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Rat Rotation Monitoring for Pharmacology Research

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WALSH, M. J. AND E. K. SILBERGELD. *Rat rotation monitoring for pharmacology research.* PHARMAC. BIOCHEM. BEHAV. 10(3) 433–436, 1979.—Much current neuropharmacological research describes drug site of action and effect in terms of rotational behavior in rats. Such studies at the National Institutes of Health led to the development of required instrumentation capabilities. The method developed for quantifying rotational behavior to the exclusion of other locomotor activity is presented. Such full turns counts can be output as mechanical counter inputs, computer inputs, user-selected time interval subtotals, summary totals, or a voltage proportional to cumulative count which provides turning-rate data when recorded on strip-chart. The latter two options are described herein.

Rotation Circling behavior Rotometer

EVALUATION of potency and site of action of dopaminergic drugs utilizes rotational (turning) behavior of rats as a quantitative measure. Animals which have a central nervous system imbalance due to unilateral lesion in the nigrostriatal pathway circle in a manner indicating the influence of such drugs [9]. In addition, rotational behavior has been produced by unilateral lesions of the raphe and by administration of serotonergic compounds [3]. Recently rotational behavior after unilateral intracerebral injection has been used to study many novel drugs [8]. Further, rotations can occur in nonlesioned rats administered dopamine agonists peripherally; this has been used to indicate striatal asymmetry [1].

Interest is focused on the direction and amount of rotation, to the exclusion of any other locomotion subsequent to drug administration. The need to record only rotational activity is based upon the need to quantify only the rotations without possibility of erroneous counts due to noncircling locomotion activity, grooming, etc. Extra partial turns are a better measure of a different behavior, and possibly depend on different neurotransmitter pathways [2]. Direction of rotation, clockwise (CW) or counterclockwise (CCW), can be matched to the site of lesion to determine ipsilateral/contralateral effect.

The observation method of early experiments prompted development of automated recording techniques. The earliest and simplest method relied upon a single switch and observer notation of direction [10]. Error can be introduced for partial turns or other random activity which makes the switch.

A more complex four photocell method followed which counts full turns and senses direction, but uses a floating reference point which is reset for each change in direction [2]. This method also provides superfluous information, i.e., extra quarter turns, which is not indicative of rotational behavior.

Yet another method uses two photocells to describe direction and count turns [6]. While it does satisfactorily determine change of direction, it does not positively determine the actual direction and is subject to one initial miscount.

The instrumentation techniques described below concisely measure only full turns and positively determine the rotational direction. They have been used reliably for over two years. The discussion is deliberately general to allow application to the technology most available and familiar to the reader. At NIH, the devices were constructed of standard transistor-transistor logic (TTL) and metal-oxide semiconductor (MOS) circuitry. For brevity, the details of hardware are omitted but are available as working drawings from the authors upon request.

METHOD

Data Derivation

Each animal is typically placed in a flat-bottomed Plexiglas cylinder of 12–18" dia. with no central obstruction for the testing period. Each is harnessed in a Velcro waistband/wire neckloop (manufactured by BRS-LVE) which connects directly via a stiff spring to a free-moving disk mounted in ball bearings in a headstage transducer. The headstage transducer is positioned centrally above the animal and supports a portion of the spring's weight. Thus, turning motion of the animal is converted to turning of the disk. It is significant that this technique does not seem to significantly alter the animal's turning behavior.

The determination of direction and full turns requires a minimum of three discrete sensors; use of fewer allows spurious counts for certain partial movements; a larger number does not increase resolution but does increase the possibility of collecting unwanted information.

Three slotted, optical-limit switches (Monsanto MCA 2-81) are fixed on the headstage perimeter as close together as possible, typically on radii of 15° separations. The rotating disk passes through the slotted switches without physical contact. These switches are integral Photodiode/Photo Darlington pair transistors which change electrical output level for completion/interruption of the light path across the slot. Thus, the movement of a cut in the rotating disk causes an electrical level shift as it passes through the switch. These three level shifts are input via cable to the logic circuitry.

This headstage transducer can be used on any shape container or cage and can be readily modified to allow use of catheters, tubing, or electrical stimulation apparatus.

Logic Interpretation

The logic circuit of Fig. 1 creates a pulse output on either the CW or CCW count line as described below.

The center optical limit switch (center switch) initializes the circuit by setting its flip-flop and resetting all others. Since the circuit must be enabled by one crossing of the center switch, that partial turn between the starting position of the animal and the center switch will be neglected by the circuitry.

A subsequent CW or CCW switch change (15° turn) is paired (logical "and") with the initializing condition to set the proper "directional sense" flip-flop, inhibiting the opposite "directional sense" flip-flop and enabling the proper count line.

Subsequent crossing of the remaining switch is logically paired with the CW or CCW switch first crossed to produce the "full turn" condition, causing a full-turn pulse which is output on the enabled count line. Completion of the rotation to the center switch reinitializes the circuit.

