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An Automated Method for Measurement of Circling Behavior in the Mouse

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TORELLO, M. W., J. CZEKAJEWSKI, E. A. POTTER, K. J. KOBER AND Y. K. FUNG. *An automated method for measurement of circling behavior in the mouse.* PHARMACOL BIOCHEM BEHAV 19(1) 13–17, 1983.—Circling behavior in animals lesioned unilaterally in one striatum with 6-hydroxydopamine is widely used to test pharmacological compounds with dopaminergic activity. Although automated techniques employed to record this behavior have previously been reported, most of these methods either limit the movement of the animal or are less reliable for various reasons. This paper describes a new method which allows the free movement of the animal in addition to recording the circling behavior automatically. Furthermore, this device provides new and unique data on the diameter and total area of the circles that are circumscribed by the animal. Consequently, the present device may prove to be a better and more reliable tool to assess the pharmacological effect of drugs on the striatal dopaminergic system.

Circling behavior Automated rotation measurement

IN response to amphetamine administration, mice with unilateral 6-hydroxydopamine-induced lesions of the nigrostriatal dopaminergic pathway will circle to the lesioned side [4, 5, 8, 10, 11]. This circling model is widely used to test pharmacological compounds with striatal dopaminergic activity [2, 3, 10].

Observational methods of recording circling behavior are tedious and time-consuming and have led to the development of automated measuring devices. A good automated system should take into account the direction of circling as well as the number of turns. In addition, partial turns should not be recorded. Moreover, other locomotor behaviors such as grooming and rearing should not interfere with the recording. Although a number of automatic measurement systems have been developed using mechanical or photocell methods or a combination of each [1, 6, 7, 9, 12], most methods have drawbacks associated with them. Some methods are unable to determine the direction of the turn, while others record random activity or partial turns as complete turns [6, 7, 9]. Finally, most methods require the restriction of the animal with a body harness or head gear [1, 9, 12].

The automated method described below records the number of 360° clockwise and counter-clockwise turns. In addition, this new system of measurement can detect partial turns, the pattern of circling behavior and the circle diameter.

METHOD

Animals

Male Swiss-Webster mice (Laboratory Supply, IN) weighing between 23–30 g were used. They were allowed free access to food (Purina Laboratory Chow and water. All animals were housed in plastic cages (4/cage).

Surgery

The mice were anesthetized with chloral hydrate (420 mg/kg, IP) and an incision was made in a longitudinal direction. Four microliters of chilled physiological saline, containing 16 μg of 6-hydroxydopamine HBr (Regis Chemical Co.) and 2.4 μg of ascorbic acid were injected into the right striatum of the mice over a 4-min interval, using a stereotaxic instrument (David Kopf) and a 10 μl Hamilton syringe. The center of the corpus striatum was determined to be 3.5 mm below the skull surface, 2.2 mm lateral to the midline and 5 mm anterior to the occipital suture [5].

Apparatus

The "Videomex Turn-Track" System (Columbus Instruments), consists of three main components, (Fig. 1). The first component is a white cylindrical circling chamber with a

