Amphetamine 0.313 mg/kg	15.7 ±5.3	20.8 ±5.5	20.3 ±5.4	13.2 ± 5.8	14.5 ±6.3	11.8 ± 3.4	79 ±21.9
Amphetamine 1.25 mg/kg	30.2 ±6.8	23.7 ±3.2	19.5 ±7.0	28.3 ± 4.1	26.3 ±3.3	11.5 ± 4.3	109 ±13.8
Amphetamine 5.00 mg/kg	23.8 ±4.8	37.8 ±8.3	53.0 ±8.7	60.8 ±10.1	53.5 ±5.6	41.7 ±11.0	247 ±21.3
Amphetamine 5.00 mg/kg + Haloperidol 0.3 mg/kg	19.7 ±5.6	15.0 ±4.1	12.2 ±5.4	12.8 ± 5.1	10.0 ±4.3	12.3 ± 4.3	62 ±25.2
Haloperidol 0.3 mg/kg	9.3 ±3.3	8.2 ±2.6	6.0 ±3.6	1.5 ± 0.7	5.2 ±3.1	0.0 ± 0.0	21 ± 8.2

Data represent mean number of "up-and-down" pen deflections determined for six animals ± SEM.

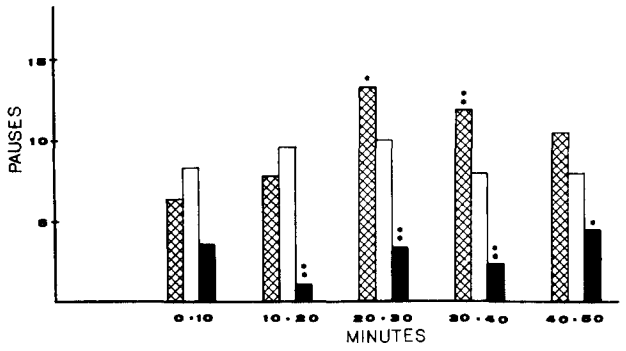

FIG. 4. Effect of morphine (5 mg/kg □), saline (□), and amphetamine (5 mg/kg ■) on "pausing." The horizontal axis indicates consecutive 10 min intervals after drug injection. The vertical axis indicates the number of pauses (number of horizontal tracings lasting more than 10 seconds), (*$p<0.05$, **$p<0.01$ rank test compared to saline; N=12 for amphetamine and N=40 for morphine).

We were unable to demonstrate a consistently preferred side in animals treated with saline (N=18, $p>0.05$) or amphetamine (N=6, $p>0.05$). Additonally, the administration of morphine significantly increased the number of net rotations compared to saline (N=18, $p<0.05$). Saline-treated animals rotated at the rate of 1.0±0.77 net rotations per hour; whereas morphine-treated animals rotated at the rate of 3.08±0.77 net rotations per hour toward the side preferred on the first day.

Figure 3 shows sample recordings of movement patterns of normal rats after treatment with morphine, amphetamine, saline, or apomorphine. One can readily distinguish which drug a given animal received by inspection of the polygraph tracing. For instance, apomorphine treatment (Fig. 3D, E) was marked by the presence of a large number of uninterrupted extended movements (long relatively smooth pen deflections), absent from the records of amphetamine-treated animals (Fig. 3B) and rare in those of morphine- (Fig. 3A) or saline-treated (Fig. 3C) animals. The extended movements observed following apomorphine were readily blocked by the neuroleptic, haloperidol (Fig. 3F).

The records from apomorphine (Fig. 3D–F) and amphetamine (Fig. 3B) treated animals always showed a large number of very rapid "up-and-down" pen deflections. These deflections, when of large amplitude (as in Fig. 5A), were due to stereotypic rearing, whereas deflections of small amplitude were due to rapid stereotypic side-to-side movements of the head and shoulders. Thus, the counting of "up-and-down" pen deflections provides a measure of the number of stereotypic movements displayed by a given animal. We quantitated "up-and-down" pen deflections by counting the number of upward deflections which corresponded to a change in body position of 20° or greater and were followed within 10 seconds by a downward deflection of the same magnitude.

Table 1 shows that amphetamine induced "up-and-down" pen deflections in a dose-dependent manner and that haloperidol diminished the effect of the highest dose of amphetamine. Table 1 also shows the time course of the effect of amphetamine on "up-and-down" pen deflections. At both the 5 mg/kg and 1.25 mg/kg doses of amphetamine the largest increase in number of "up-and-down" pen deflections was observed 30–40 minutes post injection. The number of "up-and-down" pen deflections doubled for each multiple of four increase in amphetamine dose. Neither the rearing nor the