Should direction of rotation be reversed before all three switches are sequentially made, no count is output. If the center switch is crossed a second time before the CW and CCW switches are made, the circuit is reset and the partial turn is rejected as non-rotational behavior. Because all such partial turns are rejected, the circuit will not create an erroneous count pulse for any partial turn.

Data Output

Subsequent data processing is determined by the user's needs. Count pulses can be used to drive mechanical counters directly, can be input directly to a TTL-compatible computer input for on-line recording, or can be processed further, as discussed below.

Data Accumulation

Each turn-count increments a binary counter which sums counts for the interval of interest. The maximum count per interval is 255. Two counters are required for each observed animal/data channel—CW and CCW. As many as eight, simultaneously-clocked channels have been used to date.

One approach transfers these summary counts to buffers and digital-to-analog converters (DACs) every ten sec during either five min or ten min intervals. The counters are cleared at the end of each time interval. This provides a rate-meter output when displayed on strip-chart recorder, showing the cumulative total number of turns, incremented each ten seconds. Figure 2 shows a sample of this type data derived from studies of intranigral injection of compounds active on dopaminergic and GABAergic systems (Silbergeld and Walters, in preparation). In addition, this system has been used in studying rotational behavior in kainic acid-lesioned rats [7]. This method has disadvantages because it requires a

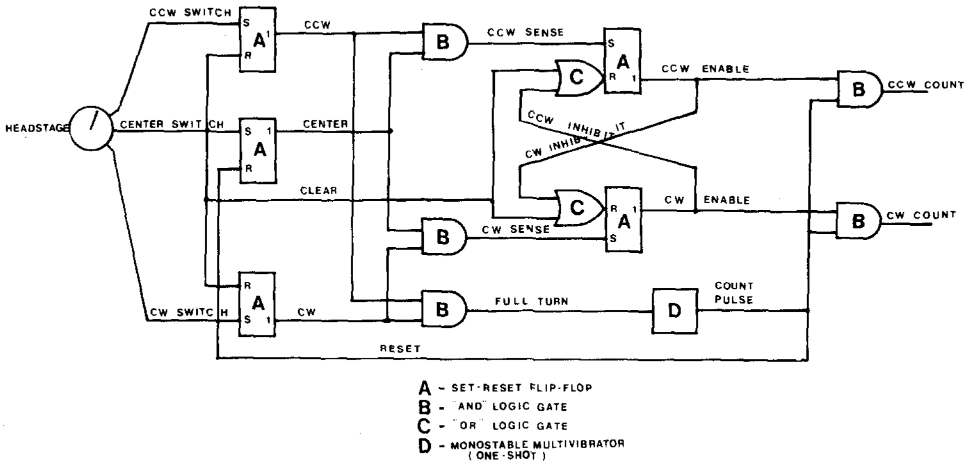

FIG. 1. Functional circuit diagram of logic for sensing direction of rotations and turn counts.

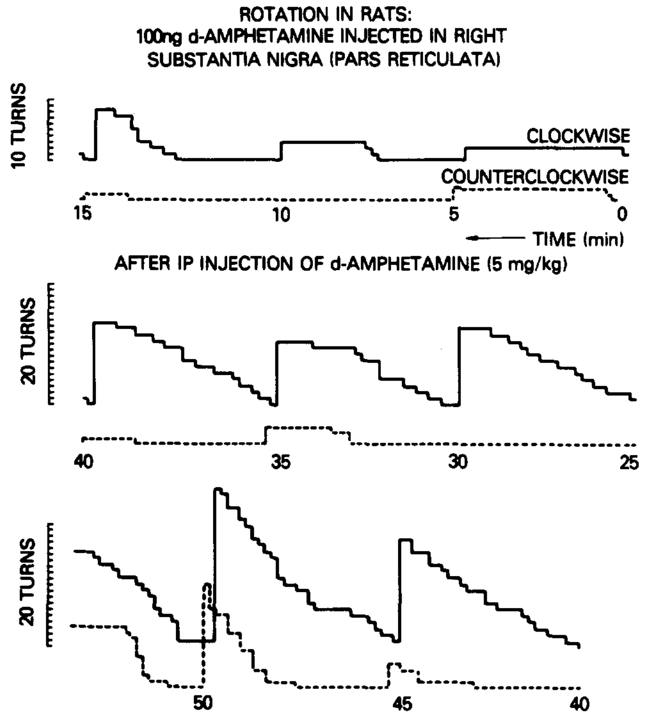

FIG. 2. Rotation-rate data recorded from a male, Sprague-Dawley rat injected unilaterally in right substantia nigra (pars reticulata) with 100 μg d-amphetamine in 1 μl saline. Contralaterally, the rat was injected with 1 μl saline. Rotations were recorded for 55 min after recovery from halothane anaesthesia; at 15 min the rat was injected intraperitoneally with d-amphetamine (5 mg/kg). Each turn deflected the recorder 1 mm; output sampled each ten seconds; reset each 5 min. (Data from Silbergeld and Walters, in preparation.)

large number of strip-chart recorders for multi-channel operation and it is difficult to read the exact number of turns from the strip-chart recording. Its advantage is an output which displays a rate of turning.