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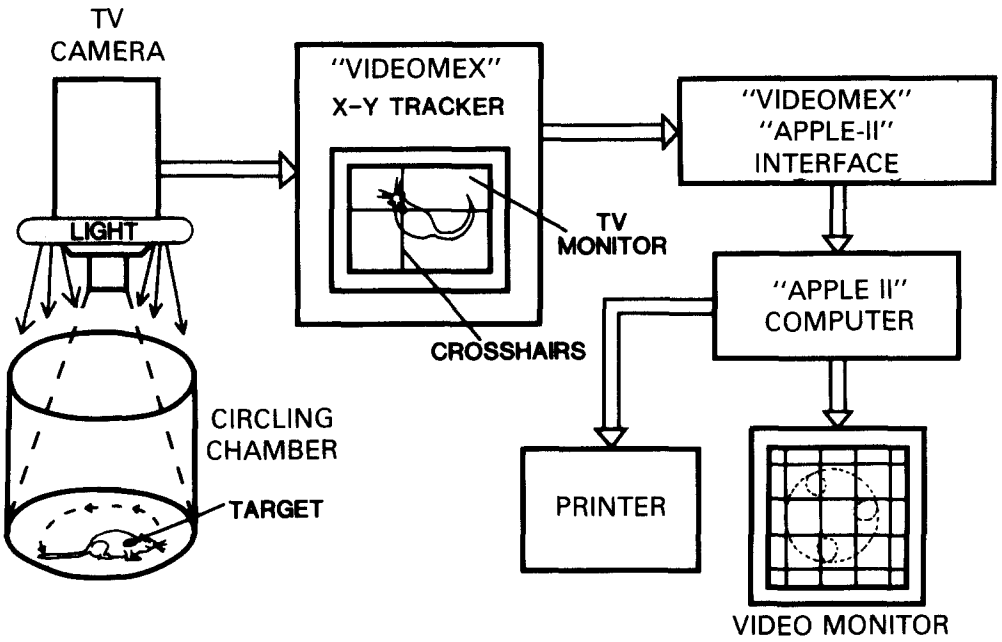

FIG. 1. Block diagram of "Videomex Turn-Track" system for automated monitoring of circling behavior.

white flat-bottom floor, measuring 34.5 cm in diameter and 37.0 cm in height. This chamber is illuminated by a circular fluorescent light positioned directly above it. The second component consists of a TV camera (Sanyo model 1610X) mounted on a tripod and directed toward the inside of the circling chamber. The camera provides a luminance (L) signal to the Videomex X-Y Tracker. Based on this L signal, the Videomex X-Y Tracker produces two analog voltages proportional to the mouse's X-Y coordinates in the camera's field of view. Crosshairs on the Videomex TV monitor "lock onto" and track any object which is different from the overall luminance in the field of view. The third component of this system consists of an Apple-II computer with video monitor and printer. The "Videomex Turn-Track" software is designed to operate on an Apple-II+, 48K system with Disk II and an Integral Data Systems Printer model 460. The computer program consists of an algorithm which combines a compiled Basic control program and an assembly language real-time interrupt program. At precise instants of time, the analog X,Y voltage inputs from the Videomex X-Y Tracker are transformed into digital information and stored in memory. The compiled Basic control program examines this digital information and determines the movement of the animal using preset criteria. These movements are then further analyzed to determine the animal's circling tendencies, utilizing a mathematical technique to remove noise and false movements such as grooming. The animal's movements are then plotted, in real-time, on the attached 12" video display (CRT) for experimenter information. The accumulated circling totals, both clockwise (CW) and counterclockwise (CCW), are also displayed on the CRT along with the totals for the five minute observation period. Each minute, the computer prints out CW and CCW totals along with the accumulated totals for the observation periods. The exact time of day is also printed enabling the experimenter to relate the data to the exact time it was collected. At the end of the five-minute period, the printer prints out the total number of turns and then copies the video data of the animal's movements (over the last minute) onto the printer (Fig. 2).

This graphic picture can be utilized to analyze the general activity of the mouse (resting, random movement) and the drug-directed activity (small or large circles). The computer then continues to monitor the animal's movement over the next minute. The compiled Basic control program repeats this process every 10 minutes until the end of the test period.

Procedure

The experiment started ten days after surgery. Initially, a black, non-toxic marker was used to mark a spot approximately 15 mm^2 on the neck of each albino mouse. This serves as a target to be tracked by the "Videomex Turn-Track" system. After a 30 minute adaptation period in the circling chamber, each mouse was injected with either saline

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                INTERVAL NUMBER 1
          THE TIME IS ***** SUN AUG 30  3:28:00 PM *****
#CCW TURNS THIS MIN=0  #CCW TURNS OVER 5 MIN=0
#CW TURNS THIS MIN=0  #CW TURNS OVER 5 MIN=0


