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# An Improved Drug Infusion Pump for Injecting Nanoliter Volumes Subcortically in Awake Rats

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IWAMOTO, E T , E. C WILLIAMSON, C WASH AND R. HANCOCK *An improved drug infusion pump for injecting nanoliter volumes subcortically in awake rats*. PHARMACOL BIOCHEM BEHAV **20**(6) 959–963, 1984.—A new drug infusion pump capable of injecting nanoliter volumes of drug solution into the brains of awake rats has been constructed which incorporates a new "turntable" commutator and a compact tubing compressor mechanism requiring no gears. Injector cannulas inserted into guide cannulas permanently mounted in the rat's skull are connected to the drug pump by spring-protected, PE 10 tubing Drug solutions are delivered when the pump rollers compress the drug-filled PE 10 tubing. An additional animal-activated switch and motorized mechanism rotates the drug pump in response to the animal's movements so that the PE 10 drug reservoir is not twisted. Testing the drug pump's performance *in vivo* with injections of $^{14}$C-nicotine into the caudate nucleus shows that drug delivery is both reliable and reproducible

Nanoliter drug injections Microinfusion pump Subcortical drug administration $^{14}$C-nicotine diffusion Central self-administration in rats

OLDS *et al* [5] introduced the technique of injecting very small volumes of drug solutions (3 mμl, or 3 nl) directly into the lateral hypothalamus of rats moving freely in an operant cage. Injector cannulas made of PE 10 tubing were fitted into guide cannulas mounted in the rat's skull and connected to a compression apparatus with PE 20 tubing. Drug solution was delivered when the PE 20 tubing was compressed between two steel rollers of the pump apparatus which was suspended above the rat. However, because of circling movements of the animal, the tubing could twist and unpredictably eject the drug solution from the PE 20 tubing.

Stein and Rodd [7] utilized Olds' model to design a geared drug pump with a brush commutator/slip ring assembly which allowed for rotation of the drug pump around the vertical axis, and which was counterbalanced to follow the animal's vertical movements. By using PE 10 tubing as the drug reservoir and 33 gauge injectors, microinjections as small as 10 nl were reproducibly made into rat brain. However, we estimate that the Hurst pump motor, transmission gears, and housing weighed about 750 g, so that the rat would have to apply a substantial amount of torque to overcome the inertia of the system.

This report represents the design of a new "turntable" commutator, and a drug pump with a compact compression mechanism. The pump is capable of injecting nanoliter quantities of drug solution into subcortical sites of rats freely moving in an operant cage An additional animal-activated switch and motorized mechanism has been added to rotate the whole pump apparatus in response to the slightest change in direction of the tethered animal. Thus, the rat is not the force rotating the pump housing, and there is virtually no twisting of the drug reservoir PE 10 tubing. The performance of the drug pump was ascertained by measuring the reproducibility of the injections of $^{14}$C-nicotine administered into rat brain by the pump.

## MATERIALS AND DESIGN

The electronic schematic diagram for the drug pump assembly is shown in Fig. 1. As the rat circles, the animal-activated switch (A) closes a circuit that activates motor B (Hurst Mfg., Princeton, IN, model PC-DA, 20 r.p.m.) which rotates the pump either clockwise or counterclockwise in the direction of the animal. After following the animal's movements, circuit A is opened and motor B is disengaged. Activation of a 12 volt d.c. regulated power supply C by operant instrumentation (Coulbourn Instruments, Lehigh Valley, PA) activates the pump motor D (Hurst PC-DA, 1 r.p.m.) which rotates the stainless steel compression roller. Both motors B and D run continuously; start and stop are con-

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trolled by a clutch (included with the motor) which engages or disengages in less than 25 msec.

Figure 2 is a photograph depicting the drug infusion pump. "A" is the rotating contact switch with swivel tube "T." The PE 10 tubing containing drug solution is inserted through T and up past the two stainless-steel rollers Set screw "S" forces the mobile arm with roller "$R_1$" against roller $R_2$, thus compressing the drug reservoir. Thus, fluid is forced out of the injector cannula when the PE 10 tubing is compressed and rolled between $R_1$ and $R_2$ during activation of motor D.

The 4 mm o.d. Tygon tubing of the injector-steel spring assembly (see Fig. 4) attaches to T. Rotation of the rat turns T causing the switch lever to contact one of two posts; this closes circuits 3-3′ or 5-5′ (see Fig. 1) resulting in activation of motor B which turns the drug pump apparatus either clockwise or counterclockwise.

