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A new intraretinal recording system with multiple-barreled electrodes for pharmacological studies on cat retinal ganglion cells

Motoharu Takao [a,*,1], Yi Wang [b,1], Tomomitsu Miyoshi [a], Ichiro Fujita [b], Yutaka Fukuda [a]

[a] *Department of Physiology and Biosignaling, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
[b] *CREST, Japan Science and Technology Corporation, and Laboratory for Cognitive Neuroscience, Department of Biophysical Engineering, Osaka University Graduate School of Engineering Science, Toyonaka, Osaka 560-8531, Japan*

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Abstract

To overcome technical difficulties associated with in vivo intraretinal recordings of cat retinal ganglion cells (RGCs) with multiple-barreled electrodes, we developed a new guide-trocar system that consisted of a small-diameter and large-diameter pipes. We also improved the method to construct tungsten-in-glass multiple-barreled electrodes suitable for intraretinal recording from RGCs. Only the small-diameter pipe was inserted into the eye ball through the sclera, through which only the taper part of a multiple-barreled electrode pass. The large-diameter pipe stably held the electrode at its trunk and remained outside the eye ball. Insertion of only the small-diameter pipe minimized damages in the eye ball and prevented the eye ball movements while positioning the electrode. The system allowed us to keep the recordings stable for more than 1 h. Iontophoretically applied L-glutamate successfully activated RGCs of both X and Y types in the cat retina. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Intraretinal recording; Retinal ganglion cell; Multiple-barreled electrode; Iontophoresis; L-Glutamate; Cat

1. Introduction

Studies using in vitro retinal preparations have provided abundant data on pharmacological and electrophysiological properties of mammalian retinal ganglion cells (RGCs) (for example Cohen et al., 1994). In such in vitro preparations, however, it is difficult to present complex visual stimuli to the retina and to maintain retinal tissues (Miller et al., 1986). Experiments on in vivo preparations are required to study the pharmacological regulation system relevant to complex visual functions of RGCs.

In vivo recordings have been performed with a specially designed intraocular manipulator system (Ikeda and Pringle, 1971; Molenaar and van de Grind, 1980). A guide-trocar was inserted for electrode advancement into the cat's eye ball through the incised sclera. The guide-trocar is used not only to protect and support fragile glass micropipettes, but also to function as a pivot at the point of the incised sclera so as to aid the manipulator to locate exactly the electrode tip in the retina without moving the eye ball.

Multiple-barreled electrodes have been frequently used for in vivo pharmacological and electrophysiological studies (Bolz et al., 1984; Ikeda et al., 1989). Though none of the previous papers gave detailed information on the construction of multiple-barreled electrodes used for intraretinal recording, the diameter of bundled glasspipettes was large, and, hence, the inner diameter of guide-trocar must be large. A large-diameter guidetrocar often caused intravitreous hemorrhage, lens luxation and abnormal miosis by unexpected mechanical injury in the uvea of the eye ball, while it was inserted into the eye ball through the incised sclera. Besides, the eye ball easily moved to hamper the receptive field plotting in coordination of visual field, accompanied by swinging the guide-trocar for electrode positioning.

* Corresponding author. Tel.: +81-6-6879-3611; fax: +81-6-6879-3619.
E-mail address: takao@phys2.med.osaka-u.ac.jp (M. Takao)
[1] These authors contributed equally to this work.

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Our recording system was designed as follows to prevent the troubles accompanied with a large-diameter guide-trocar. First, we made a special guide-trocar which was slender enough to prevent severe damages in eye ball, and to minimize its dislocation.

Secondly, we developed a new method to efficiently construct long-tapered multiple-barreled electrodes with high-recording quality. Through these devices, we succeeded in recordings from RGCs stably for more than one hour, and applications of neurochemicals onto the recorded RGCs through drug micropipettes.

2. Fabrication of the system

2.1. Construction of multiple-barreled microelectrodes

When we use an ordinary multiple-barreled glass micropipette with a short taper for in vivo retinal recordings, a large-diameter guide-trocar should be used to allow the trunk of the barrels to pass through. The problem is that a large-diameter guide-trocar requires a large incision in the sclera, and often causes deformation of the eye ball. To overcome these defects, we adopted tungsten-in-glass microelectrodes with triple glass micropipettes whose taper part was long and slender enough to pass through a small guide-trocar (Fig. 1A). Here we describe an improved method to construct tungsten-in-glass multiple-barreled microelectrodes.

