A grid system and a microsyringe for single cell recording

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The designs of two instruments are presented which have proven to be useful in single cell and chemical injection studies performed in awake monkeys. The first is a plastic grid that acts as a guide to produce parallel penetrations with either a microelectrode or microsyringe. The second is a syringe for injecting microliter quantities of a solution that also allows recording of neuronal activity.

Introduction

In a number of our recent experiments on chronically implanted monkeys, we have found it necessary to explore areas within the brain with a series of microelectrode penetrations (Komatsu and Wurtz, 1986, 1988). In order to establish that a given microelectrode penetration corresponds to a particular electrode track on subsequent histological sections, it has also been essential to devise a way to produce parallel electrode tracks within the brain. To do this, we have developed and used a grid system that attaches to a standard Evarts type implanted base (Evarts, 1966, 1968), but the principles involved should apply to any chronic recording system. This grid has been designed to work with microelectrodes, guide tubes and a recording microsyringe for chemical injections, which will also be described.

Materials and Methods

The grid system

Fig. 1 shows the components of the grid system: a plastic ‘grid’ cylinder, an ‘extender’ and an ‘implanted base’. The grid was designed to fit within the extender, which in turn can be attached securely to an Evarts style implanted cylinder base (Evarts, 1966, 1968).

The grid cylinder (Fig. 1A, B) is made from delrin to reduce weight, and is ‘well’ or ‘cup’ shaped. Its cylindrical wall (40.0 mm high), has an outer diameter (o.d.) of 19.05 mm (0.75”), an inner diameter (i.d.) of 16.80 mm (0.661”), and a floor 10.0 mm thick.

The floor (Fig. 1, B1 and B2) consists of the ‘grid’ of 72 drill holes, the centers of which are 1.0 mm apart in the x–y plane. These holes are large and deep enough to stabilize a thin-walled 23-gauge guide tube. This design holds the guide tubes with virtually no play. The guide tubes in turn, are large enough to allow passage of either a thin tungsten microelectrode or our recording microsyringes. The holes are also large enough to guide thin platinum–iridium microelectrodes.

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mounted in stainless steel tubes which we use for exploration.

The grid may be aligned either by eye or stereotaxically, then secured indefinitely within the extender using small 2–56 cone point allen screws in the upper row of holes in the extender (shown as a single upper hole in Fig. 1A). The extender sits above the implanted base and is secured to the base by the same size allen screws in the lower row of holes. In addition, the extender is notched on its bottom rim allowing alignment of the extender (and thus the grid) over a peg on the recording cylinder. In this way, the extender–grid assembly may be removed and replaced repeatedly while maintaining its position relative to the brain, and consequently maintaining the integrity of the recorded maps. There are two types of grids. The first type (Fig. 1, B1) has 235 holes placed symmetrically around the center of the grid. Along a row in either axis, the center-to-center distance between the holes is 1.0 mm. The second type of grid (Fig. 1, B2) has 188 holes that are offset 0.5 mm in one direction with respect to the symmetric grid. This offset allows for a combined resolution of 0.5 mm for penetrations in the offset direction by replacing the first type of grid with the second. For instance, by first arranging the symmetric grid so that its rows run in the anteroposterior (AP)

![Diagram](image)

**Fig. 1.** The overall dimensions of the grid, and the device used to align the grid in the extender. A: an exploded view of the grid system assembly showing the relation of the grid, extender and Evarts type implanted cylinder base. See text for an explanation of its assembly. B: detailed view of the grid cylinder. B1 and B2: bottom view of the symmetric (standard) grid, and bottom view of the offset grid, respectively. Note that the offset grid has the center hole missing in the top row as an indicator of the direction of the offset. C: the device which is used in conjunction with the stereotax to align the grid in the cylinder. The lower half shows the bottom views of the two types of aligners. Each type has pins extending from their bottoms which then fit into the holes of the grid, thus holding the grid in a particular orientation. The pins are separated from each other by 5.0 mm. The aligner for the standard grid has its pins arranged in a line, while the pins on the offset grid aligner are arranged in a 90° angle. This pin arrangement identifies the aligner as a standard or offset type.
and mediolateral (ML) directions, then aligning the offset grid with its rows in the same orientation and with the row with the missing hole facing anteriorly, a 0.5 mm offset in the AP axis will be obtained. Then rotating the offset grid by 90° will change the offset to the ML axis.

