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BRIEF COMMUNICATION

Cortical Cups for Collecting Free Acetylcholine in Awake Rats[1]

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ERICKSON, C. K., D. T. GRAHAM AND T. U'PRICHARD. *Cortical cups for collecting free acetylcholine in awake rats.* PHARMAC. BIOCHEM. BEHAV. 1(6) 743–746, 1973. A method is described for collecting acetylcholine released from the cerebral cortex of awake rats. Bilateral transparent nylon cortical cups remain usable for at least two months after implantation, and will hold sufficient Locke's solution that acetylcholine released in a 15-min period can be analyzed by the leech muscle bioassay. An increase in acetylcholine released into the cups after nicotine, 0.4 mg/kg IP, illustrates that the method is suitable for determining drug-induced alterations in neurotransmitter release in rats during on-going behavior.

Cortical cups Acetylcholine Nicotine

THE cortical cup procedure that was originated by MacIntosh and Oborin [4] and refined by Mitchell [5] is becoming an increasingly popular technique for collecting the acetylcholine that is released spontaneously from the surface of the cerebral cortex. Generally this procedure requires that the animals are either anesthetized, which affects the spontaneous release of acetylcholine [5], or immobilized with a neuromuscular blocking agent throughout the experiment. Usually, large animals such as cats [6] and rabbits [3] are used so that acetylcholine can be collected from large areas of the cortex to ensure measurable amounts of acetylcholine.

This paper describes a method for the collection of acetylcholine samples from the cerebral cortex of freely-moving rats with chronically implanted bilateral cortical cups.

METHOD

Cortical Cups

The cortical cups were designed by us and manufactured to our specifications by Plastic Products Company (P. O. Box 1204, Roanoke, Virginia 24006). The base of the transparent nylon cup is similar to an ellipse but with parallel sides (Fig. 1) in order that one cup can be placed on each side of the sagittal sinus and cover as much area of the cortex as possible, while causing minimal damage to the temporal muscles. Each cup covers 14 mm^2 and contains approximately 90 μl of solution. The transparent nylon enables the level of fluid in the cup to be seen, while the upper inside shoulders of the cup are rounded to help prevent air bubbles from collecting in the corners. The problem of air locks forming within the cortical cup during filling can be reduced further if the interior of the cup is sprayed with Teflon before implantation.

Solutions are injected from a microliter syringe into the cup through an internal cannula assembly (Fig. 1) that is inserted into the 22 gauge stainless steel guide tube which passes through the top of the cup. Another hole in the top of the cup allows air to escape from the cup during filling. The perfusate is collected at intervals by suction into a collection vial situated between the internal cannula assembly and a 5 ml syringe. The internal cannula is removed between collections so that the rat is completely unrestrained. Between experiments the cup is sealed by screwing a dust cap over the top (Fig. 1).

Implantation Procedure

Female Sprague-Dawley rats, weighing 240–270 g at the time of surgery were used. Each rat received atropine sulfate 5 mg/kg IP, 20 min before ether anesthesia. The head of the anesthetized rat was held in a rat stereotaxic instrument and the skin was cut along the sagittal suture from the eyes to the base of the skull. The skin was retracted and the