The commutator (TC) is a turntable design consisting of 5 circular contact strips milled from the copper conductor of a printed circuit board (Cu). Pins 1′ through 5′ (see Fig. 1) are in contact with the circular strips 1 through 5 of the commutator (Fig. 2, panel III). Thus, this commutator is a horizontal rather than a vertical slip-ring design, and, in our experience, is less apt to come apart after prolonged usage.

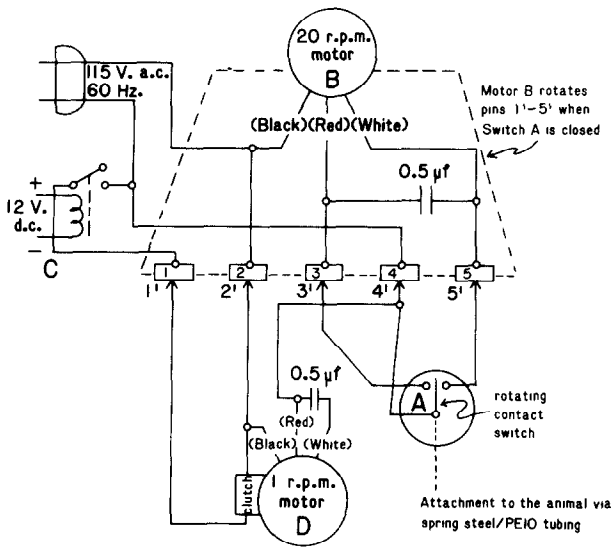


FIG 1 Schematic diagram of the circuitry for the drug infusion pump Circling movements of the rat closes switch A activating motor B which rotates the drug pump in the direction of the animal Activation of power supply C activates motor D resulting in compression of the drug delivery tubing

## METHOD

Sprague-Dawley rats weighing approximately 300 g are permanently implanted unilaterally into the caudate nucleus with a 25 gauge thin-walled stainless-steel (Small Parts, Miami, FL) guide cannula depicted in Fig. 3 using the surgical methods outlined by Cooley and Vanderwolf [4]. The 25 gauge thin-walled stainless steel tubing is inserted and lightly soldered to a one-half inch length of stainless steel type 316 tubing (Small Parts) that has been threaded on one end to accept the cannula cap and on the other end to form a grip with the dental acrylic used to permanently mount the cannula. After adjusting the stereotaxic nosebar so that the skull landmarks lambda and bregma were in the same horizontal plane, the site of implantation was 8 2 mm anterior to lambda; 2.7 mm lateral to midline, and 4 mm from the top of the skull. Stylet wires 3 mm in diameter (Small Parts) and 1 mm longer than the guide cannulas keep the guides patent while the animal recovers in its home cage. The plastic cap protects the guide and stylet between experiments.

Injectors made of 31 gauge stainless steel tubing are cut electrolytically to a predetermined length 1 mm longer than the guide cannulas (Fig. 4). Mild heating and stretching of the PE 10 tubing (Clay-Adams) assures a tight junction with the 31 gauge injector. After threading the PE 10 tubing through the steel spring (Plastic Products, Roanoke, VA) with the aid of an appropriate length of 31 gauge wire, the tubing is washed with 95% ethanol and distilled water before filling with the desired drug solution The PE 10 drug reservoir, plugged by a short length of 3 mm diam. wire, is compressed and rolled between the stainless-steel rollers of the drug pump. The injectors are then inserted into the guide cannulas, and secured by the injector-steel spring assembly depicted in Fig. 4.

The suppliers and catalog numbers of the cannula-injector materials are as follows. Plastic Products Company, P.O. Box 12004, Roanoke, VA 24022, (703) 989-4511. Self-tapping stainless steel mounting screws 0-80×$^3/_{32}$″. Small Parts Inc., 6901 N.E Third Avenue, P.O. Box 381736, Miami, FL 33138, (305) 751-0856· Stainless steel tubing, 31 gauge (No. HTX-31) and 25 gauge thin-walled (No. HTX-25 TW); stainless steel type 316 tubing (No. CTX-6220); 0.011″ stylet wire (No. SWX-011), 1–72×$^1/_8$″ machine screws (No MX-172-2FL). Bioanalytical Systems, 2701 Kent Avenue, West Lafayette, IN 47906, (317) 463-4527; $^1/_8$″ o.d.×0 032″ i.d. plastic tubing (No. MF 1036).

