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A simple method for constructing microinjectrodes for reversible inactivation in behaving monkeys

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Abstract

A method for constructing a simple, durable injection-microelectrode (injectrode) is described. The injectrode can record neuronal activity, stimulate neuronal tissues, or inject substances locally through its tip. The injectrode is lightweight and is easy to construct from commercially available parts, and it can be used repeatedly for multiple recordings and injections. Since dura penetration can damage fragile electrode tips, a reliable method to pass the injectrode through an intact dura matter is described.

Keywords: Injection-microelectrode; Reversible inactivation; Head-unrestrained; Cortex; Monkey

1. Introduction

The combination of neuronal recording, stimulation, and reversible pharmacological activation/inactivation of neural tissues has yielded invaluable information about the functions of the brain (e.g. Malpeli and Schiller, 1979; Schiller and Malpeli, 1979; Maunsell et al., 1990; Dias et al., 1995; Lee et al., 1988; Hupé et al., 1999). An injection-microelectrode, referred to as 'injectrode', that is capable of recording and stimulation as well as delivering substances to the brain for placing anatomical tracers or markers is thus an important tool for neuroscientists. In the past, most studies using the injectrode technique in primate neurophysiology have focused primarily on head-restrained monkeys (Lee et al., 1988; Maunsell et al., 1990; Dias and Segraves, 1997), and little has been done to adapt the injectrode technique for studies of head-unrestrained, behaving monkeys. When the monkey's head is unrestrained, the factors that affect use of injectrodes increase dramatically, and the standard injectrode technique needs to be modified.

We have developed an injectrode for use in head-unrestrained monkeys. The injectrode permits electrical stimulation and monitoring of neuronal activity at the tip of the injection needle before and during the infusion of solutions. The quality of recording achieved by our injectrode can be comparable to that achieved by standard microelectrodes used for single-unit recording. In addition, the injectrode can be used repeatedly without damaging the electrode tip. Our injectrodes are lightweight (less than 1 g in total). The parts used for construction are readily available from commercial sources and easily assembled.

2. Materials and methods

2.1. Injectrode

The injectrode and its connector are illustrated in Fig. 1A. The central part of the injectrode is a three-way tubing connector (Small Parts, Inc., U-TC-20/3) which has cannulas at the top, the side, and the bottom. The top cannula connects the recording electrode to a preamplifier (Fig. 1B). The side cannula connects to a Hamilton microsyringe (1 μl total volume) via polyethylene (PE) tubing. The bottom cannula supports

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a recording electrode and stainless steel injection tubing that delivers fluids (Fig. 1C, D).

The connector cannulas (i.d. = 0.584 mm) are fitted to 25G stainless steel tubing (o.d. = 0.508 mm, i.d. = 0.254 mm, Small Parts, Inc., U-HTX-25). The top and side cannulas are fitted to a 5-mm piece, and the bottom cannula to an 80-mm piece. The junctions are sealed with either silver solder or Epoxy (5-minute Epoxy, Devcon).

The recording electrode is a fine-tip, Epoxylite-insulated tungsten microelectrode (Fig. 1B, FHC, Inc., catalog: UEWLCESE1PNE; length = 14 cm). The extremely fine shank diameter of the tungsten wire (bare wire = 87 μm) easily fits inside a 32G stainless tubing (o.d. = 0.229 mm, i.d. = 0.102 mm, Small Parts, Inc. U-HTX-32). The impedance of the electrode is approximately 0.5–1.0 MΩ, measured in physiological saline at 1 kHz. An additional layer of insulation is provided to the electrode by threading it through Polymide tubing (Microprobe, Inc., MIL-33, length = 95 mm; Fig. 1C). Both ends of the Polymide tubing are sealed with Epoxy. The upper end of the electrode is crimped to a male gold pin connector (WirePro, Inc., catalog: 220-P0200-100). To provide reinforcement, heat-shrinkable tubing is sleeved outside of these fragile parts.

