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A reversible system for chronic recordings in macaque monkeys

Ivan N. Pigarev [1], Hans-Christoph Nothdurft *, Sabine Kastner [2]

AG Neurobiology, Max Planck Institute for biophysical Chemistry, P.O. Box 2841, 37018 Goettingen, Germany

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Abstract

We propose a system for head fixation and neuronal recording that minimizes surgery for implantation. Fixation is obtained by posts which are attached to the opposite sides of the skull and are connected by a rigid frame around the animal's head. As forces are counterbalanced and distributed around the head, the system does not need to be implanted into the skull, and thus allows for continuous adjustment to the growing skull in young animals. Except for small incisions for the posts, the skin over the skull is left intact. Recording is achieved through small bone holes which are easily reached by means of conical guide tubes. The system provides perfect stability of recording, allows flexible access to various areas of the brain and can be easily removed during longer pauses in experiments. The use of this system may also decrease the number of laboratory animals needed. © 1997 Elsevier Science B.V.

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1. Introduction

The method of painless head fixation for electrophysiological experiments (Evarts, 1960; Hubel, 1960; Evarts, 1968; Noda et al., 1971) is now widely used in neuroscience. It allows the application of stereotaxic recording techniques in behaving animals. Depending on the actual requirements of a given task, various types of pedestals for head fixation are used in different laboratories, usually in combination with an implanted chamber for neuronal recording (Li and Jasper, 1953; Davis, 1956).

Along with obvious advantages, this technique has also several negative features. The firmly implanted recording chamber does not allow for an easy change of recording sites. The walls of the chamber restrict the angle of electrode penetrations. Also, chambers are often used with large bone holes which may lead to unstable recording due to brain pulsation. These instability problems become especially serious when the animal makes large movements or changes head orientation during the experiment. The dura mater under the recording chamber grows fast and needs to be scraped before new penetrations. Also, microelectrode penetrations through a largely exposed dura are often connected with deformations of the brain; in particular cases this may even lead to a temporary inactivation of neurons.

To eliminate these difficulties some modifications of this method have been proposed (Pigarev, 1977; Sirota et al., 1988; Beloozerova and Sirota, 1993). The surface of the skull was mainly left intact, and the head was instead fixed to a surrounding frame by small metal posts. These posts were screwed into the skull, and fixed to the frame by cement. In some cases these posts were long enough to pass through incisions of the skin. Microelectrodes were inserted into the brain through small holes in the skull. To reach these holes, either the skin over the recording zone was opened (Pigarev, 1977), or microelectrodes were guided through small

* Corresponding author. E-mail: hnothdu@gwdg.de

[1] On leave from the Institute for Problems of Information Transmission, 101447, Moscow, Russia.

[2] Present address: Laboratory of Brain and Cognition, LPP, NIMH 49/1B80, Bethesda, MD 20892, USA.

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tubes inserted through the skin (Sirota et al., 1988; Beloozerova and Sirota, 1993).

In recent experiments on two juvenile macaque monkeys (*Macaca fascicularis*) we have recorded from extrastriate areas under head fixation while animals performed a behavioral visual task. For head fixation, we have applied the system proposed by Sirota et al. (1988) with minor modifications. The main difference was in the shape of the fixing frame, which in our case was constructed from four separate elements and was located at a rather low level, close to the equator of the skull. We also reduced the number of fixing posts, and instead of the recommended ten posts we used eight in one animal, and only six in the other one. With this number of posts the system provided good stability of the frame for 9 months in the first animal, and for 6 months in the second one. After that time stability of the frame was not sufficient for microelectrode recording.

When trying to improve stability of the frame, we found another way of fixation, which does not require any additional screws or holes in the skull. This new system allowed us to continue neuronal recording for another year with no stability problems during that time. While in our experiments the new system was applied only in parts and added to the already implanted frame, it is obvious that this method of head fixation can be even better used without any preliminary implanted screws.

In this paper we illustrate the principle of this new system, describe its main elements, and give a simple construction of externally applied guide tubes, which we used for microelectrode penetrations through the skin and through small holes in the skull.

