Pharmacology Biochemistry & Behavior, Vol. 6, pp. 595–597.

BRIEF COMMUNICATION

Simple and Compact Cannula System for Mice[1]

LARRY KOKKINIDIS, LOUIS RAFFLER AND HYMIE ANISMAN

Carleton University, Ottawa, Ontario K1S 5B6, Canada

(Received 3 May 1976)

KOKKINIDIS, L., L. RAFFLER AND H. ANISMAN. *Simple and compact cannula system for mice.* PHARMAC. BIOCHEM. BEHAV. **6**(5) 595–597, 1977. – A small and simple device is described for applying substances to the brain of freely moving mice. The effectiveness of the technique was evaluated by intraventricular injections of d-amphetamine sulfate. It was observed that intraventricular injections of d-amphetamine (50–200 μg) produced a dose dependent increase in activity.

Intraventricular injections d-Amphetamine sulfate Electrode implantation Induced locomotor excitation

IN MICE implantation of chronic electrodes or cannulae is achieved only with great difficulty. Owing to the gracility of the skull and the small cranial area, a limited number of anchor screws can be used, resulting in a disproportionately high loss of skull caps. Moreover, the small cranial area available to work with does not lend itself readily to placement of more than one cannula. Finally, the weight and size of standard cannulae may result in awkward mobility among mice.

In a recent report [1] an elegant technique was described in which a single jeweler's screw served both as an anchor for the skull cap and retainer for the cannula system. While this technique is adequate for rats, preliminary observations from this laboratory revealed that this device was too large for use in random bred or smaller inbred mice. Furthermore, use of several cannulae proved to be virtually impossible within a single animal. In the present paper, a small and inexpensive modified version of this device is introduced, which lends itself nicely for single or multiple placements in mouse brain, with minimal skull cap loss. In addition, this system may substitute as a device by which chronic lesion experiments in freely moving animals may be carried out. The adequacy of this technique was examined by inducing locomotor excitation following intraventricular injection of 3 dosages (50, 100, 200 μg) of d-amphetamine.

METHOD

Twenty-four Swiss Webster mice, approximately sixty days of age, were anesthetized with sodium pentobarbital (60 mg/kg). One half of the mice were stereotaxically implanted with a cannula to the left lateral ventricle, while the remaining half received implantation to the right lateral ventricle. (A = 1.0 mm, L = 0.7 mm from bregma). Placements were calculated from Wahlsten, Hudspeth and Bernhardt [3], together with preliminary examination of brain sections in this laboratory. The cannula system (see Fig. 1) consisted of a flathead stainless-steel jeweller's screw (OD = 0.035 in.) with a hole drilled through the centre (0.020 in.). A precut blunted 26 guage stainless-steel needle was inserted through the screw and soldered into place such that 2.7 mm of the stylet protruded below the screw, and 2.6 mm protruded above. The cannula was held firmly to the skull by four rotations of the screw (pitch = 0.2 mm), thus making up the required vertical depth of 3.5 mm from the skull to the ventricles. The cannula was held to the skull with the application of dental acrylic cement. Thus, the screw served as an anchor for the cement, and retainer for the stylet.

Following the hardening of the acrylic, a premade polyethylene cannula cap was fitted over the 1.5 mm barrel protruding from the skull cap. The cap (see Fig. 1) consisted of a polyethylene tube (ID = 0.0154 in., OD = 0.043 in.) and a premeasured, precut stainless-steel wire (0.01 in.) which passed through it. The wire was heat-sealed to the polyethylene tube and when inserted into the stylet descended to the bottom of the implanted barrel. The cannula cap and wire served to keep the barrel free of fibrotic material, while requiring a minimal amount of skull

[1] Supported by Grant A-9845 from the National Research Council of Canada to H. Anisman. Appreciation is extended to Smith, Kline and French (Montreal) for the gift of d-amphetamine sulfate. Appreciation is extended to Dan McIntyre for his helpful suggestions and assistance. Requests for reprints should be addressed to Larry Kokkinidis, Dept. of Psychology, Carleton University, Ottawa, Ontario, K1S 5B6.