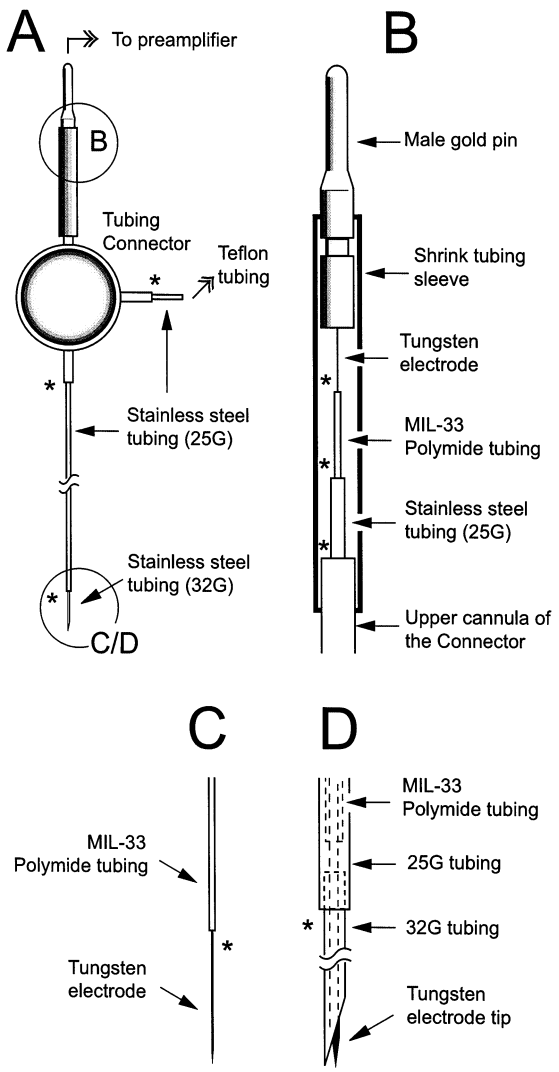

Fig. 1. Assembled injectrode (A) and configuration of the parts in detail (B–D). Asterisks indicate the places where a soldering job or epoxy glue is needed in order to seal the gaps at the junction.

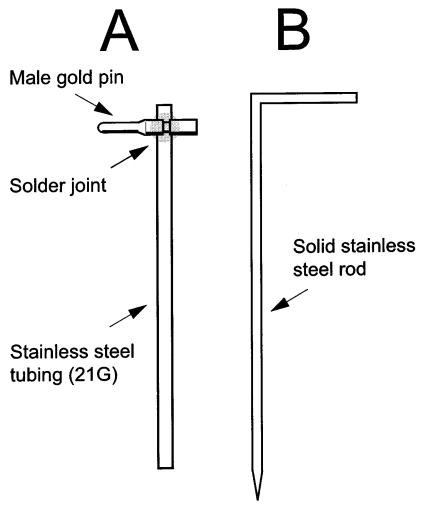

Fig. 2. Removable stainless steel guide tube (A) for fitting the injectrode, and dura-puncturing rod (B).

Fig. 1C, D illustrate the configuration of the electrode, Polymide tubing and beveled 32G tubing. In order to reduce the size of the tubing which enters the brain, we fit a 25G tubing to a 10-mm piece of 32G tubing. The 32G tubing is small, yet easy to work with, and it is less likely than 25G tubing to damage brain tissue. The reason for choosing 25G tubing as the main body of the injectrode cannula is that 25G tubing fits tightly within a guide tube (21G, Small Parts, Inc., U-HTX-21TW, o.d. = 0.813 mm, i.d. = 0.584 mm; see Fig. 2A) and thereby centers the 32G tubing/microelectrode relative to the guide tube. The lower end of 32G tubing is beveled to smooth the transition of the injectrode through the dura. The electrode tip is positioned flush with the tip of the injectrode to avoid possible damage when penetrating the dura.

Note that the diameter of polymide tubing is approximately the same as that of 32G tubing. Hence, the electrode, if sleeved by polymide tubing all the way to the tip, will not fit into 32G tubing. The polymide tubing goes only half way near the tip, allowing the rest of the electrode to be fitted with 32G tubing. A solder (or epoxy) seal is applied at the junction between the 25G and 32G tubing. The overall length of an assembled injectrode as described is approximately 10.5 cm.

A critical step in the injectrode construction is to ensure that the junctions between the stainless steel tubings are sealed airtight. Also, the solder (or epoxy)

seal should be smoothed so that excessive solder or glue will not catch in the guide tube. It is helpful to do the sealing under a microscope (e.g. power of 15×35) to better eliminate gaps.

