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BRIEF COMMUNICATION

A Simple Chronic Microinjection System for Use with Chemitrodes

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CRISWELL, H. E. *A simple chronic microinjection system for use with chemitrodes.* PHARMAC. BIOCHEM. BEHAV. 6(2) 237–238, 1977. – An inexpensive, electrically operated microinjection system is described which is small enough to be mounted on the animal side of a standard Mercury commutator. This eliminates the troublesome swivel joint needed for chronic microinjection in freely moving animals. This system allows simultaneous electrical stimulation or recording and chemical stimulation via a chemitrode using a standard 4-channel Mercury commutator.

Microinjection system Electrical stimulation Chemitrode Freely behaving animal

A NUMBER of chemitrodes are available which permit simultaneous injection of chemicals and electrical stimulation or recording at discrete brain sites [2, 3, 4]. A factor which has hindered the use of these systems in freely behaving animals is the lack of a simple and reliable method of combining an electrical commutator for stimulation and recording with a swivel mount for chemical injection. Without some provision to allow their rotation, the cables containing the wires and tubing rapidly become twisted and long-term stimulation or recording is impossible. This is especially true when the stimulation produces circling behavior. The present paper describes a simple scheme to eliminate the need for a swivel for microinjection. This is useful when microinjections must be carried out over a prolonged time period and when concomitant electrical stimulation or recording is desired. The system is easily constructed from commonly available laboratory supplies and, as it is operated by simply passing a constant current through the apparatus, it is easily automated.

The elimination of the troublesome swivel joint which typically does not turn easily or leaks is accomplished by using an electrically operated injection system small enough to be mounted on the animal side of a standard Mercury commutator. Such a system is shown in Fig. 1. The miniature injector is shown in the insert (B). It consists of the tip and last cm of a disposable tuberculin syringe. Two 27 gauge silver wires are inserted into the syringe and sealed in place with dental acrylic. These wires are then soldered to a connector (A) which will mate to the commutator. At this point, a second injector may be built and both bonded to the connector with dental acrylic as shown in Fig. 1, or one injector can be bonded to the connector leaving the other two leads for electrical stimulation or recording. Forming a seal between the syringe and Silver wires has not

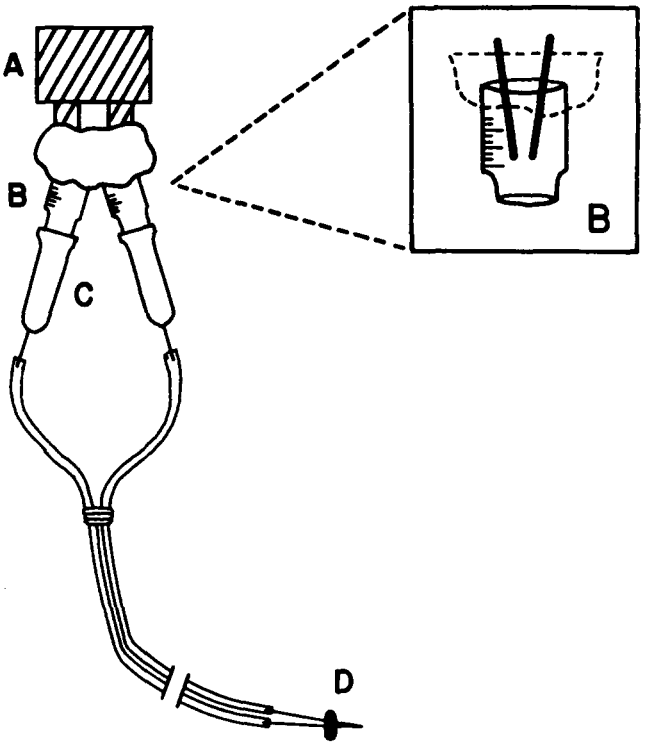

FIG. 1. Schematic diagram of a dual injection system consisting of a 4 pin Jones Plug (A) which attaches to a 4 channel Mercury commutator, two electrolysis cells (B) and two disposable 27–30 gauge blunt hypodermic needles (C). The needles are press fit into 30 gauge tubing (PE-10) which lead to an implantable cannula or chemitrode (D).