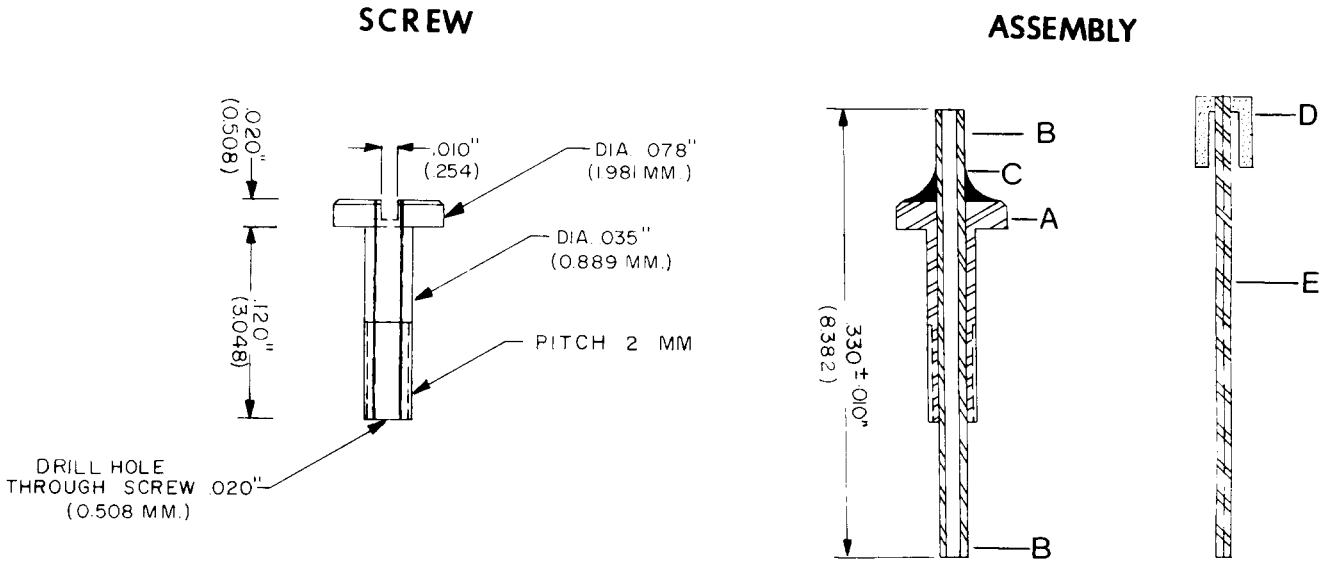

FIG. 1. Schematic representation of the cannula system. A – Screw, B – Stylet, C – Solder, D – Polyethylene Cap, E – Wire.

surface. Because of the relatively small size of the device, a number of cannulae could be implanted to various regions of the brain.

Seven days following surgery mice were intraventricularly injected with either physiological saline (10.0 μl) or one of three dosages of d-amphetamine sulfate (50.0 μg, 100.0 μg, 200.0 μg all in a 10.0 μl volume). d-Amphetamine sulfate was dissolved in a physiological saline. In addition, several animals were intraventricularly injected with 10 μl of cresyl violet in order to determine the distribution within the ventricular system [3].

The injection procedure was carried out as follows. The polyethylene/stainless steel wire cap was removed thus exposing the barrel of the cannula (see Fig. 1). A microlitre syringe with a 26 gauge needle was fitted with a 5.0 mm length of polyethylene tubing. The needle and tubing were in turn fitted to the exposed barrel of the cannula and the drug was slowly injected.

Immediately following injection mice were placed in one of six identical circular black anodized aluminum chambers, 30 cm in diameter and 30 cm high. The roofs of the chambers consisted of two layers of 0.32 cm red Plexiglas thereby reducing the illumination. Six infrared photo-electric relay units were situated 7.85 cm apart such that three of the units were perpendicular to the remaining three units. If the mouse broke a beam, then this beam could not be activated again until a second beam was broken, thus precluding movements such as head bobbing or tail thrashing from being recorded. Activity (photocell counts) was recorded and printed every 10 min over a one hour period.

RESULTS AND DISCUSSION

Following testing, subjects received an overdose of sodium pentobarbital and were perfused intracardially with 0.9% saline followed by 10% of formal saline. Frozen coronal sections were cut at 40 μm, and stained with cresyl violet. In all animals, the lateral ventricles were penetrated by the stylet. In 2 cases hippocampal damage was observed, while the stria terminalis were damaged in three cases. Figure 2 shows that the spread of dye was confined to the ipsilateral ventricle (lower portion of Fig. 2). However, as it progressed caudally (upper portion), evidence of distribution to the anterior hippocampal commissure, as well as slight spread to the contralateral ventricle was observed.

Analysis of variance of the photocell crossings revealed significant Drug treatment and Block main effects $F(3,20$ and $5,100) = 24.22$ and 9.15, $p < 0.01$, 0.001, respectively. The mean square crossings for each group over six blocks is depicted in Fig. 3. Consistent with earlier reports utilizing intraventricular injections [2] Newman-Keuls multiple comparisons revealed that treatment with d-amphetamine produced a dose dependent increase in activity. Over the 1 hr session the between group differences in activity decreased.

Whereas saline animals exhibited a sharp reduction in activity after ten min of testing followed by a gradual monotonic decrease over the remaining 50 min, treatments with 50 and 100 μg of d-amphetamine entirely eliminated the abrupt decline in activity. 200 μg of d-amphetamine, on the other hand, produced locomotor excitation during this time interval.

It is noteworthy that the two higher dosages of d-amphetamine (100 and 200 μg respectively) elicited circling behavior. Although not objectively quantified, ipsilateral turning behavior was observed immediately following injection for approximately 1–2 min followed by a brief period of sedation, after which rotational behavior contralateral to the site of injection was observed. Additional descriptions of this behavior, and effects in other tasks are presented in Kokkinidis and Anisman (in press).

