METHOD

Subjects

The Ss were 12 male Long-Evans and 3 Sprague-Dawley rats ranging in age from 4 to 12 months, with a mean weight of 530 g. They were selected from the available stock in the California State College, Los Angeles, animal colony and were allowed ad lib food and water for the duration of the experiment. All Ss had been subjected to behavioral testing, but were intact, and had not been subjected to pharmacological manipulation.

Procedure

Animals were given subcutaneous injections of CPPS in the loose skin at the back of the neck. Dose levels of 0.2, 0.5, 1.0, 2.0, and 5.0 cc/kg of body weight were tested. The first level of treatment was continued for 3 days, at which time one randomly selected S from each group was sacrificed, and one S had its dose switched from a low- to high-dose group, or vice versa. All Ss were treated at the new levels for 3 more days, at which time another S from each group was sacrificed. The procedure for any given S during treatment was as follows: The S was weighed, lightly anesthetized with ether, carefully examined around the site of the previous injections, and given its next injection. Food and water intake and the condition of the drop pans were also observed at this time.

After the Ss were sacrificed they were autopsied. Special attention was given to the inspection of the area of injection and to a number of internal organs, particularly the liver. A blood sample from one S treated with a 5.0-cc/kg dose for 6 days was examined and no anomalies were noted.

RESULTS

It is most notable that all Ss survived until they were sacrificed for examination. In no case did there appear to be any sign of an adverse reaction to treatment with the foreign protein. The animals ate and drank normally and showed no overt sign of ill health. One S, apparently suffering from a respiratory ailment at the beginning of the experiment, recovered in 2 days. The Ss' weight, usually a good indicator of well being, held constant throughout the period of the experiment. The autopsies revealed no sign of physical malfunction. There was a very slight discoloration of the skin around the site of the injection in most cases. Those Ss with high dose levels showed the presence of slight blood clots around the site of the injection, and several Ss showed an apparent increase in blood vessels serving the injection site. Examination of the liver and other internal organs revealed no anomalies of any sort.

DISCUSSION

We have concluded that CPPS may be safely administered to rats without short- or long-term side effects. It is important to note that most of the Ss participating in this experiment were treated for 6 consecutive days. If CPPS were to be used to facilitate recovery from surgery, there would probably be no more than two doses given, and these on a single day, before and after the surgery. This research indicates that there is nothing to contraindicate the use of CPPS in rat surgery or in treating the other conditions in which it has been shown to be effective in dogs. CPPS has been used in this laboratory as a routine pre- and postsurgical treatment for stereotaxic surgery in rats. While no systematic figures are available, there has been a much higher rate of survival from lengthy surgical procedures; however, a planned comparison of survival rates of rats with and without CPPS treatments has not been performed.

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NOTE

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An inexpensive surface-mounted cannulae for the study of spreading cortical depression[1]

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The literature concerned with methods and surgical procedures used in the study of spreading cortical depression with rats manifests a number of inconsistencies and inadequacies. Specifically involved are a number of saliently important factors. These include dura mater dehydration, cranial exposure to possible infection, awkward requirements for suturing skin areas surrounding and covering the trephine hole placements, loss in ability to accurately quantify the amount of the depressing agent (typically KCL), and the magnitude of its corresponding effect, temporal limitations for postoperative recovery, and needless sacrificing of animals. The latter results in time loss and sometimes necessitates tampering with the experimental design (Marshall, 1959; Tapp, 1962).

Burešová and Bureš (1958, 1963, 1964, 1965), while controlling for possible brain edema with a postoperative injection of procaine penicillin, employed a loose suture technique to gain access to the trephine placements prior to experimental hemispheric depression. Using a similar procedure, Travis (1964) was forced to discard Ss that, prior to depressant application, manifested cortical surface discoloration. Generally, the methods and surgical procedures in this area of research required topical application of the depressing agent with soaked cotton swabs.

METHOD

Special cannulae were designed in an effort to eliminate some of the problems outlined in the introduction. These surface-mounted cannulae were produced from standard aluminum inflation needles (as used on footballs and basketballs and available at most sporting-goods stores). The needles were cut off at the base; the bases were then placed in a vise, and the centers drilled out with a 2.5-mm drill. The same size trephine was then used for trephining the skull. A fine metal file was used to flatten the surface contact area of the cannulae (Fig. 1) in such a way as to form a lip extending from the bottom about ½ mm.