proven to be a problem using Duzall dental acrylic. However, Duzall does not bond well to polyethylene or Silver and to minimize the possibility of leakage care must be exercized to insure that at least a 5 mm length of the syringe and wire is covered and that stress is not applied to the seal during use. At the beginning of an experimental session, the injector (B) is filled with isotonic saline and a 27–30 gauge blunt hypodermic needle (C) is filled with saline and mated to the injector with care taken to eliminate any air bubbles in the system. A length of PE-10 tubing is then mated to an injection cannula or chemitrode (D) and filled with the substance to be injected. A small air bubble is introduced into the open end of the tubing and it is press fit onto the needle (C). The air bubble prevents diffusion between the substance to be injected and the saline solution in the injector and the occurrence of an injection can be verified by monitoring movement of the bubble. The microinjection cannula is then inserted into a guideshaft which has been previously implanted into the animal's brain and the experimental session is begun. When a microinjection is desired, a current is passed between the two silver wires. AgCl will be formed on one and H_2 evolved at the other. The rate of formation of Hydrogen gas is entirely dependent upon current with one molecule of H_2 formed for each two electrons. Thus, in an ideal cell at 20° C 1 ma for 1 sec will evolve 0.12 μl of H_2. In practice, the formula $\mu l = 0.12 \times ma \times sec$ holds within 10% between 100 μA which will inject 1 μl over a period of 83 ± 8 seconds and 2 ma which will inject one μl over a period of 4.2 ± 0.4 sec. At higher currents, local heating causes a temporary increase in H_2 volume and at lower currents, the slow reversibility of the Ag AgCl-H_2 battery which is formed by the reaction causes a significant decrease in the volume of H_2 evolved. The injection system can be used at lower rates but each cell must be empirically calibrated. At rates above 1 μl/4 hours injection volumes are repeatable within 10% for a given current-time combination once original calibration has been performed. Lower rates were not tested.

The slow loss of H_2 in the injector due to chemical recombination can become a problem if multiple injections are administered at long time intervals. This problem can be eliminated if a current of 5 μA is maintained through the injector between injections. This current is sufficient to maintain the volume of gas originally generated without generating significant new gas. Of 10 injectors tested, the maximum increase in volume over a 24 hr period at 5 μA was ½ μl. This injection volume is probably less than the amount of substance which would diffuse out through a 30 gauge needle over the same time period. In no case was a decrease in volume observed at this current.

As with any microinjection system, the injection pressure depends upon the compressibility of the substance being injected and the elasticity of the total injection system. It is, therefore, important to remove any unnecessary air bubbles from the system and use as short a piece of tubing as is practical. Each injection introduces a volume of gas equal to the volume of the injection and this places an upper limit upon the number of injections which can be made before the injection pressure drops to a point where the injection volume is unreliable. We have found that injection volume variability is under 10% for the first four injections of a given volume into rat or mouse brain. Beyond that number, variability increases rapidly as injection pressure is relatively low.

It is important that the Hydrogen electrode be clean at the time of injection as impurities on the electrode may react with the nascent Hydrogen and prevent the evolution of gas. Cleanliness of the electrode can be insured by filling the injector with saline solution and passing a 1 to 2 mA current through the electordes for 20 sec prior to each injection session. The gas formed must then be flushed out with more saline before use and for best accuracy to be maintained, a constant 5 μA current should be maintained through the injector until the time of the injection. As the system is used, one electrode is gradually converted into AgCl. This process is rather slow and several hundred microleters may be injected before electrode deterioration becomes a problem. At that point, the chlorided silver wire must be replaced.

If a freely turning Mercury commutator is used, 2 lengths of PE-10 tubing as shown in Fig. 1 provide sufficient stiffness to turn the commutator when the animal turns. If a single length of tubing is employed a 27 gauge wire can be taped to the tubing to provide added rigidity and prevent twisting of the tube. This system is light and flexible enough that it can be used with young rats or mice and substances can be introduced into the vascular system via the mid-sagital sinus [1] as well as into the brain.

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