Different volumes of injected drug solution are obtained by varying the time of activation of power supply C, and hence, the pump motor D. A solid-state universal timer (Coulbourn Instruments, Lehigh Valley, PA) is used to vary the on-time of the drug pump. The drug pump is calibrated with a solution of radiolabelled compound, in this case, $^{14}$C-nicotine (pyrrolidine-2-$^{14}$C-nicotine, New England Nuclear, 60 mCi/mmol) dissolved in an artificial cerebrospinal fluid. The pump is activated for 0.2, 0.4, 0.8, or 1.6 sec and the $^{14}$C-nicotine collected in liquid scintillation vials containing 100 $\mu$l of water, to which is added 10 ml of a water-miscible scintillation cocktail (Aquassure, New England Nuclear) and counted to 2% sigma error, or 10 min. The time required to deliver 25 nl of drug solution is calculated by the following formula:

$$t\ (\text{sec}) = \frac{(\text{dpm } ^{14}\text{C-nicotine in 1 } \mu\text{l of injection solution})}{(\text{dpm } ^{14}\text{C-nicotine delivered by the pump in 1 sec})} \times \frac{1}{40}$$

In order to demonstrate the reproducibility of the drug pump delivery system *in vivo*, rats implanted with guide cannulas for at least one week were briefly anesthetized with halothane-$O_2$, injected with varying amounts of $^{14}$C-nicotine solution using the drug pump, and sacrificed at varying intervals after injection. Brains were quickly removed (care was taken when lifting the guide cannula from the brain), blocked in the coronal plane, frozen on an object disc on the freezing stage of a cryostat-microtome, and cut in the coronal plane. Slices 100 $\mu$ thick were collected at least 3 mm