2. Head fixation

Head fixation is provided by a frame that is firmly fixed to the skull by means of eight posts. These posts are, however, not screwed into the bone but touch it on opposite sides of the skull. They are held in position by the frame to which they are firmly fixed (Fig. 1A). The frame itself is made of two arcs, one in front and one behind the head, which are interconnected by two longitudinal bars. An optional third bar somewhere on top (not shown in Fig. 1) may be added to improve rigidity of the system. Construction of these bars and arcs allowed us to change the size of the frame and the relative position of individual parts.

The system shown in Fig. 1 is made of aluminium and weighs less than 100 g including posts and fixation patches of dental cement. Posts are made from plastic, with aluminium heads. All screws are made of stainless steel. While we used the same material in our prototype, it may be reasonable to replace the material in future models by plastic, thus allowing for nuclear magnetic resonance imaging (MRI) of the animal's head even when the frame is fixed.

Fig. 2 presents a schematic drawing of fixing elements superimposed on a sagittal (A) and a frontal (B) MRI scan of monkey's head. For clarity, posts are shown as if they were located within these planes; in reality, however, they are located at intermediate orientations (Fig. 1). Note that the optional third longitudinal bar is shown in Fig. 2A. For head fixation during experiments the frame is fixed to a head holding system by means of four stainless steel bars with conical ends (Fig. 1B, Fig. 2B) that fit into holes in the frame. To protect recording tubes (see below) and connectors, we recommend to cover the frame with a helmet attached to the fixation frame which is easily removable for experiments.

Posts (Fig. 3) are passed through incisions in the skin down to the surface of the skull. They need to be attached to the frame at their other end; in our prepara-

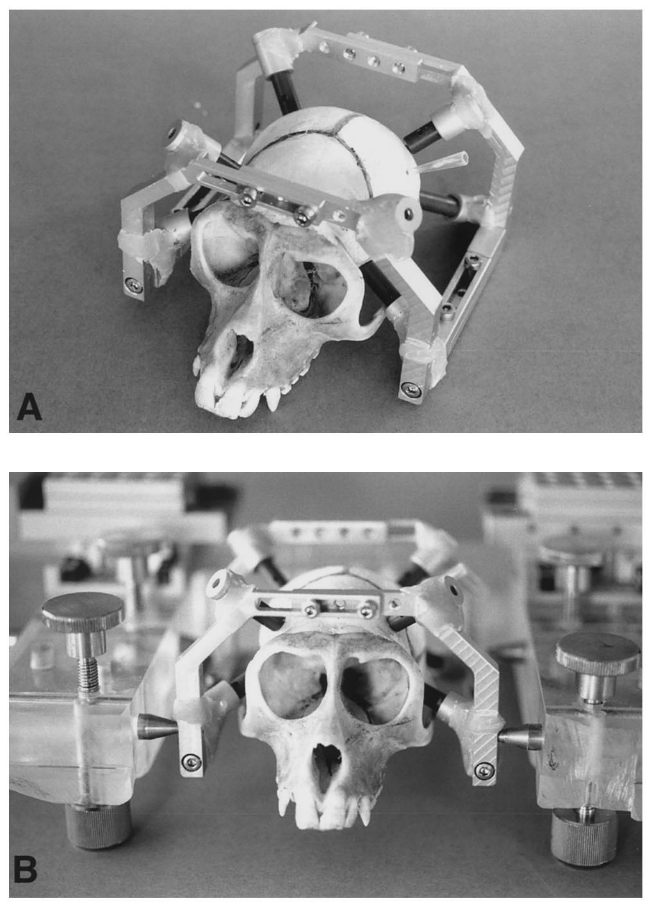

Fig. 1. Photographs of the proposed head fixing system attached to a monkey's skull; (A) alone, and (B) when fixed to the head holding device. Stability of the system is provided by an outer frame fixed to the skull by eight posts that touch only the surface of the bone. Arcs and longitudinal bars can be adjusted in size. During experiment, the frame itself is held by four conical bars. Note the recording tube over the left hemisphere.