We combined two previously reported methods. First, an assembly of three glass micropipettes and a tungsten wire were made according to the method of Li et al. (1990). A micropipette of 10 cm length without an inner filament (o.d. 2 mm, i.d. 1 mm, A-M systems, USA) and two micropipettes of 5 cm with an inner filament (o.d. 1 mm, i.d. 0.58 mm) were tied together with heat-shrinkable tube. A tungsten wire (o.d. 0.3 mm, length 7 cm, Inter Medical, Japan) was etched with an alkaline solution (Levick, 1972) until the diameter of the tip became 2.5 μm at 25 μm from its end. The etched wire was inserted into the longer mi-

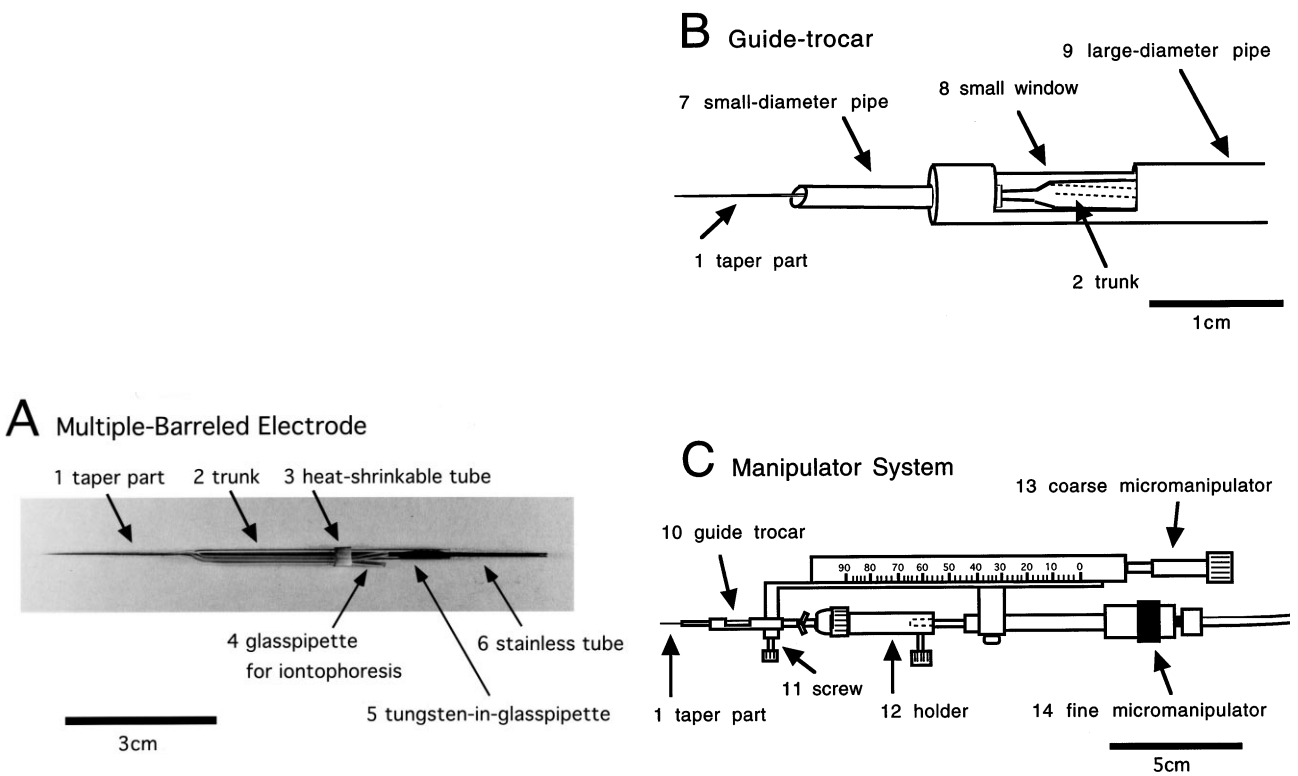

Fig. 1. Intraretinal recording system: multiple-barreled electrode (A), guide-trocar (B), manipulator system (C). A, Three glasspipettes are tied together with heat-shrinkable tube (3), one tungsten-in-glasspipette (5) and two glasspipettes for iontophoresis (4), to fabricate a tungsten-in-glass multiple-barreled electrode. The taper part (1) and the trunk (2) of this electrode are long (about 3 cm) enough to pass through guide-trocar (10 in C). The tungsten wire and the stainless tube (6) are fastened together in the tungsten-in-glasspipette (5), so as to lead the electrical activity easily to the amplifier. B, The guide-trocar consists of two stainless pipes of different diameter. The small-diameter pipe (7) and the large-diameter one (9) are connected and cemented with each other. A small window (8) made in the large-diameter part of guide-trocar allows us to see the tip and the taper of electrode and to insert easily into the small-diameter part (7). C, The large-diameter part of the guide-trocar is fixed to a coarse micromanipulator (13) with a screw (11). A multiple-barreled electrode and the fine micromanipulator (14) are connected to each other by the holder (12). A taper part of multiple-barreled electrode (1) is introduced through a guide-trocar (10) and advanced onto the retina with the aids of two micromanipulators, one coarse (13) and the other fine (14).

cropipette, and the assembly was placed in the heating coil of a microelectrode puller (PE2, Narishige, Tokyo) with the tip of tungsten upward. The coil was made of nichrome wire of 1 mm thick, had six to seven turns, and was 6–7 mm in height and 8.3 mm in outer diameter. The switches of the puller for automatic control of heat and magnet force were made ineffective. The assembly of tungsten and micropipettes was heated (current intensity, ≈ 17 A), while the lower pulling pole was held in position to prevent from its falling. After the glass of the micropipettes was melted sufficiently, the assembly was twisted by 180–360°, and the heating current was switched off to let the glass cool down and the three micropipettes adhere together. The assembly was then reheated, and pulled apart by allowing the pulling pole to naturally fall down by its weight. The diameters of the taper part of the whole assembly were 300, 400, 700, and 900 μm at 0.5, 1, 2, and 2.8 cm, respectively.