In summary, the cannula system described permitted accurate placement of substances into brain tissue of freely moving mice. The usefulness of this system was demonstrated through intraventricular injections of d-amphetamine and resulting changes in locomotor activity over time.

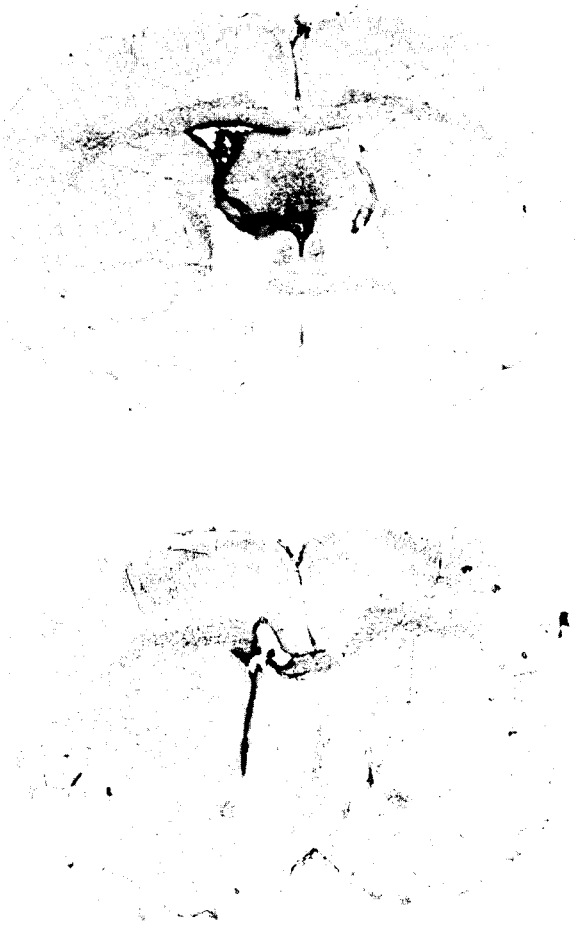

FIG. 2. Representative slices of a brain in which cresyl violet (10 μl) was injected into the right ventricle. In the lower section the cannula tract entering the ventricle is evident. In this anterior section injectate is restricted to the right ventricle. In the more posterior section (upper portion) distribution of injectate is less restricted with stain being visible in anterior hippocampal commissure, and to a lesser extent in the left ventricle.

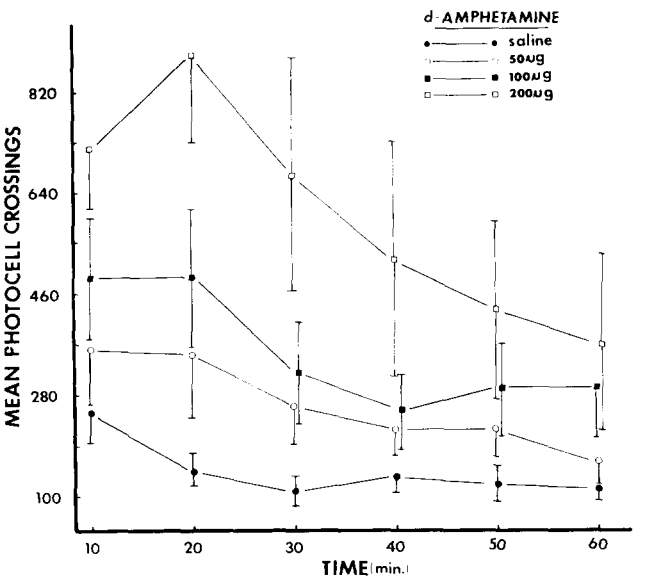

FIG. 3. Mean photocell counts over 10 min periods after intraventricular injection of d-amphetamine.

REFERENCES

1. Edmonds, H. L. and L. G. Stark. Application of substances to the brain of small animals. *Res. Communs chem. pathol. Pharmac.* 7: 641-644, 1974.
2. Kokkinidis, L. and H. Anisman. Perseveration and rotational behavior elicited by d-amphetamine in a Y-maze exploratory task: Differential effects of intraperitoneal and unilateral intraventricular administration. *Psychopharmacology* in press, 1977.
3. Myers, R. D. Methods of chemical stimulation in the brain. In *Methods in Psychobiology: I,* edited by R. D. Myers. New York: Academic Press, 1971.
4. Segal, D. S., C. McAllister and M. A. Geyer. Ventricular infusion of norepinephrine and amphetamine: direct versus indirect action. *Pharmac. Biochem. Behav.* **2:** 79–86, 1974.
5. Wahlsten, D., W. Hudspeth and K. Bernhardt. Implications of genetic variations in mouse brain structure for electrode placement by stereotaxic surgery. *Comp. Neurol.* **162:** 519–531, 1975.