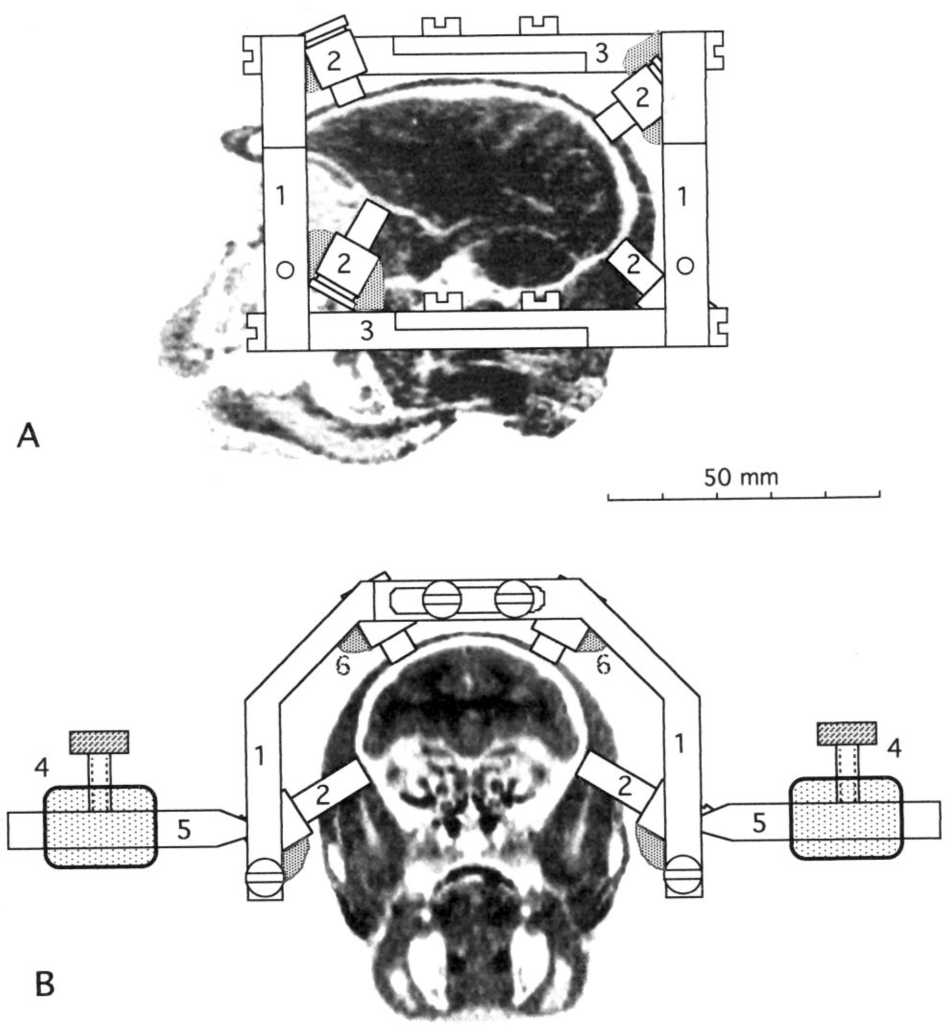

Fig. 2. Schematic illustration of head fixation by posts which touch the skull from opposite sides. The drawing is superimposed on MRI scans (negatives) of a monkey's head, (A) sagittal, (B) frontal views. (1) Fixing arks; (2) posts; and (3) longitudinal bar. The system is attached to an outer head holding device (4) by means of four conical bars (5). Posts are fixed to the frame with dental cement (6).

tion we used dental cement which allowed us to fixate posts in any orientation. Each post can be changed in length by rotation of an inside screw so that the pressure on the skull can be varied once posts are fixed to the frame. A small silicon plate at the tip distributes this pressure.

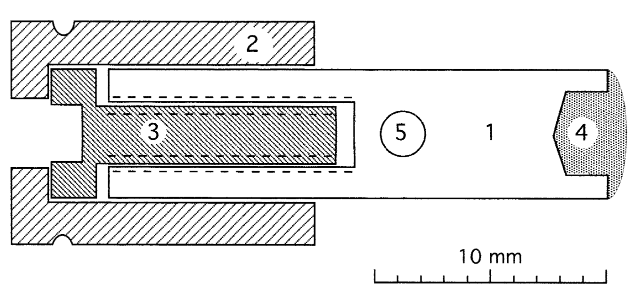

Fig. 3. Construction of the post with adjustable length. (1) Plastic post; (2) aluminum head; (3) screw for length adjustment; (4) silicon plate; and (5) hole to hold the post during adjustment.