An alternate method (shown in block diagram, Fig. 3) inputs the CW and CCW counts to binary counters for summary over the interval of interest to the user. Data is accumulated for a selectable time interval; a range of 5 to 60 min in five-min increments has been used. At the end of the timed interval, the summary counts are stored for later retrieval and all counters are cleared.

The storage memory (Fairchild F3539) is a 256×8 static, random-access, MOS integrated circuit. Two devices are operated in parallel to store separate CW and CCW data and to allow paired simultaneous writein/readout.

The memory has a common Input/Output structure, permitting a quiescent data readout mode which allows the user to output CW/CCW data from memory to LED displays for experimental record on a nearly continuous basis. The memory address of the data displayed is selected by either thumbwheel switches or a manually-stepped, LED counter display for each trial interval and channel. Thus, interval summary counts can be copied during the experiment or at its conclusion.

At the end of each time interval, the data read/write circuit overrides the readout mode for a write cycle to store data. The entire cycle is accomplished in a few milliseconds and occurs almost unnoticed. The write mode multiplexes memory address from readout control (i.e., selected data display interval and channel) to writein control (i.e., current trial interval and channel selected by the write circuit) and shifts the memory from read to write mode.

Next the tri-state outputs of the binary data counters for each channel are sequentially enabled. As each channel is addressed, the CW/CCW counts are transferred and written into the proper memory location. After all channels have been accessed, the memory is returned to readout mode and memory address control is switched back to user control.

At the end of 32 intervals, the circuit disables the timing so that no further write cycles are possible and the data in memory is protected from inadvertent overwrite.

A memory clear feature is included, which requires a three-step sequence to prevent inadvertent memory erase. Clear is accomplished by applying a high-speed pulse train as interval input, equivalent to running the entire 32 interval trial in three sec and writing zeros into the data locations of memory.

This data accumulation technique has been used to determine the effect of opiates on nigrostriatal dopaminergic activity [4] and to analyze the mechanisms and sites of action of opiate alkaloids and opioid peptides in modulating motor behaviors [5].

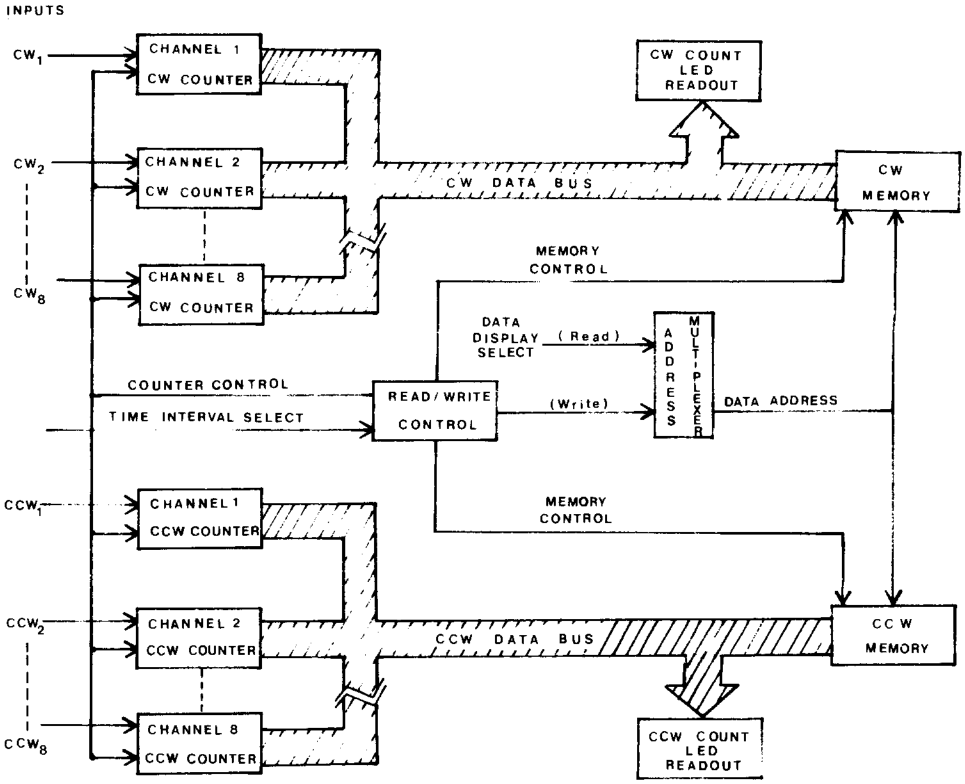

FIG. 3. Block diagram of eight-channel data accumulation storage, and readout processes for interval summary counts.

DISCUSSION

The instrumentation techniques described successfully monitor the rotational behavior of rats in pharmacological studies by quantifying animal turning behavior and its rotational direction. Count and direction data can be output consistent with the experimenter's needs. Two processing techniques currently in use are described: the first provides a rate of turning output; the second provides accurate time interval summary counts of rotational behavior.

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