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A New Injection Technique for Intracerebral Drug Administration in Behaving Animals

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STEIN, E. A. AND D. RODD. *A new injection technique for intracerebral drug administration in behaving animals.* PHARMAC. BIOCHEM. BEHAV. 12(5) 815–817, 1980.—A new fluid pump and technique has been described for drug administration into either ventricles or directly into brain tissue of awake, freely behaving animals. The novel apparatus and procedures eliminate most of the common methodological obstacles to injecting nl volume amounts into freely moving animals.

Intracerebral drug administration Behaving animals Fluid pump Drug self-administration

MANY areas of neuroscience research require the delivery of small quantitites of chemicals or drugs into localized regions of the brain. One of the major problems encountered by many investigators, however, is how to accurately and repeatedly deliver these small (μl or less) quantities, especially when trying to use "freely behaving" animals.

In the past, individuals have resorted to microliter syringes driven either by hand or by syringe pumps [1, 2, 3]. However, when using behaving animals, one must also interpose a fluid slip ring into the delivery apparatus. These are normally leaky with large hysteresis characteristics that tend to act as fluid integrators, i.e., rather than permitting discrete micro-injections, they result in delayed onset and offset of fluid movements.

This communication reports the design and construction of a pump which can accurately deliver a predetermined amount of fluid (down to the nl range) into the brain of freely moving rats. With the proper behavioral control equipment, these injections may be programmed to be delivered automatically. The unique feature of this assembly is the reliable delivery of these small quantities as well as the mounting of the entire pump via electrical slip rings. This permits the entire pump to rotate and thus eliminates the need for a fluid commutator when using freely moving animals.

DESIGN AND CONSTRUCTION

The pump assembly is shown in Fig. 1.

The pump consists of a series of step-down gears driven by a small Hurst motor (Allied Electronics No. 811-0920, PCDA Series, 20 rpm). This motor starts within 20 msec and is clutched to stop within 1/5°. Connected to these gears are two stainless steel rollers which can be tightened together with two thumbscrews (see Fig. 1). A long piece of polyethylene tubing (PE 10) runs between the two rollers and is prefilled with the drug of interest. When the motor is activated, the rollers crush the PE 10 tubing between them forcing the fluid to move forward through the tubing and out the distal end, much like toothpaste squeezed from a tube. This whole pump assembly is mounted above the animal via a brush commutator/slip ring assembly and can move 360° with the animal. It is also arranged on a counter balance arm allowing it to follow vertical movements of the animal as well. Thus full range of motion of the animal is preserved while preventing twisting and kinking of the PE tubing. The physical arrangement of the complete apparatus is shown in Fig. 2.

OPERATION

The pump may be operated manually by the experimenter, under automatic programmed control or operantly by the animal, and has been successfully used in behavioral, self-administration, and neuropharmacological studies in our laboratory for several years [5].

The test animal is surgically prepared with a 28-ga stainless steel guide cannulae aimed to end 1 mm dorsal to the site of interest. At the time of the experiment, a 33-ga injection cannula, 1 mm longer than the guide, is lowered through the guide and locked in place with a screw cap. The other end of the injection cannula is glued (Eastman 910) to the PE tubing which goes through the pinch rollers. The surgical implantation and guide cannulae assembly is not significantly different from that previously described [3,4].

The quantity of drug delivered by the pump is determined by the speed of the motor, the gear tooth ratio, the size of the PE tubing and the duration of time the motor is activated. We have chosen to vary the activation time of the pump and keep all the above constant. On-time is set via an accurate solid state one shot.

CALIBRATION

The pump is calibrated daily prior to use. This is necessary due to changes in diameter of the PE tubing within a given batch. To calibrate, the 33-ga injection cannula is placed into one end of a 5 μl capillary sampling pipette (Corning No. 7099-U) graduated in 1 μl divisions. Since the delivery time is in constant time increments, one can follow