For installation of the system, the head of the monkey must be fixed in a conventional stereotaxic holder while the new frame is placed around the head, and arcs and longitudinal bars are adjusted to optimal positions ($\approx$ 0.5–1.0 cm above the skin). Once good positions for the posts are found, small incisions into the skin are made, soft tissue is moved apart and posts are inserted until they reach the surface of the skull. The heads of the posts are then attached to the arcs of the frame by dental cement. When firmly fixed, the lengths of the posts are adjusted by gentle rotation of the regulating screws to provide rigid fixation of the head within the frame. This pressure might be slightly reduced in intervals between recording sessions.

In our experiments, surgery was performed under general anesthesia with pentobarbital (Nembutal®, Sanofi, 5–8 mg/kg per h i.v.) in aseptic conditions, with post-surgical antibiotic (clindamycin, Sobelin® Solubile 300, Upjohn, 20–40 mg/kg three times a day) and analgesic treatment (metamizol, Novalgin®, Hoechst, 0.2 ml i.m.). Guide tubes for recording (see below) were implanted under ketamine hydrochloride (Ketanest®, Parke-Davis, 10–25 mg/kg per h). All procedures were carried out under institutionally approved protocols and conformed to the NIH Guidelines for the care and use of animals.

Our animals lived well with a permanently installed frame without any signs of discomfort for 2 years. No special requirements to the construction of the cage was needed. The monkeys soon included the frames into the scheme of their body and were never seen to touch the cage with the frame or were never caught by the implanted system on the sides of the cage.

3. Neuronal recording through small holes in the skull

Because the frame is arranged around the monkey's head, the entire surface of the skull could be accessed for microelectrode penetrations. We have left the skin covering the head intact and performed neuronal recording through small holes (1–1.5 mm in diameter) through the skin and the skull.

To allow electrodes to pass easily through skin and the bone hole, we installed guide tubes which were of conical shape design to allow for an easy change of microelectrodes. We found transparent single-use polyethylene tips for chemical micropipettes quite useful for this purpose. Before implantation, the tube was filled with melted bone wax up to half of its length (Fig. 4). Depending on the actual diameter of the hole and the planned tilt of the tube, the tip was exactly adjusted in size by cutting off parts of its conical shape. In order to produce an optimal fit, we used a similar hole in a metal plate which had the same thickness as the skull (Fig. 4A). The length of each tube was measured for reference in later electrode penetrations.

For implanting a guide tube, first a small incision into the skin was made and the soft tissue underneath was moved aside. When the skull could be reached, a small hole was drilled using a slowly rotating machine (e.g. a hand drill with flexible shaft). The hole should be drilled with precise diameter; for this purpose, drilling bits for metal are better than the spherical drills used by dentists. If no big blood vessels were found in the dura underneath the hole, the tube was firmly pressed into the skull and fixed by dental cement to the frame or to a microdrive platform (see below). The dura itself was left intact.

For recording, we used varnished tungsten microelectrodes on which we had previously marked the length of the tube. Electrodes were inserted into the bone wax and moved, by hand, along the wall of the tube down to the mark indicating the length of the tube. When an electrode was installed, a micropositioning device was adjusted for external control of further penetration. At the end of the experiment, the microdrive was disconnected, and electrodes were removed or even left in the brain. We obtained stable recordings sometimes for more than 2 months with the same microelectrode in one penetration. When electrodes were removed, the electrode tracks through the bone wax in the tube should be closed, for example by melting the wax with a piece of heated wire.