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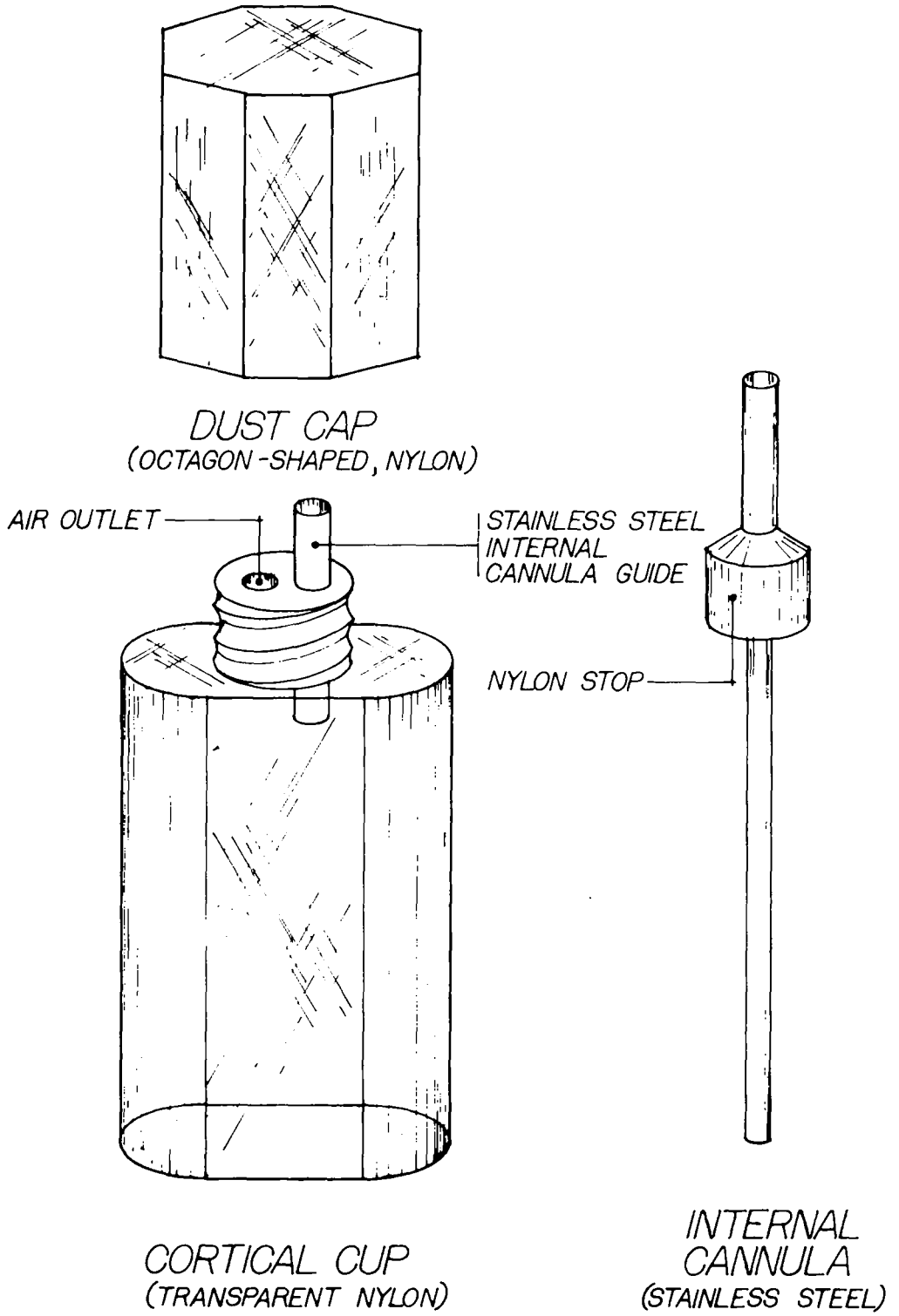

FIG. 1. Diagram of transparent nylon cortical cups used to collect acetylcholine from the cerebral cortex in rats.

cranium was scraped clean of tissue. An outline of the base of the cortical cup was traced on the skull, on both sides of the sagittal suture. A dental drill was used to drill along the outline and, when the bone was pliable, the area of bone inside the outline was removed. The dura was left intact. Three holes (two rostral and one caudal to the cortical cup sites) were drilled in the bone and 1/8 in × 080 stainless steel screws were turned into the skull. The cortical cups were placed in position to rest lightly on the dura, and dental acrylate was spread over the skull, around the base of the cups and over the screws. The acrylate was allowed to dry for 20 min, the area was dusted with an antibiotic powder, and the rat was given 30,000 units of procaine penicillin IM to retard infection. The cups were filled with Locke's solution containing penicillin G (100 units/ml) and amphotericin (2 μg/ml) to prevent bacterial overgrowth on the dura. The rats were housed individually in an air-conditioned room.

Experimental Procedure

Thirty minutes before the start of an experiment, the Locke's solution that contained the antibiotics was replaced with Locke's solution containing physostigmine, 100 μg/ml. During the experiment, the solutions in the cortical cup were removed and replaced with fresh solutions every 15 min. The samples from the two cortical cups for each collection period were pooled. The acetylcholine content of the collected samples was determined using a leech muscle bioassay [3]. The agonist was identified as acetylcholine because: (a) the activity on the leech muscle was abolished if physostigmine was omitted from the Locke's solution; (b) the agonist was unstable when the samples were stored at room temperature; and, (c) earlier work by Szerb [7] has shown that such activity seen in cerebral tissue perfusates is probably due to acetylcholine.

After determining the control levels of acetylcholine from the cerebral cortex of 6 rats in two 15 min collec-