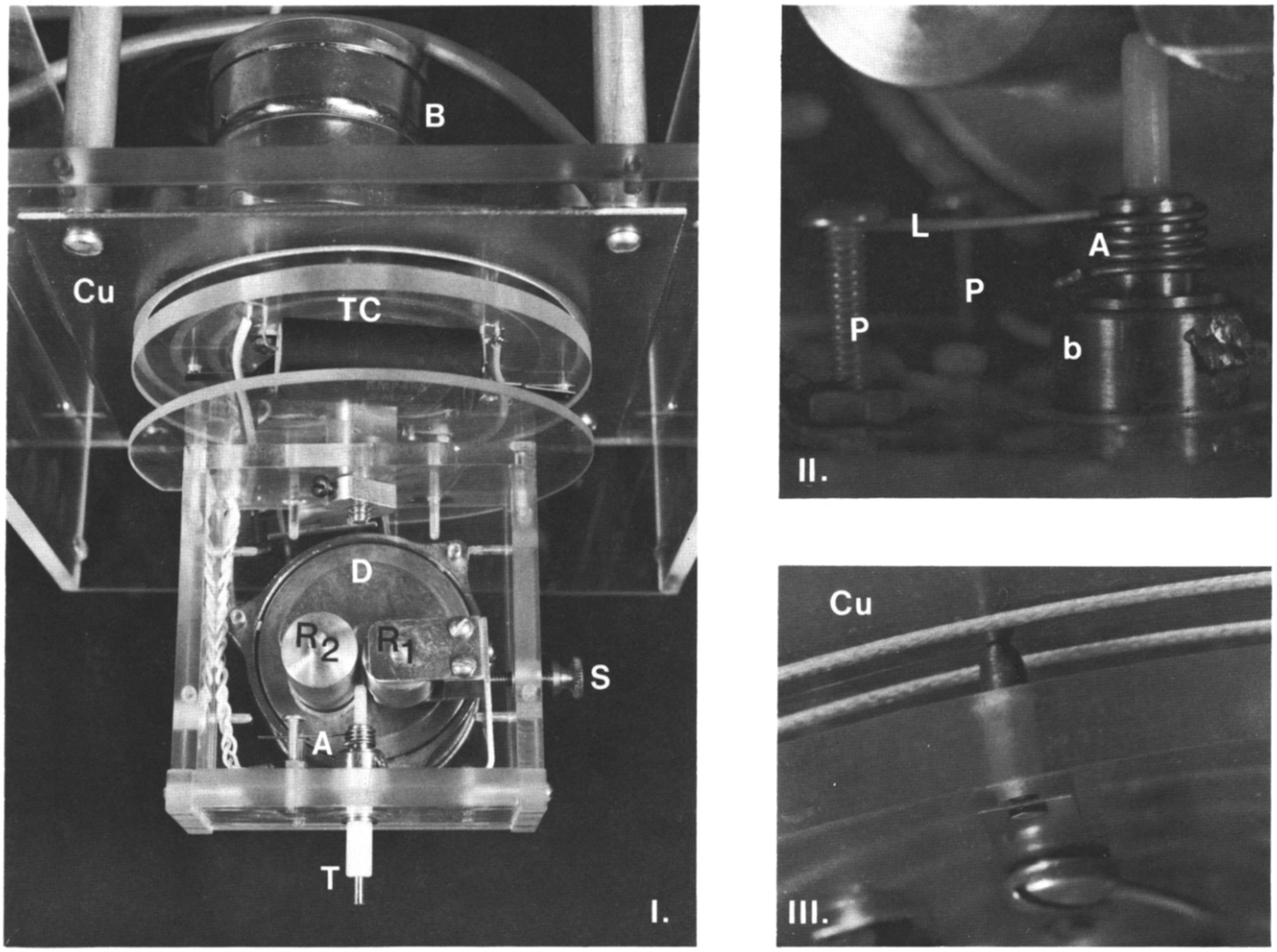


FIG 2 Photograph of the drug infusion pump Panel I. A, rotating contact switch, B, 20 r.p m motor, Cu, copper circuit board with 5 concentric contact strips, D, 1 r.p m motor, $R_1$ and $R_2$, stainless steel compression rollers; S, setscrew, T, swivel tube; and TC, "turntable" commutator Motor D rotates $R_2$ Motor B and the Cu are stationary. Panel II. Close-up of A with switch lever L and electrical contact posts P. T is directly coupled to A which is set in ball-bearing "b" Panel III. Close-up of one of the contact strips of the Cu and a copper contact pin of the turntable commutator

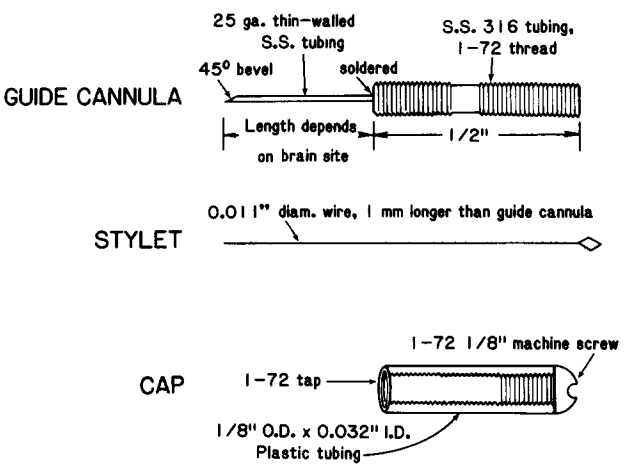


FIG. 3 Schematic of the 25 gauge thin-wall stainless steel (S S) guide cannula, the stylet and plastic cap

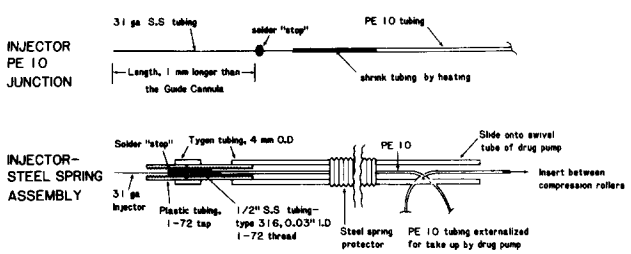


FIG 4 Schematic of the 31 gauge S.S. injector cannula, PE 10 tubing drug reservoir, and the injector-steel spring assembly

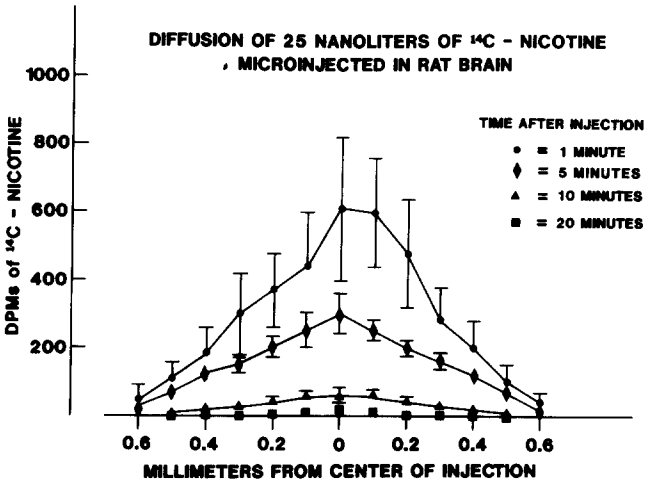


FIG 5 The diffusion of 25 nanoliters of $^{14}$C-nicotine at 1, 5, 10 and 20 minutes after microinjection into the rat caudate nucleus Bars denote ±S E.M. N=6–8 for each group