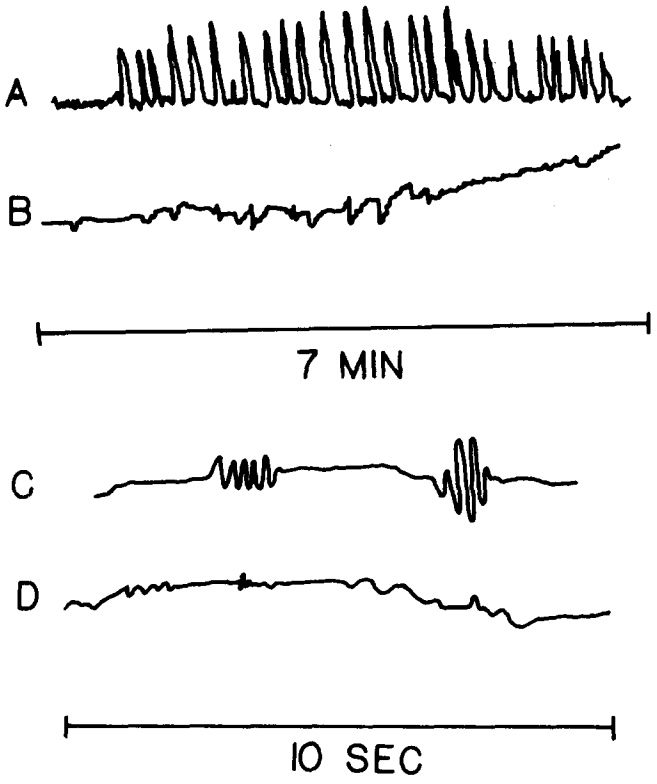

FIG. 5. Wave forms of selected behaviors monitored by the RoSe. (A) Rearing (rapid "up-and-down" pen deflection of large amplitude), (B) grooming (note repetitive and rounded deflections of medium amplitude), (C) shaking (very rapid and large pen deflections), (D) licking and sniffing (note very rapid deflections of small amplitude).

side-to-side movements were made by morphine- or saline-treated animals, whose records showed few of these rapid "up-and-down" pen deflections (Fig. 3A, C).

The records of morphine-treated animals were typified by a characteristic blocked or squared pattern, which corresponded to discontinuations of movement. We quantitated this "pausing" by counting the number of occurrences of horizontal pen tracings lasting more than 10 seconds. Figure 4 shows the time course of the effect of various treatments on pausing; morphine increased whereas amphetamine decreased pausing relative to saline.

Visual comparison of the behaviors of animals in the RoSe to the continuous output from the polygraph has revealed several discrete patterns which correspond to distinct stereotypic behaviors (Fig. 5). Rearing produced a very rapid "up-and-down" pen deflection of large amplitude. Grooming corresponded to regular and repetitive pen deflections of moderate frequency and amplitude. Episodes of shaking resulted in periodic bursts of rapid pen deflections. Licking and sniffing corresponded to regular repetitive pen deflections, which were very rapid, and had very low amplitude.

DISCUSSION

The objective of this paper was to describe a newly developed rotation sensing device (RoSe), and the application of this device to the automated monitoring of rotational asymmetry, various stereotypic behaviors and other indices of psychomotor function. Using the RoSe, we were able to replicate the observation that morphine enhances the rotational asymmetries of normal animals in a manner which is consistent from day to day [4], and the finding of Iwamoto and others [6] that morphine induces a slow ipsiversive rotation in 6-OHDA-lesioned animals. Furthermore, we have demonstrated that morphine-challenged animals show increasing rotational asymmetry with time after 6-OHDA lesions.

By continuously recording the position of the animal, the RoSe precisely monitored rotational behavior, both qualitatively and quantitatively. In fact, we found that a single rotation could serve as a behavioral "fingerprint" for several psychoactive drugs. In addition to rotation, we have demonstrated two other measures of behavior that the RoSe quantitatively monitors. Counting the frequency of "up-and-down" pen deflections provided a means of quantifying stereotypic movements, while horizontal pen deflections were counted to measure bouts of discontinuation of movement (pausing). In conclusion, the RoSe provides a convenient means of simultaneously monitoring a number of measures of behavior. Quantitation of three of these behavioral measures provided a means of distinguishing among various drug treatments which are known to stimulate central dopaminergic neurotransmission by different mechanisms.

The RoSe compares favorably with other automated monitors of behavior that are currently available. First, while the radar and capacitance field devices are able to measure stereotypic movements, they have a very limited ability to distinguish among types of stereotypic movements, and are not able to measure the lateralization of locomotor movement (rotation). Previously described rotometers measure laterality, but are not able to measure stereotypic movements. The RoSe distinguishes among types of stereotypic movements and simultaneously monitors rotation. Finally, the RoSe is commercially available at a considerable cost advantage compared to the competing technology. For some applications, the RoSe may be at a disadvantage, compared to the more elaborate devices which monitor stereotypic movements, in that the animal is placed in a highly artificial environment and that interactions between animals cannot be monitored.

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