Screw caps were made from standard soft plastic valve-stem caps (automobile) by cutting off the bottom one-third of their length (Fig. 2). These caps, when shortened and placed securely on the cannulae, will not come off inadvertantly and provide an easy-to-manipulate, waterproof, and relatively air-tight cap. The use of this combination provides easy access to the trephine holes, makes dressing changes unnecessary, and holds infection and dural drying to a minimum.

Procedure

After the initial incision has been made, the cranial area should be thoroughly scraped and dried. An elliptical lateral incision appears to be the most practical for this method rather than the typical medial incision. The cannulae (with caps attached) should be placed on the skull and their positions marked for drilling of the trephine holes and screw sets. Two anchor screws are usually sufficient, one anterior and one posterior to the cannulae placement.

Cranial openings are made with a dental drill, using a 2.5-mm metal bit for the trephines and whatever size bit is appropriate for the set screws. Care should be taken to avoid breaking through the dura.

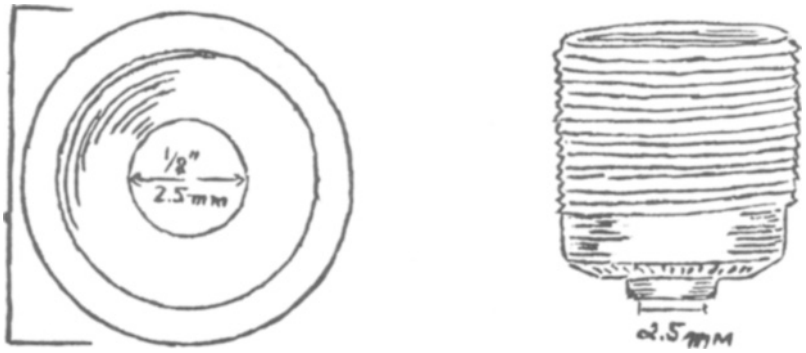

Fig. 1. (a) Top view of drilled-out cannula. (b) Side view of drilled cannula showing effects of filing and formation of surface contact, lip of ½-mm length.

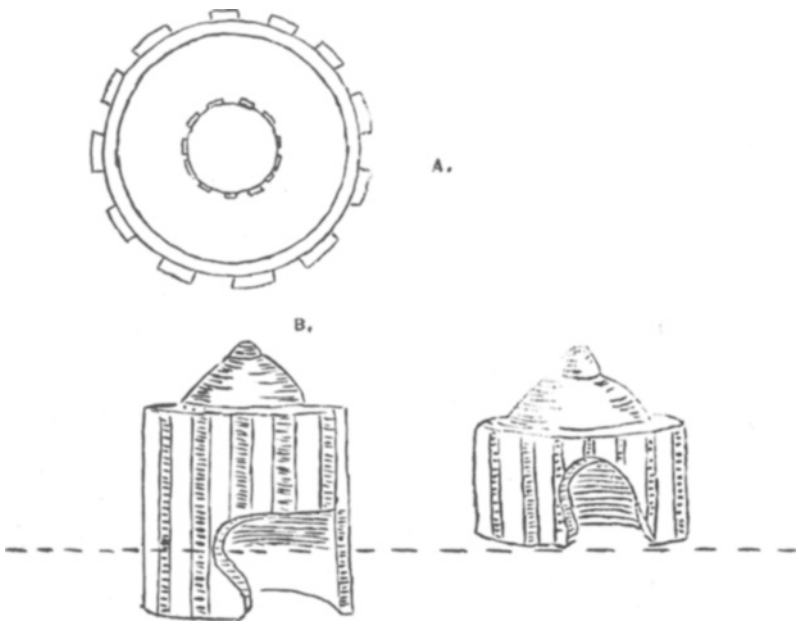

Fig. 2. (A) Top view of screw cap. (B) Cutaway of unaltered screw cap. (C) Cutaway of screw cap shortened to bottom thread (about two-thirds of original length).

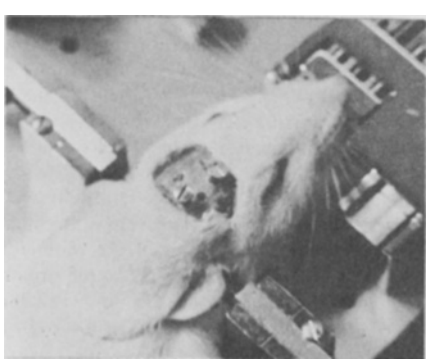

Fig. 3. The screws are set in place with bilateral trephine holes located ½ mm anterior to bregma.