In contrast to Li et al. (1990), we intentionally allowed a small amount of the melted glass to coat the tip of tungsten wire. This could be accomplished by adjusting parameters such as the electric current for the heating coil and the length of tungsten wire above the coil. In Li et al.'s method, the parameters were selected so that the glass broke at the tip of tungsten wire to obtain a bare tip. In our hands, however, it was difficult to control the size of the electrode tip with a reasonable success rate. This was because any slight change in the above mentioned parameters led to varying distances of the breaking point of the glass. In our method, trial-by-trial changes in the parameters can be tolerated to a large degree by allowing glass to cover the tip of the tungsten wire.

The excess glass at the tip was then removed by using the method developed for single tungsten-in-glass electrodes (Merril and Ainsworth, 1972). A proper amount of solder glass powder (GSP990A510, Narishige) was heated on a platinum wire loop installed on a microphorge (MF77, Narishige, Tokyo). The temperature of the loop was adjusted so that the solder glass formed a softened but not melted bead, appearing red, not bright orange (current intensity, 6–7 A). The assembly was held by a micromanipulator, and the glass-covered tip of the tungsten wire was slowly pushed into the bead under a microscope. As soon as the tip was submerged in the bead up to the level the glass should be removed, the heating current was switched off. The bead was cooled down, and the excess glass was broken off by shrinkage of the bead at their junction. This procedure yielded a clear-cut fracture of the glass at a desired position of the tungsten tip. In addition, the tips of the other two glasspipettes for iontophoresis were neatly formed, and they were closely attached to the tip of the tungsten electrode. Furthermore, by repeating the process, the length of the tungsten tip and the tip size of the micropipettes could be readjusted.

The method described above markedly raises the efficiency to obtain high-quality multiple-barreled tungsten electrodes, with a successful rate up to 80%. In our experiments on RGCs in cats, the exposed tip of the tungsten wire was 5–7 μm long and less than 1 μm in diameter. The diameter of the tip of a single iontophoretic micropipette was 2–3 μm.

2.2. Construction of guide-trocar

The guide-trocar system is a key apparatus for the in vivo intraretinal recording. This must work as a pivot for rotation of an electrode manipulator at the sclera, a trocar for protection and support of a fragile electrode while recording, and a guide for smooth advancement of the electrode. For intraretinal recordings with multiple-barreled electrodes, a guide-trocar should have two contradictory characteristics. Firstly, the tip part of the guide-trocar inserted into the eye ball through the sclera must be as small in diameter as possible lest the eye ball should be damaged so much that intraretinal recording and visual stimulation could not be done, and lest the ocular position of eye ball should dislocate while rotating the manipulator around the pivot at the sclera. Secondly, the other part of the guide-trocar must be large in diameter enough for the insertion of multiple-barreled electrodes.