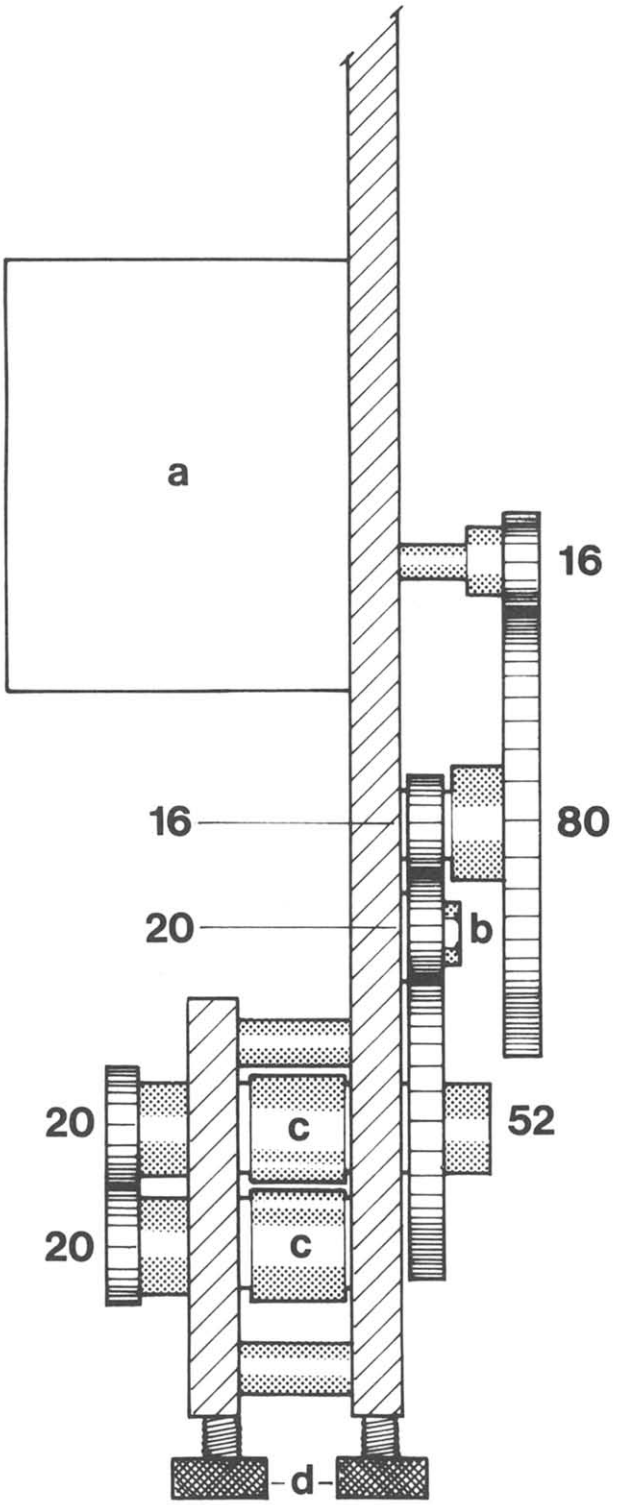

FIG. 1. Schematic drawing of pump assembly: a—Hurst PCDA motor (20 RPM). b—Clutch. c—Stainless steel rollers. d—Thumbscrews to tigten rollers. Numbers indicate number of teeth per gear.

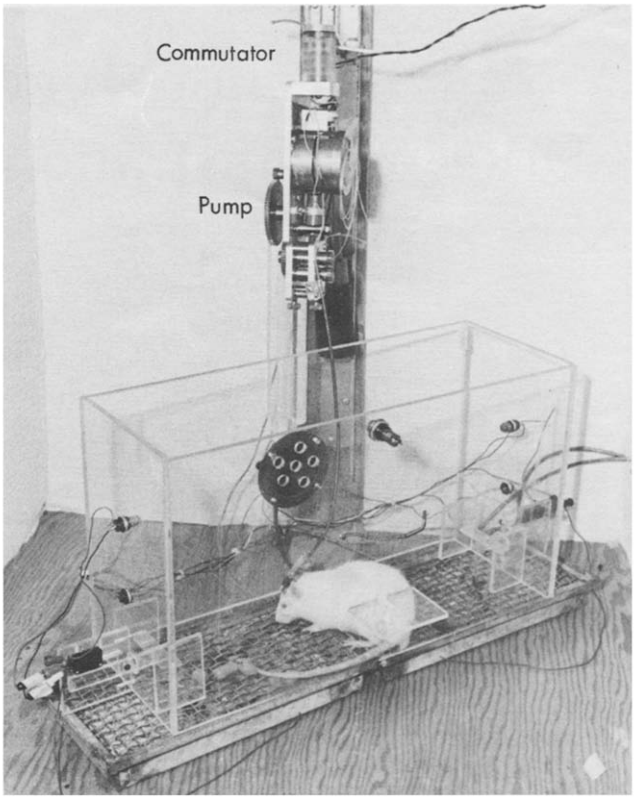

FIG. 2. The complete, assembled apparatus. Note the electrical slip ring commutator directly above the pump allowing for rotational movement. The entire apparatus is then mounted on a counterbalance arm allowing for vertical movements. Note the stainless steel spring connecting the rat to the pump. The PE 10 tubing runs through the spring and is thus protected from the rat.

the movement of the miniscus under a microscope as the pump is activated. It is then a simple matter to count the number of times the pump must be activated for the fluid to move 1 μl. Dividing by this number yields the average injection volume (e.g., if 100 activations equal 1 μl, each pump activation = 10 nl). In our experiments, our one-shot time for the above is approximately 280 msec.