Following the drilling, the cranial area is washed with normal saline solution and the skull surface dried. Short set screws are then placed in the appropriate holes extending approximately 1 mm above the cranial surface (Fig. 3).

Dental cement is then placed on the skull around the trephine holes and anchor screws. The cannulae are then placed over the trephine holes and held in place while the cement hardens sufficiently to hold them steady. Care should be taken to see that the trephines remain clear of cement and any cement finding its way into the opening should be cleaned out as soon as possible. As soon as correct positioning of the cannulae has been affirmed, a final application of dental cement is made to insure stability (Fig. 4).

The entire area is then sprayed with zephrine chloride solution and an injection

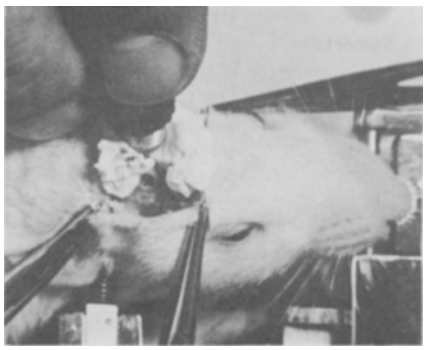

Fig. 4. The cannula is centered over the trephine hole and held in place while applying dental cement. It is allowed to set slightly and the other cannula placed using the same procedure. The second cannula is allowed to set and the hemostats removed. A finish coat of cement is applied to form a smooth surface.

of procaine penicillin is administered as insurance against disease and infection.

The screw caps are removed and the trephines washed with normal saline solution. Small wads of cotton soaked in saline solution are then placed in the cannulae and the caps replaced (Fig. 5). The finished product should appear as in Fig. 6.

DISCUSSION

The method under discussion was designed for laboratory rats of 200-300 g. However, this method, with certain adjustments, could be used effectively with other species. Smaller animals may be used, but the cranial area anterior and lateral to bregma is very small on Ss under 200 g, forcing very close placement of the twin cannulae to the midline suture and making removal of the screw caps very difficult.

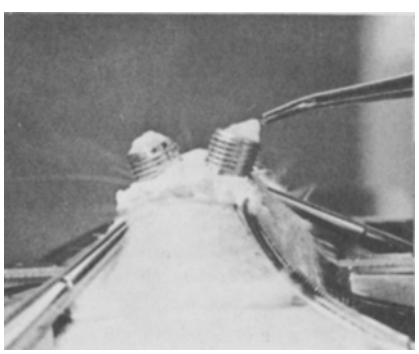

Fig. 5. Screw caps are removed from cannulae and the exposed cortex is masked with normal saline solution. Saline-soaked cotton wads are placed in each cannula and the caps replaced.

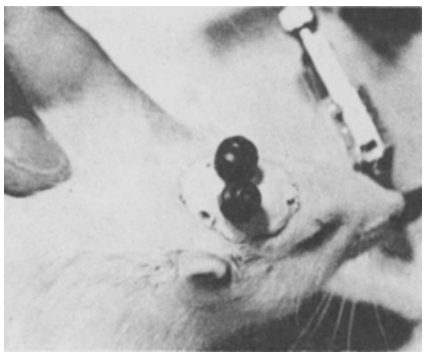

Fig. 6. Completed surface-mounted cannulae cap in place.

Also, growth of the cranial area is sufficient to loosen the cap if it is left in place for any length of time. Further, the position of the trephine holes with relation to the cannulae may be altered significantly. Animals larger than 300 g demonstrate increased growth and development of the sternoclidomastoid muscle group as well as extension of cranial muscles with insertion posterior to the occular ridge and extending back over the parietal region. Such muscle growth requires more extensive surgery to clear an area adequately for placement of the cannulae.

Observations of three experimental animals were made at the end of an 8-week period. With cannulae removed there remained no manifest signs of any reaction to the aluminum material.