2.2. Dura penetration

A common problem in introducing any electrode into the brain is penetrating the dura without damaging the fragile tip. This problem is often exacerbated by the fact that the dura inside the recording chamber becomes thickened over time with the growth of tough granulation tissue. The situation may worsen from tissue scars caused by repeated penetrations.

We have developed a guide-tube/puncturing rod tool (Fig. 2) to help solve this problem. The tool consists of a 21G stainless steel tubing (Fig. 2A, Small Parts, Inc., U-HTX-21TW, o.d. = 0.813 mm, i.d. = 0.584 mm, 65 mm in length) and several sharpened, solid stainless steel rods (Fig. 2B, Small Parts, Inc., GWXX-190-30, o.d. = 0.483 mm, length = 66–67 mm from the bent point to the tip). The 21G tubing serves as the guide for injectrodes, and the rod is used to penetrate the dura. The tip of the rod is beveled into a conical shape. A male gold pin connector is soldered at the side of the 21G tubing, 2 mm from the tip. The male pin serves as the handle for the guide tube, and it can be used as a reference ground for physiological recording. The lower end of the guide tube is cut straight and smooth to assure that the guide tube stays flush with the dura and does not penetrate it. The stainless steel rods fit tightly within the guide tube. The rods are bent at different points so that, when inserted all the way through the guide tube, each will protrude out of the guide tube by a different, known length.

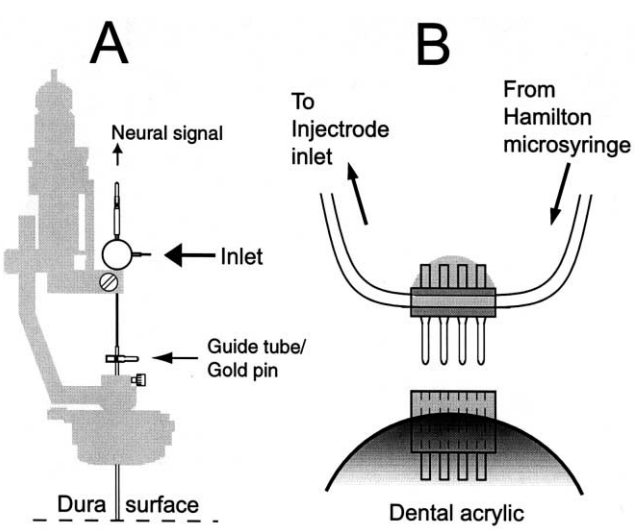

Fig. 3. Injectrode (in outlined contours) inserted through the guide tube (in outlined contours) using a micromanipulator (in silhouette) (A), and removable, tube-anchoring connector (B).

Before recording, a Kopf XY-positioner (Fig. 3A, David Kopf Instruments, Inc., Model B608, weighted approximately 32 g; for review see Lemon, 1984) is secured to the recording chamber. The guide tube is inserted through the plastic guide (Kopf Instruments, Inc., D608) of the XY-positioner. The lower end of the guide tube is pressed against the surface of the dura, and the guide tube is secured by a set screw at the plastic O ring of the XY-positioner. The dura-penetrating rod is inserted through the guide tube and pressed against the dura. By exerting pressure from the top of the stainless steel rod, the dura can be penetrated without much effort. In our experiments, a rod that protrudes out of the guide tube by 1.5 mm is sufficient to puncture through the dura in most cases. We often start by using rods that protrude 1.0 mm, then switch to the ones with longer protrusion as needed. We have used rods protruding from 1.0 to 2.0 mm.

Before and after the experiments, the probes and tubings should be thoroughly flushed with disinfectant solution (Nolvasan, Fort Dodge Co.) and distilled water. Ethanol or warm water may be used in the case of tubing clogs. It is preferred that the injectrode is sterilized in a gas sterilizer (Anprolene, AN74) before each usage.

When ready for recording, the injectrode is marked by ink or a piece of clay on the surface of its tubing to indicate the length of the guide tube. The injectrode is then inserted through the guide tube. A hydraulic slave cylinder (David Kopf Instruments, Inc., Model 608, weighted approx. 52 g) is then attached to Kopf XY-positioner. The injectrode is held by the Kopf manipulator so that the marking point stands at least 1 mm above the top of the guide tube. This places the injectrode tip at least 1 mm above the surface of the dura. Then, the injectrode is advanced through the already punctured surface of the dura by a remote-controlled hydraulic micropositioner (David Kopf Instruments, Inc., Model 650).