                INTERVAL NUMBER 2
          THE TIME IS ***** SUN  AUG 30  3:29:00 PM *****
#CCW TURNS THIS MIN=0  #CCW TURNS OVER 5 MIN=0
#CW TURNS THIS MIN=0  #CW TURNS OVER 5 MIN=0


                INTERVAL NUMBER 3
          THE TIME IS ***** SUN AUG 30  3:30:00 PM *****
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                INTERVAL NUMBER 4
          THE TIME IS ***** SUN AUG 30  3:31:00 PM *****
#CCW TURNS THIS MIN=0  #CCW TURNS OVER 5 MIN=0
#CW TURNS THIS MIN=3  #CW TURNS OVER 5 MIN=3


                INTERVAL NUMBER 5
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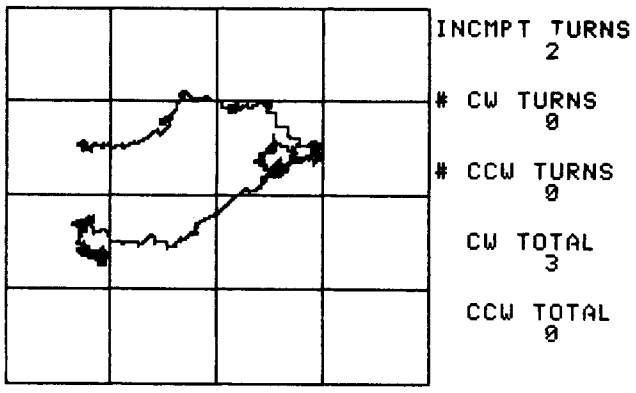

FIG. 2. Sample printout showing performance of one saline animal for one five minute observation period. The grid is the graphical representation of the test chamber floor displayed on the video monitor. Each grid is 9.6 cm².

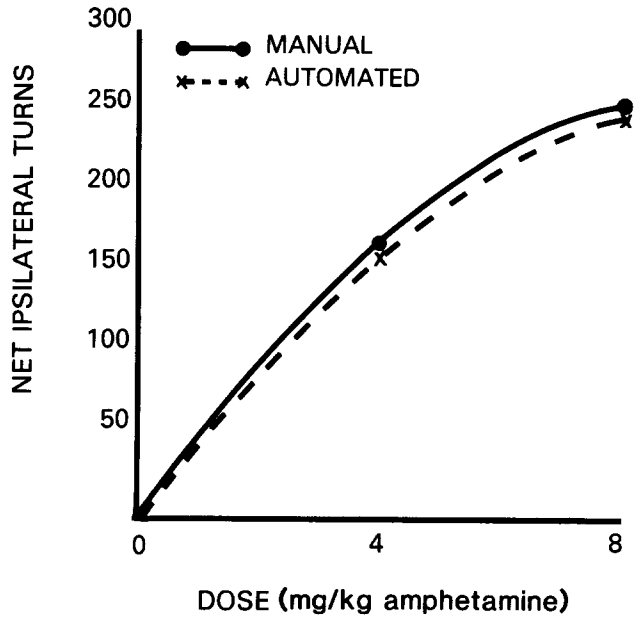

FIG. 3. Dose/response curve for manual versus automated measurement of circling behavior for the average of all five of the five minute observation periods. (N=4 animals/group). Standard errors of the means for the manual group are 3.3, 71.0 and 45.5 respectively and similarly for the automated group 1.6, 67.8 and 40.2.

or amphetamine (4 mg/kg IP, or 8 mg/kg IP). Ten minutes after injection, the number of 360° turns (clockwise or counter-clockwise) were manually and automatically counted for 5 minutes. In this experiment, in order for a circle to be counted, the mouse has to complete it within 5 seconds. The experimenter can increase or decrease this 5 second criteria by changing one variable contained within the basic control program. These measurements were repeated at 20, 30, 40 and 50 minutes post-injection. Computer printouts were generated containing subtotals of the number and direction of circles for every minute of recording. No data acquisition was lost during the printing of this information. A picture printout of the animal's activity for the fifth minute of observation was also generated by the computer.