To fulfill these two contradictory demands, our new guide-trocar system consisted of two different stainless pipes, a small-diameter part and a large-diameter part (Fig. 1B). The small-diameter pipe (i.d. 1.5 mm, o.d. 2.0 mm) and the large-diameter one (i.d. 3.5 mm, o.d. 4.0 mm) were connected and cemented together. Though the diameter of the trunk of a multiple-barreled electrode was about 3 mm, a room (about 0.5 mm) between the large diameter pipe and the trunk of the electrode was needed for a smooth and fine control of electrode advancement. A small window was made in the joint between the two pipes for a sight control of directing and positioning of the electrode taper into the small diameter pipe via large-diameter pipe. This window and the open end of the guide-trocar were sealed by a sticky clay to prevent leakage of vitreous from these parts while recording.

2.3. Manipulator system

The coarse and/or fine micromanipulators are indispensable for neuronal recording studies. We used the special manipulator system originally designed for intraretinal recording in in vivo cat eye ball (Fukuda et al., 1984). The manipulator system, as shown in Fig. 1C, consisted of two micromanipulators, one coarse and the other fine (Narishige MO-8). The large-diameter pipe of guide-trocar was fixed to the stay of coarse micromanipulator with a screw, not to move while the

electrode was advanced. The fine micromanipulator was also connected to the coarse one with a screw. A multiple-barreled electrode and the fine micromanipulator were connected to each other by the holder. An electrode whose taper-part was introduced through the small-diameter pipe carefully not to damage the tip of electrode, was advanced with the aids of combination of these micromanipulators. This manipulator system enabled the electrode to advance easily but finely onto the retina across the vitreous body (about ≈ 2 cm).

3. Applications

3.1. Animal preparations

All experimental procedures were in accordance with the institutional and NIH guidelines for animal care and treatment. Adult cats were initially anesthesized with ketamine hydrochloride (32 mg/kg i.m.). After being mounted in a stereotaxic head holder, the animal was maintained for neuronal recordings by artificial ventilation of a mixture of halothane (1.0–1.5%), nitrous oxide (1.5 l/min) and oxygen(1.5 l/min). The animals were paralyzed by intravenous, continuous injection (5.0 ml/h) of 0.3% gallamine triethiodide in a mixture of 20% glucose and physiological saline. Secretions were minimized with atropine sulfate (0.4 mg/body i.p.). Electrocardiogram and the proportions of inspired and expired oxygen, carbon dioxide, nitrous oxide and halothane were monitored throughout the surgery and the recordings.

The eye ring was sewn on the limbus of the cornea and was held with the head holder, to minimize eye movement. A small incision was made with an ophthalmic scleral knife (20G) in the temporal upper part of the sclera ≈ 8 mm behind the limbus. The small-diameter part of the guide-trocar was inserted intravitreously through the incision. The large-diameter part of the guide-trocar was fixed to the manipulator system with a screw, as mentioned above (Fig. 1C). This manipulator system was attached to a rotator which was fastened on the overhead girder, propped by the pillars specially fixed on the stereotaxic apparatus (see Molenaar and van de Grind, 1980). The guide-trocar could thus rotate at the point of the scleral incision as a pivot, accompanied by a rotation of this manipulator system. This rotator functions as a fulcrum to control the rotation of the guide-trocar precisely at the scleral pivot.

As mentioned above, a tungsten-in-glass multiple-barreled electrode was introduced through the guide-trocar and advanced onto the retina with the aid of manipulator system (Fig. 1C). The inserting angle of the electrode onto the retina could be adjusted with the rotator.

3.2. Recordings of RGC activity and drug applications by iontophoresis

For recordings of RGC activities and drug applications by iontophoresis, the multiple-barreled electrode was connected to the input of a preamplifier. One of the two glass barrels was filled with 0.1 M L-glutamate (Sigma, USA) adjusted to pH 7.0 with 1 N NaOH to excite RGCs, while the other was filled with 0.9% NaCl to balance the current. They were connected via silver wires to an iontophoresis unit (Microiontophoresis Dual Current Generator 260, World Precision Instruments, USA).