One difficulty that we have encountered with the guide tube setup is loading the injectrode into the back end of the guide tube. To avoid damage during loading, we use overhead, binocular magnifying glasses (Edroy Products, Inc., power of $200 \times$) with an illuminating light. With the help of the magnifying glasses, we have had little difficulty inserting the injectrode into the guide tube. For the deep penetrations when the guide tube extends through the dura, this no longer becomes an issue, because one can leave the injectrode in the guide tube during dura penetration and not worry about the electrode tip being damaged.

It is worth noting that one needs *not* sedate the monkeys in order to set up the XY-positioner, hydraulic manipulator and the injectrode in behaving monkeys. However, the monkey's head needs to be restrained for this purpose. We surgically implant a

head bolt over the monkey's head (Everts, 1966; see Lemon, 1984 for review). During the recording, the monkey is seated in a primate chair. A head-holding rod is locked in one end to the head bolt of the monkey, and is secured in the other end to a horizontal bar on the top of the primate chair. This places a restraint on the monkey's head movement. As soon as the injectrode is in place and the recording cables are connected, the head-holding rod is carefully unlocked and removed, so that the monkey's head is free to move. This procedure is routinely performed during daily recordings using the injectrodes or standard electrodes.

2.3. Injectrode used in head-unrestrained monkeys

When the injectrode is used in head-unrestrained monkeys, there are additional considerations. For instance, PE tubing may become entangled with other cables as the monkey rotates its head. Tangled tubing could also pull the PE tubing off the injectrode or interrupt fluid flows. Therefore, a mechanism that prevents this from happening is important.

One solution to this problem is to relieve the pulling force by anchoring the injection tubing to the top of the monkey's head. Instead of connecting PE tubing straight to the Hamilton microsyringe, we glue part of the PE tubing to the surface of a miniature four-pin Nylon connector (Fig. 3B, Microtech, Inc., GM-2, male). The male pin connector is plugged into a female-pin connector that is permanently cemented next to the recording chamber. In this way, we reduce the risk that the connection between PE tubing and the injectrode will be pulled off during the experiment. Moreover, the anchoring setup makes meniscus monitoring much easier if the meniscus is centered on the horizontal (instead of the vertical) portion of the tubing.

3. Discussion

Early development of injectrodes has focused on adapting the technique for head-fixed animal experiments, and the injectrodes have been made of glass pipettes or a combination of pipette and metal tubing (Malpeli and Schiller, 1979; Crist et al., 1988; Dias and Segraves, 1997; Malpeli, 1999). Although the early techniques have offered satisfactory convenience, the injectrode made of glass pipettes would not be adequately durable for the head-free animal experiments, in which parts can break easily as a result of movement. The ideal injectrodes designed for later experiments should consist of the following: (1) all-metal (or metal–plastic) construction; (2) lightweight; (3) secure tubing connections; and (4) superior recording capability to that of standard electrodes.

The injectrode described here has been designed to meet some of the demands. It is constructed with metal–plastic materials, and it is lightweight and durable enough for repeated use. Its components are readily available from commercial sources, and thereby are easy to assemble. We have successfully used the injectrodes for injecting a GABAergic agonist, muscimol (0.5–1.0 μl of 1 μg/μl; Goffart et al., 1999), in a reversible inactivation study of cerebellar fastigial nucleus in awake, behaving monkeys. In these experiments, the injection was done by pressure using a 1-μl Hamilton microsyringe in 0.1-μl steps per minute. The injectrode was filled with muscimol at the start of the day, loaded into the brain later, and then the target sites were examined for unit activity and effects of microstimulation. When microstimulation confirmed the site as being of interest, microinjection was then conducted at that site. We have used one injectrode for weeks of experiments, and we have isolated units well even after weeks of penetrations.

It is worth noting that, in practice, occasionally when the injectrode is inserted into the brain, air compression or pressure of tissue fluid can push the injection fluid slightly backwards. Hence, the amount of injection must be adjusted to compensate for the misalignment of the meniscus before and after the injectrode enters the brain. We have rarely seen the meniscus move forward as the injectrode enters the brain. If that happened, one must suspect a leak in the tubing connections or the injectrodes.

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