In addition, the grids may be aligned in stereotaxic planes with the use of a device (Fig. 1C) which attaches to the stereotaxic manipulator. This alignment tool fits within the grid well and has pins on its bottom surface, which fit in the grid holes. It is designed to fit onto the adapter used to position the monkey’s head holder in stereotaxic coordinates. This ensures that the grid holes are aligned in the AP and ML axes. There is a separate aligner for each type of grid. The implant base and extender are generally similar to those originally described by Evarts (Evarts, 1966) and are made from grade 316L stainless steel.

The guide tube-electrode assembly

Each guide tube (Fig. 2A) is flared at its top which facilitates the top loading of an electrode into it and which, more importantly, controls the depth of the guide tube in the brain. This is accomplished by encasing the guide tube in a

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**Fig. 2.** A: the guide tube assembly. B: an exposed view of the entire grid, guide tube and electrode assembly as used in a long-term chronic (permanent) setup. The spacer (shown but not labeled) is cemented onto the anchoring plate, while the electrode and guide tube are in turn cemented against the top of the spacer. However, the grid system is usually used without the cement and anchoring plate, which allows for the easy removal or replacing of guide tubes. C: the chronic electrode anchoring plate.
stainless steel sleeve or 'spacer' (20.5 gauge tubing), which is wider than the grid holes, and that does not allow passage of the guide tube's flared top. The spacer then holds the guide tube at a predetermined depth, and prevents it from penetrating any further. The guide tube has a sharply beveled bottom which allows it to penetrate the dura with a minimum of dimpling.

In addition, each guide tube has a matching insert tube (28 RW) which fits within the guide tube, is the same length as the guide tube, and has a matching bevel at its bottom. A notch is placed at the top of the guide tube which holds the bent top of the insert. This prevents the insert from rotating within the guide tube and keeps the beveled bottoms in register (similar to the design of a spinal needle). When the guide tube is not in use, it is plugged with the insert and the top of the assembly is covered with an antibiotic cream. This prevents tissue and blood from blocking the bottom of the guide tube, and also aids in preventing infection. The guide tube assembly can therefore be left chronically in place within the recording cylinder, or repeatedly taken out and replaced.

Placement of a chronic (permanent) electrode in the grid is achieved with the use of a stainless steel anchoring plate (Fig. 2C). This plate consists of holes that are the same size, and in register with those in the grid. It is held in place by a screw (000–120) which is self-tapped into a grid hole. A guide tube assembly can be inserted through the plate and grid, and held in place with dental acrylic as shown in Fig. 2B. The plate is desirable because it provides a surface onto which the acrylic can hold. In contrast, the dental cement will not adhere to the grid's Teflon-like delrin surface. A tungsten electrode can then be lowered through the guide tube to the desired depth, and cemented in place at the top of the guide tube as shown in Fig. 2. This design prevents the electrode from moving either up or down relative to the brain. Removal of the electrode is accomplished by drilling away the dental acrylic at the base of the spacer, and removing the entire electrode and guide tube assembly. The plate is designed to allow the placement of several guide tubes.

The microsyringe

Our recording syringe (Fig. 3) is a modified Hamilton syringe (10.0 µL, 701RN series, screw type) onto which an extending recording barrel, (30RW stainless steel tubing, All Tube, New York, NY) is attached.

The recording barrel is 102.0 mm long, has an o.d. of 305.0 µm (0.012"), and an i.d. of 150.0 µm (0.006"), and contains a fine enamel-insulated stainless steel wire (California Fine Wire, Grover City, CA, SS304, diameter = 0.002") This wire is

![Diagram of recording system](Fig. 3. An exploded view of the syringe assembly. The various tube widths are not drawn to scale, and the exposed length of the recording wire is exaggerated to show that it extends beyond the tip of the syringe.)
thin enough to allow the flow of relatively high viscosity solutions through the syringe barrel. For instance, it has been used successfully for injections of WGA–HRP (7%), Fast blue (4%) and Diamidino yellow dihydrochloride (2%) (Stanton, Segraves and Goldberg, unpublished experiments). The recording barrel has a 10.0 mm notch at one end to match a notch made on an intermediate tube (25TW gauge) approximately 40.0 mm long and having an i.d. matching the o.d. of the recording barrel (3050 µm). This produces a hole when the two tubes are placed together, through which the wire enters the barrel. The most difficult step in manufacturing these modified syringes is in avoiding damage to the wire insulation as it traverses this hole.