Visual responses of RGCs were evoked by a stationary light spot of the same diameter as the receptive-field center, which was projected onto a tangent screen from a slide projector. RGC activity was recorded, while $+20$ nA iontophoretic current was applied as a backing current to the L-glutamate micropipette. Following the recording of the visual evoked unitary activity from RGCs as a baseline, L-glutamate was applied iontophoretically onto the same RGC to investigate its effects on the visual evoked unitary activity of RGC. The intensity of iontophoretic currents was usually -10 to -50 nA. When the band-pass filter was used from 300 to 3000 Hz, the signal/noise ratio of single action potentials was above 10 under good recording conditions. The recordings could be successfully continued stably more than 1 h when the recording conditions were good.

Examples of extracellular recordings of ON-X type (ON center brisk-sustained) and ON-Y type (ON center brisk-transient) of RGCs are shown in Fig. 2. For both cell classes, L-glutamate activated the maintained (data not shown) and the light-evoked activities. The X cell activity was obviously increased by -20 nA of L-glutamate. By contrast, the Y cell activity was only slightly increased by -20nA. This result is probably not a fundamental difference between the two cell classes, but simply reflects the different size of their dendritic fields (Bolz et al., 1984). The dendritic field of the Y cell was 1.7 times the area of the X cell so that iontophoretically applied L-glutamate did not appear to have diffused uniformly over the large receptive field of the Y cell.

4. Discussion

In the present study, we developed a new guide-trocar system including multiple-barreled electrode for in vivo intraretinal recording of single RGC activity in cat. This guide-trocar system was successfully employed in extracellular recording from RGCs and microiontophoretic application of L-glutamate onto them. Iontophoretically applied L-glutamate enhanced the visual evoked unitary activity of both X and Y cells. These

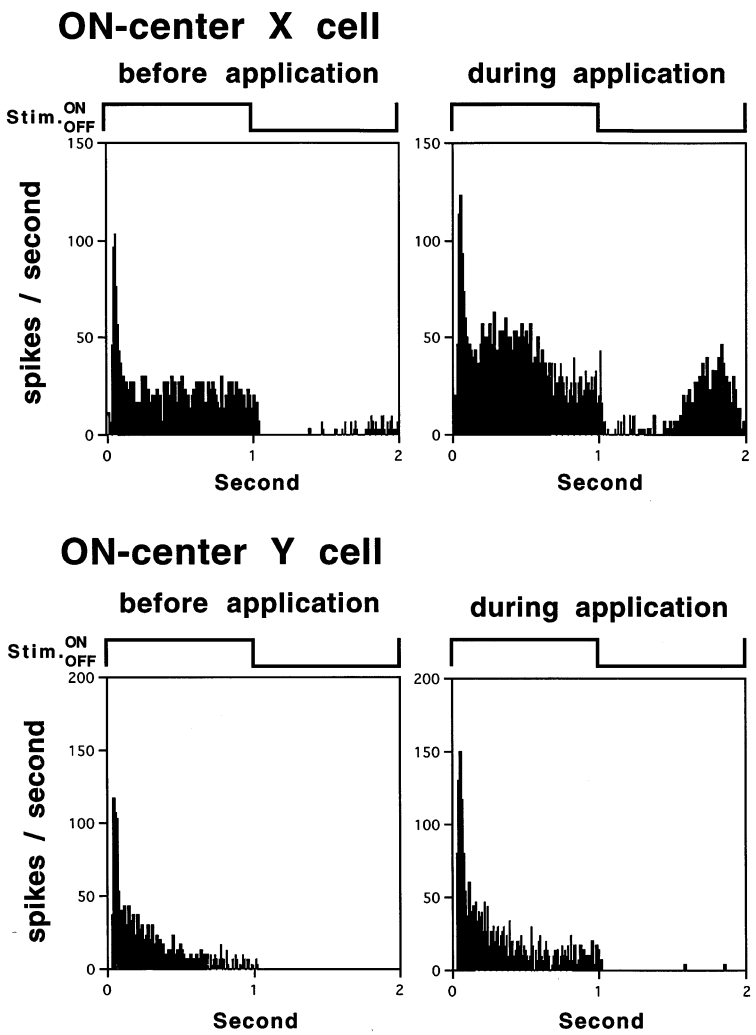

Fig. 2. Effects of L-glutamate on the activity of two types of RGC: ON-center X and ON-center Y cells. The cells were stimulated with a light spot in the center of their receptive field flashed for 1 s at 2 s interval. Post-stimulus time histograms (PSTHs) before and during application of L-glutamate with −20 nA are shown for each cell. PSTHs were collected at 20 ms bin during 30 consective stimulus presentations. Traces above PSTHs indicate ls periods when light was ON and OFF, respectively. The visual evoked unitary activity was enhanced by application of L-glutamate.