All mice were tested for circling behavior between 12:00 noon and 6:00 p.m. at 23±2°C in a quiet environment. The administration of either saline or amphetamine was counterbalanced between animals and over days.

RESULTS

The "Videomex Turn-Track" system was found to accurately and reliably measure circling behavior of the mouse when compared to manual recording (Fig. 3). The difference in the measurements, obtained by both methods (manual vs. automatic), for the saline group was not significant, $t(3)=0.42$, $p<0.35$, ns. Similarly, there was no difference for the amphetamine-treated groups for a 4 mg/kg dose, $t(3)=1.92$, $p<0.07$, ns and for an 8 mg/kg dose, $t(3)=1.10$, $p<0.18$, ns.

The Videomex system provided a printout of the pattern of circling behavior during the fifth minute of each observation period. Thus, the total area traversed by each animal could be calculated for a representative minute of observation. Furthermore, information obtained from the computer allowed for precise determination of the diameter of a typical 360° turn circumscribed by the animal. This data is shown in Fig. 4. The average diameter of circles circumscribed by the animals in both drug-treated groups was 56±3 mm. The pattern of rotational behavior between both drug groups was

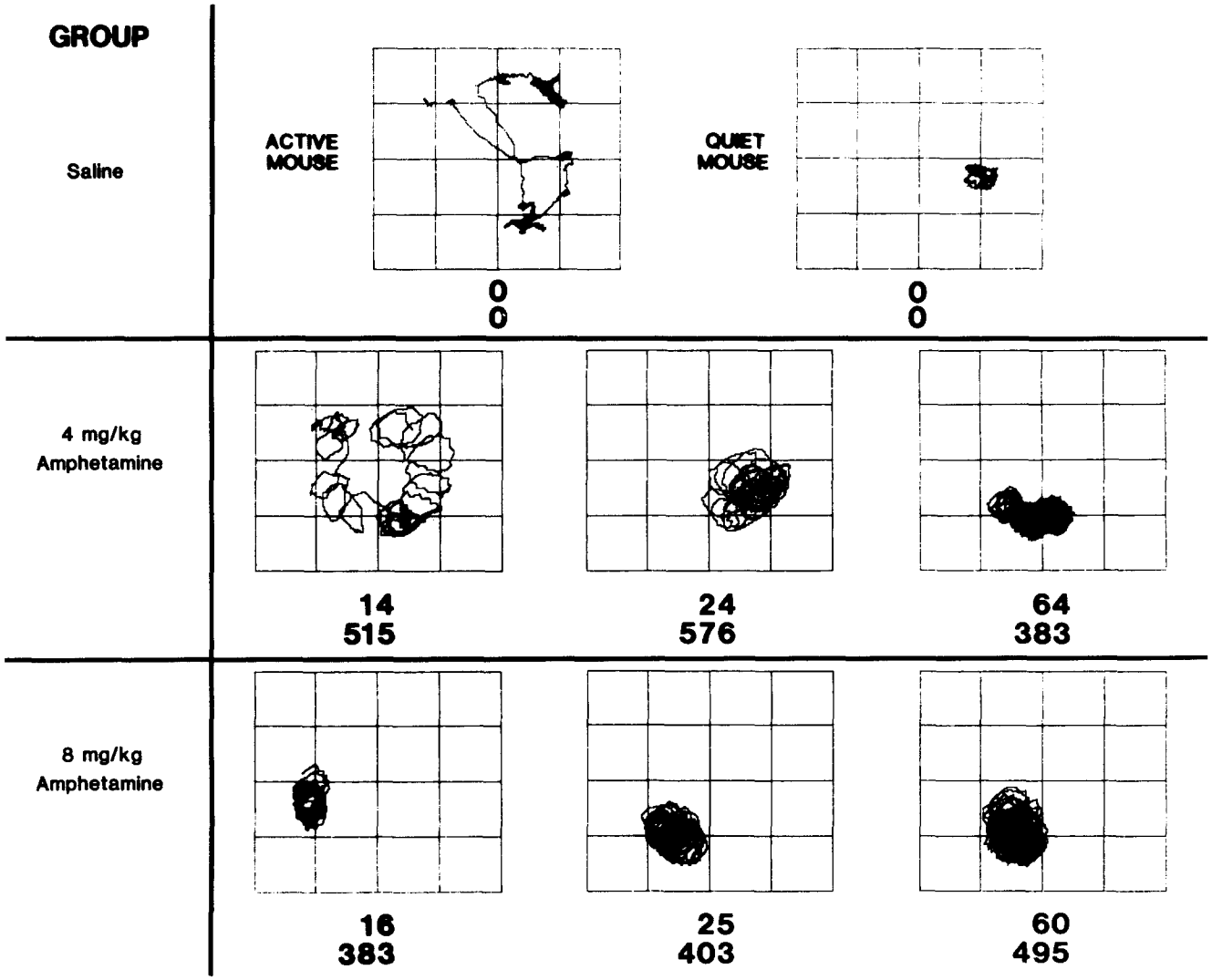

FIG. 4. Typical behavior patterns of representative animals during the last minute of a five minute observation period. The first number below each grid shows the number of circles made during this minute and the second number represents the total area covered (mm^2) during this circling behavior.

quite similar for animals that turned at a moderate or fast rate (Fig. 4). However, animals which turned at a relatively slow rate (approximately 1 turn every 4 seconds) showed marked differences, not in the total area covered during the circling, but in the pattern of circling. The slow-turning animals in the lower dose group (4 mg/kg) circumscribed a larger circle as they moved in smaller circles. In comparison, animals in the high dose group (8 mg/kg) turned in smaller circles in a somewhat smaller area without circumscribing larger circles. (Precise measurements of the diameters of the circles produced by each animal can be made by examination of the computer printouts.) This difference in the observed circling behavior may reflect unique dose-dependent effects on the dopaminergic system with amphetamine administration. Further studies are required to elucidate this finding. Figure 4 also shows the random behavior of an active and inactive animal from the saline group as a marked contrast to the animals in the amphetamine-treated groups.