The wire passes through the barrel and extends 750.0 µm beyond the tip of the tube. This was designed so that the recording tip of the wire was far enough away from the large syringe barrel that tissue displacement would not interfere with the recordings, yet not far enough to place the wire at risk to damage (the length of exposed wire shown in Fig. 3 is exaggerated). At the recording end, the wire is cut straight across (perpendicular to its length) with a pair of scissors, in order to minimize the surface area of exposed wire and produce a resistance suitable for unit recording. We have measured wire impedances in a range of 0.3–1.0 MΩ at 1000 Hz, and have been able to record single units with wire resistance in this range.

The original syringe barrel (Fig. 3) is cut to an approximate length of 21.0 mm. The recording barrel, with the attached intermediate tube, is connected to the original syringe barrel by a stainless steel coupling sleeve (21TW gauge). All pieces are cemented in place with a non-conducting epoxy (Locitite Fast Cure epoxy 45) around which an exposed portion of the wire is wrapped. Finally, a conducting epoxy (Acme, E-Solder 3021) is set around this portion of the wire. This results in an air-tight, long, yet thin syringe shaft which is insulated from the recording wire. The wire can then be connected to the recording preamplifier by clamping the input lead to the conducting epoxy.

The microsyringe can be held and advanced with a microelectrode microdrive, with the shaft of the syringe clamped by a modified electrode carrier. We typically clamp the syringe anywhere along its intermediate tube, coupling sleeve or the original shaft (where the tube is sturdiest). We then support the weight of the syringe body by taping it to the microdrive. Depth of the syringe tip is then indicated by the microdrive depth scale. The location of the injection sites can be marked by passing current through the recording wire (20 µA for 60 s works well for long-term studies). The recording wire of the syringe typically records multi-unit activity, however we have frequently been able to isolate single cells.

The syringe is best filled by repeatedly unscrewing the barrel, loading the body of the syringe and injecting the solution through the barrel until the dead space is removed from the syringe. Then the syringe can be loaded from its tip as is done with a normal syringe. The syringe can be cleaned the same way as is done for an unmodified Hamilton syringe. The lifetime of this syringe has varied from one use, to several successful recordings and injections. Care must be taken not to bend the exposed wire at the tip of the syringe.

Discussion

Using the grid system, we have been able to make penetrations 0.5 mm apart and still reconstruct the individual tracks from histological sections. The thickness of the grid floor provides a guide for the electrodes, and produces more parallel penetrations than does the conventional Evarts system we have used previously. Furthermore, since the grid can be removed and replaced, parallel penetrations can be made over many days of recording. We have been able to reliably reconstruct the grid-like array of electrode penetrations after a period of several months of chronic recording (Komatsu and Wurtz, 1988). In addition, we have placed as many as 8 guide tubes in the grid at one time, over a period of several weeks, in order to study receptive fields of cells at various locations within the middle temporal area of the superior temporal sulcus (MT) in the monkey (Yamasaki and Wurtz, 1987). We have noticed on occasion, possible shift of brain tissue as noticed
by a shift in receptive field location after placing a guide tube 1 mm away from an existing guide tube. However, it is reasonable to assume that tissue displacement is an inherent result of guide tube use in general.

The dimensions of the grid have been designed to allow introduction of a syringe barrel and to allow us to accurately reach such deep lying structures as MT (Dursteler et al., 1986; Komatsu and Wurtz, 1986; Yamasaki and Wurtz, 1987) and superior colliculus (Stanton, Segraves and Goldberg, unpublished experiments). When the syringe and grid were used together, we have been able to place an ibotenic acid injection in MT on one day, return to the same location the following day to repeat the injection, and verify physiologically and histologically that the two injections overlapped. In addition, the syringe can be heat sterilized for use during surgery.

References