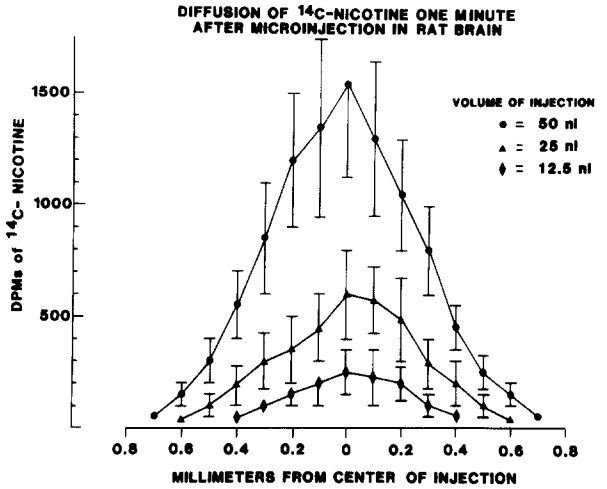


FIG 6 The diffusion of $^{14}$C-nicotine one minute after microinjection of 12 5 nl, 25 nl, and 50 nl into the rat caudate nucleus Bars denote ±S E M N=6–8 for each group The 25 nl group is the same as that depicted in Fig 4

TABLE 1

RECOVERY OF $^{14}$C-RADIOLABEL AFTER MICROINJECTION OF $^{14}$C-NICOTINE INTO THE CAUDATE NUCLEUS OF RAT BRAIN

| Injection Volume | Survival Time | DPM Recovered | DPM Injected† | Percent Recovery | N |
|---|---|---|---|---|---|
| 50 nl | 1 min | 10956 ± 2835 | 32290 ± 3865 | 33 9% | 8 |
| 25 nl | 1 min | 3844 ± 535 | 15195 ± 929 | 25 3% | 7 |
| 25 nl | 5 min | 2029 ± 276 | 15195 ± 929 | 13 4% | 6 |
| 25 nl | 10 min | 231 ± 74 | 15195 ± 929 | 1 5% | 6 |
| 25 nl | 20 min | 32 ± 9 | 15195 ± 929 | 0 21% | 7 |
| 12.5 nl | 1 min | 1426 ± 702 | 6722 ± 792 | 21 2% | 6 |

*DPM expressed as Mean ± S E M.

†Averaged from 5 aliquots taken both before and after the microinfusion into the rat caudate nucleus

anterior and 3 mm posterior to the tract left by the cannula, the presumed site of injection The tissue slices were moistened with 100 $\mu$l of water, dissolved in 500 $\mu$l of a tissue solubilizer overnight (Soluene-350, Packard), 10 ml of scintillation cocktail added (Permablend III, Packard) and counted to 2% sigma error, or 20 min (low count cut-off, 30 dpm). The counter efficiency using internal standardization for $^{14}$C was 95 percent (Packard Instruments, model 460)

In another experiment, rats were not anesthetized but connected to the drug pump using the normal injector assembly depicted in Fig 4 Over a period of 20 min, 22 injections of $^{14}$C-nicotine each 1 sec in duration (22×25 nl) were delivered at regular intervals The animals were sacrificed 5 min after the last injection, and the radioactivity contents of the brain slices surrounding the cannula tract determined in the manner just described.

## RESULTS

An example of the reproducibility of a pump calibration is given by the following typical experiment. The amount of $^{14}$C-nicotine solution (105,840 dpm/$\mu$l) delivered in 200, 400, 800 and 1600 msec was 552±100, 1023±99, 2091±151 and 4204±206 dpm (mean±S.E.M., N=5 for each mean). It can be interpolated that 2640 dpm was delivered by the pump in 1 sec. Therefore, 1 sec of pump activation delivers 25 nl of drug solution.