DISCUSSION

The results demonstrate that the "Videomex Turn-Track" system can be used, in place of tedious observational recording, to reliably measure the rotational behavior of mice. The difference between manual and automatic recording represents a total error of 4%. This was determined by summing all the observations for each animal across all groups for the manual condition (60 observations) and similarly for the automated condition (60 observations). One reason for this error discrepancy may be due to human judgement involved in determining if an animal has completed a 360° turn and if it has been completed within five seconds. This judgement was most difficult for slow turners. Indeed, the error for slower turners between man and machine was up to 10% while the error for fast turners was less than 2%. Judgement error of this kind is eliminated when an automated system is being used, since the computer reliably executes a preset criteria in detecting a 360° turn within 5 seconds.

Additional information provided by this system concerning circle diameter, total area covered during rotational behavior and printouts of the pattern of rotation represents a unique and significant improvement over other measurement systems currently available. Studies can now be designed to determine whether the pattern of circling is drug-dependent or dose-dependent. Differences in the diameter of circles or area travelled during circling may reflect differential responses of the dopaminergic system to various pharmacological compounds or may represent damage to different areas of this system.

This system can be expanded with multiple TV cameras to sequentially sample the behavior of up to sixteen animals. In addition, this instrument can provide on-line data analysis as in this study. Furthermore, if the behavior is recorded on videotape, off-line data analysis can be performed as well.

The "Videomex Turn-Track" system could also be used in other experiments involving small animals in an open field environment where precise measurement of the animal's pattern of behavior is required. In conclusion, the present system represents a substantial improvement in monitoring the circling behavior of 6-hydroxydopamine-lesioned animals.

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