To guarantee that each pump activation results in a single drug injection and that the two events are directly temporally related, great care is taken to visually follow the movement of the fluid miniscus under the microscope. It must move in discrete on-off steps with no back flow or forward "oozing" after the pump is inactivated. Air bubbles in the PE 10 or insufficient roller pinch pressure may cause either of the above. In addition, it is crucial that no fluid movement occur due to movement of the animal or PE tubing when the animal is actively moving and the pump inactive. Daily calibration of the system and visual monitoring as described above prevents such possibilities.

VERIFICATION

In order to verify that there are no leaks into brain or loss of fluid during movement, we initiated a rigorous quality control procedure utilizing a tritiated tracer agent (^{3}H-2-Deoxy D-Glucose (2DG) in this case). The ^{3}H 2DG was

TABLE 1
SYSTEM CALIBRATION — STANDARD CURVE

# Pressed into counting vial	CPM
10	520
20	1706
40	4654
60	6623
100	7725
1 μl ^{3}H	8140
Diffusion Controls	
1. Urethane anesthetized rat (no. 1)	170 CPM
Urethane anesthetized rat (no. 2)	170 CPM
2. Injection cannula in counting vial	130 CPM
3. Active rat—20 hr—no pump activation (no. 1)	180 CPM
(no. 2)	130 CPM
(no. 3)	303 CPM

supplied by Amersham and had a specific activity of 18.8 curies/mmol. It had a concentration of 1 mCi/ml and was used undiluted in the system.

The experiments performed were as follows: (1) A standard curve was determined by placing the injection cannula directly into a series of counting vials containing scintillation fluid. The pump was activated for 20, 40, 60 or 100 pulses per vial. Each pulse was calibrated with the above procedure to deliver 10 nl of 2DG. One μl was also delivered into a counting vial with the capillary pipette used for calibration.

To control for passive diffusion. (2) Animals previously implanted with guide cannulae were anesthetized with urethane and had injection cannulae filled with 2DG placed in their brains for period exceeding 12 hr. The pump was never activated. (3) 2DG-filled injection cannulae were allowed to sit in a counting vial overnight, again without the pump activated. (4) Active animals; freely moving rats were hooked up to the delivery system with 2DG in the cannula. They were allowed 20 hr to move about the chamber without pump activation and served to check for fluid leakage from the pump during movement. To be sure that fluid was being delivered into brain tissue, we also manually delivered 40 presses into the brain of a freely moving rat. Brains of all animals were removed and placed into TS-2 tissue solubilizer (Research Products International) to digest brain tissue and counted for 10 min.

RESULTS

Table 1 summarizes our system calibration results. A regression analysis performed on the standard curve gives an $r=0.946$, $p<0.05$. In addition, the 1 μl delivered during the calibration process coincides within 5% of the 100 presses into the counting vial, i.e., 1 μl ^{3}H=8140 cpm; 100 pulses into vial=7725 cpm. This indicates the pump assembly is accurately and reproducibly delivering 10 nl of the desired fluid. When allowing for the small diffusion error, this results in a reasonably linear curve.

The cpm of the active, behaving animals were 180, 130 and 303 cpm, which is not significantly different from the diffusion controls into anesthetized animals (170 cpm) or simply placing the cannula into a vial of counting fluid (130 cpm).

DISCUSSION

The above experiments indicate that our nl pump is an accurate and reliable apparatus for multiple discrete administration of small, predetermined amounts of drug into the brain of either an anesthetized or freely moving animal.

When the cannula tip was left either in a counting vial or in an anesthetized rat, the cpm was 130 and 170, respectively. That there was some minimal diffusion is not surprising since our labelled glucose tracer was diluted in saline and therefore would enable natural fluid exchange at the interface. The amount of drug leakage over a 20 hr period was the equivalent of less than 3 presses, i.e., 30 nl/20 hr.

Of greatest importance perhaps is the fact that there was no difference in drug leakage between the animals free to move about the chamber and the anesthetized ones. This indicates that under normal circumstances shaking, or otherwise movement of the PE 10 tubing above the rat does not cause inadvertent spurious injection of drug.

In light of the above findings, we feel that this pump can play a unique role in drug effects on behavior that requires awake, freely behaving animals and the administration of multiple discrete drug injections.

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