results showed the validity of our guide-trocar and multiple-barreled electrode, for intraretinal recording from RGCs in cat. We discuss hereinafter our successful improvement of multiple-barreled electrode and the advantages of our guide-trocar for intraretinal recording in in vivo cat eye ball.

We improved the techniques to fabricate tungsten-in-glass multiple-barreled electrodes. Our method to construct multiple-barreled electrodes was based on the previously reported one (Li et al., 1990). According to their method, the tungsten tip of multiple-barreled electrode was bared by the puller weight. Such inaccurate control causes a variation in size of the bare tips (1–3 μm in diamter; 5–30 μm in length). The electrode tip for the recording from cat RGCs need to be fine and accurate, because the majority of cat RGCs, X cells, are small in soma size, and they are difficult to be recorded with coarse electrode tips (Levick, 1972; Stone, 1973; Levick and Cleland, 1974). We improved the processing of tungsten tip in multiple-barreled electrodes, based on the Merril and Ainsworth (1972). We adapted their method for the fabrication of single-barrel electrode to multiple-barreled electrodes. As a result, a bare tip of tungsten could be precisely adjusted to a desired length (5–7 μm: Stone, 1973; Levick and Cleland, 1974). The precise adjustment of a bare tip of tungsten-in-glass multiple-barreled electrodes led to the successful recording of X cell activities.

In addition, our method of construction of multiple-barreled electrodes enables one to prepare different sizes of exposed tungsten tip for different experimental needs. In another series of experiments, we used electrodes with the tungsten wire tip of 5–15 μm in length and 3–6 μm in diameter for recordings from neurons in

the monkey inferior temporal cortex. We obtained a stable recording for several hours, and efficient application of γ-aminobutyric acid and its antagonist, bicuculline, through iontophoretic micropipettes of 2–3 μm in diameter (data not shown; see Wang et al., 1996).

We used the multiple-barreled electrode which has a profile of long taper and large-diameter trunk. As mentioned in 'fabrication of the system', another possibility would be to use slender multiple-barreled electrodes which could pass through a single, small diameter guide-trocar. For our guide-trocar (i.d. 1.5 mm), the diameter of a trunk of multiple-barreled electrode must be, of course, under 1.5 mm. Such electrode would be constructed using very slender pipettes (in case of three barreled electrode, each pipette must be smaller than 0.75 mm o.d.). Such electrode must, however, be fragile and difficult to deal with. Moreover, it is very difficult to construct the assembly of more pipettes, five or more (in case of a five barreled electrode, each pipette must be less than 0.5 mm o.d.). On the contrary, our system using large-diameter pipettes (in case of three barreled electrode, the maximum size of each pipette is 1.5 mm o.d.), is very easy to handle.

We tried guide-trocars whose small-diameter pipes were over o.d. 2.5 mm. In many cases, they were unsuccessful for the unexpected injury in uvea of eye ball, which caused intravitreous hemorrhage, lens luxation and abnormal miosis. We adopted o.d. 2.0 mm (i.d. 1.5 mm) stainless guide-trocar for easy control of insertion of electrode taper, and for thickness and toughness of the guide-trocar wall. Boos et al. (1990) and Ikeda et al. (1989) also utilized o.d. 2.0 mm and i.d. 1.2 mm guide-trocars, respectively, for in vivo iontophoretic studies of cat RGCs. Owing to our new guide-trocar, the operated eye could be kept normal and the RGCs' activities could be recorded from the same eye for more than 12 h.

Our new guide-trocar system including new multiple-barreled electrode has another advantage of successfully stable unitary recording of RGCs. An electrode needs to be held at two points so as to support stably an electrode itself for advancing and keeping its position. Our guide-trocar was also devised to sustain a tough trunk at the inner wall surface of guide-trocar so as to settle this electrode by surface contact. In effect we could keep steadily a fragile taper of multiple-barreled electrode in the eye ball while recording unitary responses of single RGC more than 1 h.

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