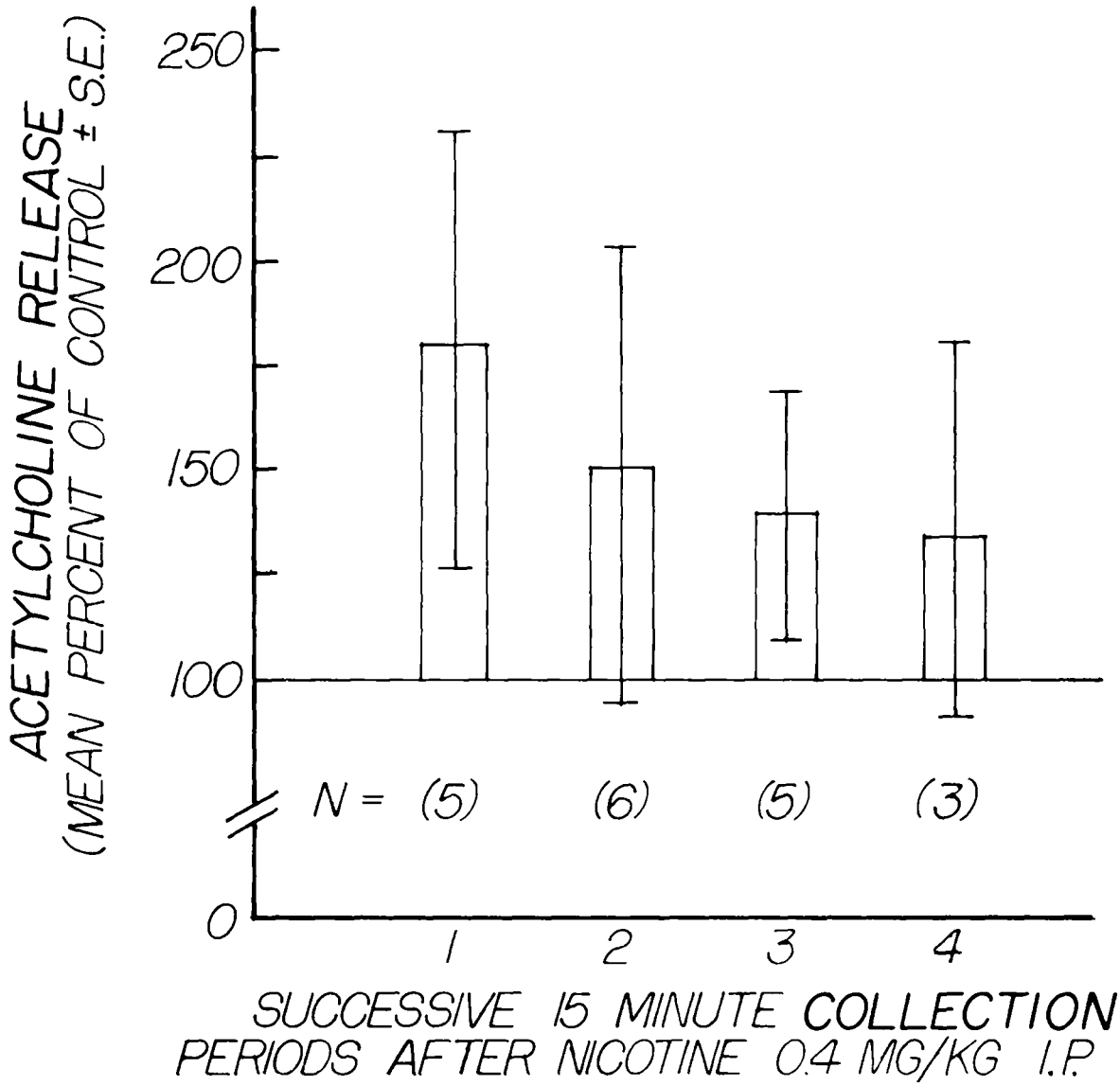

FIG. 2. Nicotine-induced alteration of acetylcholine collected from the rat cerebral cortex.

tions, nicotine (0.4 mg/kg) was injected IP to stimulate the release of acetylcholine, as reported by Armitage *et al.* [2]. Four 15 min samples were collected after nicotine administration.

RESULTS

Transparent nylon cortical cups implanted as described above rarely became dislodged and most were still usable two months after implantation.

The mean control level of acetylcholine from the cerebral cortex of 6 rats was 1.53 ± 0.17 ng/cm² /min (± S. E.). Nicotine produced an increase in free acetylcholine, with the highest level occurring during the first 15 min after injection, and decreasing levels every 15 min thereafter for the remainder of an hour (Fig. 2). Acetylcholine levels after nicotine were plotted as mean percent of control.

DISCUSSION

Studies on the release of putative neurotransmitters from the cerebral cortex of rats are not plentiful in the literature. Aquilonius *et al.* [1] have reported on the use of a unilateral Perspex cup in rats to collect acetylcholine in awake rats, but no detailed description of the cups was given. In the present experiment we have shown that sufficient acetylcholine to be measured by the leech muscle bioassay can be collected from the cortex of rats, and that the levels of cortical acetylcholine in rats can be modified by drug action. The rat is the most popular animal for behavioral studies and this technique will be useful for measuring drug- or environment-induced changes in the levels of free acetylcholine during on-going behavior. In addition, the technique is potentially useful for collecting other putative neurotransmitters released from the cortex of the rat, when combined with appropriate chemical or bioassay methods.

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