The *in vivo* capability of the drug pump is exemplified by the following experiments. The distribution of 25 nanoliters of $^{14}$C-nicotine injected by the drug pump into the rat caudate nucleus at 1, 5, 10 and 20 min after microinfusion is shown in Fig 5 Over 95% of the radioactivity at any of the four time points was recovered within 0.5 mm of the center of the injection site. Within 1 min after injection, over 75% of the $^{14}$C-nicotine was lost from the site of injection, presumably by uptake into the blood. Ten min after injection, less than 2% of the original radioactivity remained at the injection site (Table 1)

The extent of diffusion of $^{14}$C-nicotine one min after infusing three different injection volumes using the drug pump is

shown in Fig. 6. The recoveries of $^{14}$C-nicotine 1 min after injection ranged from 21.2 to 33.9% of the originally injected radioactivity (Table 1). After injecting 12.5, 25 and 50 nl of labelled nicotine, over 99% of the radioactivity spread to hypothetical spheres approximately 0.8, 1.0 and 1.2 mm in diameter. The theoretical dimensions of 12.5 nl is a sphere 0.30 mm in diameter, 25 nl is a sphere 0.37 mm in diameter and 50 nl is a theoretical sphere 0.46 mm in diameter. Thus, after injection by the drug pump into brain tissue, the diffusion of $^{14}$C-nicotine is rapid and extends to spheres larger than theoretical. The variability of the DPMs of $^{14}$C-nicotine recovered from brain tissue was the greatest after the 12.5 nl injection volume.

In one experiment, 25 nl of $^{14}$C-nicotine was injected into the caudate nucleus of dead rats, and the radioactive contents of the brain slices determined as before. One minute after completion of the injection, 93%±6 S.E.M. (N=4) of the $^{14}$C-nicotine was recovered.

In another experiment, six unanesthetized rats were connected to the drug pump and 22 injections of $^{14}$C-nicotine (10,177 dpm±342 S.E.M. per each 25 nl injection) were delivered over a period of 20 min. The total amount of $^{14}$C-nicotine delivered in 20 min was approximately 224,000 dpm. Five min after the last injection, the total radioactivity contents of slices taken from the brain region 3 mm anterior and 3 mm posterior to the guide cannulas were determined to be 13,507 dpm±1817 S.E.M. (N=6). The individual dimensions of the anterior-posterior spread of 98% of the recovered radioactivity were 2.8, 2.4, 3 4, 2.2, 3 8, and 2 4 mm. These data are indicative of the reproducibility that is possible using the drug pump injection technique

## DISCUSSION

Accurate and reproducible administration of the $^{14}$C-nicotine in both anesthetized and unanesthetized rats was made possible by the drug delivery pump just described. After practice, one can remove the cap and stylet of a guide-implanted rat, insert and secure the injector cannula, place the animal in an appropriate cage, and attach the PE 10 tubing/spring-steel drug reservoir to the drug pump, all in less than one minute. With this pump system, we have studied the effects of agents administered into the ventricles or brain tissue either acutely or intermittently over a period of a week with the drug delivery activated either operantly or manually without the complications of tangling the PE 10 tubing drug reservoir by the rat. Although it is theoretically possible that a rat will tangle the PE 10 tubing if it is ambulating faster than the 20 r.p.m. motor, it is unlikely since animal movement within the Skinner box is somewhat restricted. After habituation to the attachment of the injector/spring protector to the guide cannula, rats are usually observed to go to sleep in the Skinner box within 1 to 2 hours. Thus, it is possible to microinject drugs into the brains of animals without disturbing their ongoing behavior.

Another technique of central drug administration into the ventricles or brain tissue in the awake animal includes the use of microliter syringes to infuse the drug solution [1, 2, 6]. However, as pointed out by Stein and Rodd [7], the fluid slip rings usually used in the delivery apparatus are prone to leakage and therefore the precision of drug delivery is lessened. Another method utilizes the gas pressure of hydrogen generated by the electrolysis of water to force drug solution from an air-tight reservoir through injector cannulas [3]. This method promises to be a viable alternative to conventional modes of remote drug delivery.

The results indicate the microinfusion of $^{14}$C-nicotine into the rat caudate nucleus is characterized by rapid disappearance from the site of injection. After microinjection 0.11 nmol of $^{14}$C-nicotine in 25 nl (Table 1) over 75% is cleared from the injection site within the first min and disappearance is essentially complete by 20 min after the injection. In unanesthetized rats, over 94% of the $^{14}$C-nicotine injected over a 20 min period had dissipated from the site of injection within 5 min after the last injection. The nicotine is cleared from the brain tissue presumably by uptake into the blood.

In conclusion, we have presented the design of a reliable and reproducible drug pump, commutator and rotation mechanism which allows the subcortical administration of nanoliter amounts of drug solution into the ventricles or brain tissues of awake rats without disturbing the animal's ongoing behavior.

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