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NOTE

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An inexpensive brine shrimp dispenser for fish[1]

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Various methods of dispensing small amounts of food to reward operant responses in fish have been described. Some deliver powdered or granulated dry food (Deterline, 1956; Mark, 1967). Commercially manufactured food pellets and dispensers could be used, but the smallest available pellet is 20 mg (e.g., P. J. Noyes Co., Lancaster, New Hampshire), which is a large bite for a small fish. Smaller pellets or pills are difficult to make. Longo and Bitterman (1959) used pellets of a special mixture of fish food. Most investigators have worked with live or with fresh food (Hogan & Rozin, 1961; Longo & Bitterman, 1963; Haralson & Ralph, 1966; Northmore, 1968). Recently described methods of pumping preset amounts of preserved food or of freshly prepared soft foods through a catheter to a feeding place in the experimental tank (Ames, 1968; Holmes & Bitterman, 1968) would seem to have certain advantages.

Figure 1 is a diagram of a brine shrimp dispenser that we have used for 2 years in investigations of operant behavior in goldfish. It is based on the principle of the mariotte bottle (see Niederl & Niederl, 1942), which acts as a constant-pressure reservoir.[2] In our application, tap water from the reservoir drains through a pipette that contains a slurry of brine shrimp. Momentary activation of a control valve releases a drop of water with several shrimp into the experimental tank.

A volumetric pipette is used because its shape facilitates movement of shrimp into a tube from which drops can be formed, and it is a readily obtainable item (cost, approximately $3.00). The pipette tubes are cut 2.5 cm from the body and fire-glazed. The tip of the output tube is further heated and rotated in an oxygen flame to reduce the diameter of the opening to 3-4 mm. Narrower openings frequently become clogged with shrimp, and wider ones form irregular drops. The polyethylene stopcock and twistcock connector (Cole Parmer Co., Chicago, Illinois; Stock Nos. 6074 and 6395; approximately $3) ease the job of filling and cleaning the pipette.

The solenoid valve is a two-way, normally closed valve obtained from Skinner Precision Industries, Inc., New Britain, Connecticut (Stock No. V52DA2200, 24-V dc coil; approximately $9). The valve is connected

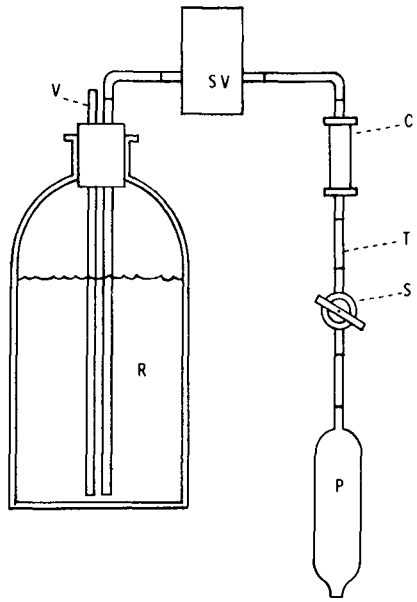

Fig. 1. C, twistcock connector; P, 50-ml volumetric pipette; R, water resevoir; S, stopcock; SC, solenoid valve; T, vinyl tubing; V, glass vent tube. Shrimp are placed in the pipette. When the twistcock connector and the stopcock are open, activation of the solenoid valve for 20 to 30 msec releases a few shrimp in a drop of water, which is replaced by water from the resevoir.

to 3/16-in.-i.d. and 5/16-in.-o.d. vinyl tubing with polyethylene adapters, ¼-in. MIPT, obtained from Cole Parmer Co. (Stock No. 6450-2). We activate the valve with a monostable multivibrator, or one-shot, and a relay driver of a BRS-Foringer, DigiBit system.

A reservoir is simply a 1-gallon bottle. A two-hole rubber stopper holds the drain tube and the vent tube, which admits air as the water is removed. When the apparatus is first set up, water is siphoned through the valve to obtain a continuous column in the drain tube to the level of the twistcock connector. Thereafter, water flows instantly on each operation of the valve and only when the valve is open; there is no afterflow.

The pressure in the system can be adjusted by varying the height of the reservoir above the pipette. Water flows at a steady rate until the level falls below the tip of the vent tube. The vent tube must extend below the surface of the water for uniform drop formation; the simple siphon which otherwise results is a workable reservoir but control of the drop size is difficult to maintain. There are similar devices for delivering liquid in which the control valve is on the air-vent side of the reservoir instead of the liquid-output side. In our feeder, regulation of the air input results in very sluggish control of the water output.