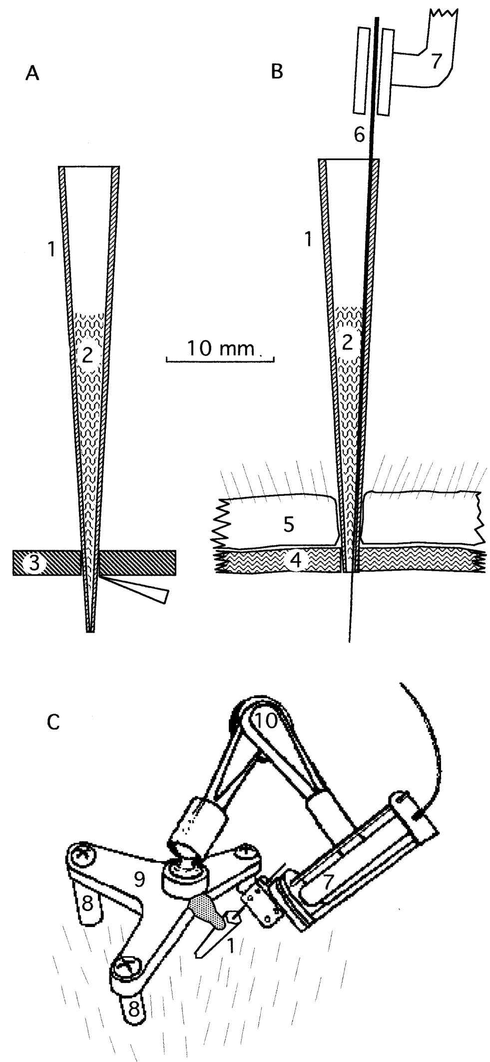

Fig. 4. The guide tube system. (A) Adjustment of tube length in the model of the skull; (B) schematic drawing of the installed guide tube; (C) drawing of the complete system, with platform, attached micromanipulator, and guide tube. (1) Polyethylene guide tube; (2) bone wax; (3) metal plate; (4) skull; (5) skin; (6) microelectrode; (7) micromanipulator; (8) posts; (9) platform; and (10) holder for micromanipulator.

To vary the trajectories of penetrations through the same tube, electrodes could be guided along opposite sides of it's conical wall. For larger variations, a new tube in another tilt could be installed at the same hole. However, for a strong tilt, one side of the hole must be previously enlarged. The use of steeply tilted tube positions allowed us to make almost tangential penetrations through the cortex and to study neurons in superficial areas as far as 15 mm away from the site of the hole.

For more than 1 year of daily recordings through one hole we had had no problems with penetrations through the dura. In one monkey, recording tubes were always oriented orthogonal to the surface of the skull and recordings were made through different holes. Usually, after some weeks of recording through a new hole, it was not possible to locate the previous holes, which apparently were closed with new bone.

We assume that microelectrodes would be moved best by a small microdrive fixed directly to the recording tube. In our experiments, however, we used conventional hydraulic microdrives which, for the sake of stability, were later attached to a separate platform (Fig. 4C) that was independent of the main frame. This platform was fixed directly to the skull by means of three thin posts passing through the skin and screwed into the bone. These posts were located far from each other at places not to be used for recording. Since the microdrives were only installed under head fixation and removed after each experiment, and since the platform itself was hidden under the helmet and thus not exposed to external forces, this fixation was sufficient to provide long-lasting and stable recordings. If recording tubes were also fixed to this platform, instead of the main frame for head fixation, the system provides perfect stability of recording even when the main frame is not rigidly fixed and minor displacements of the skull occur.

4. Reconstruction of cortical topography in stereotaxic coordinates

The approach described here is useful if recording sites can be localized exactly by their stereotaxic coordinates or easily be verified according to physiological properties of recorded neurons, as is often possible in investigations of deep subcortical structures. Cortical areas, however, are usually referred to by anatomical landmarks such as sulci and gyri. Pictures of sulci and gyri differ from animal to animal, which makes it impossible to use exact stereotaxic coordinates for localization. It is obvious that the small openings through the skull do not provide the possibility to define recording sites from visual inspection of the surface of the brain. But this information can be obtained using MRI.

In order to link MR images to the system of stereotaxic coordinates we made a special plastic stereotaxic device to fix the head of the animal during scanning. Along the axis of ear bars, and along horizontal and vertical holders, long channels of 1 mm in diameter were drilled. When filled with water, these channels became clearly visible under MRI and thus provided a precise location of stereotaxic planes. An example of a frontal MRI section of one monkey's head at the anterior–posterior coordinate 0 is shown in Fig. 5A; the water marks are clearly visible.

MRI was performed under ketamine anesthesia at 2.35 T (Bruker Biospec). Gradient-spoiled 3D FLASH images (TR/TE = 15/4.4 ms, flip angle = 20, 2 averages) were acquired using non-selective radio frequency pulses and oversampling in the longitudinal direction to avoid aliasing (Frahm et al., 1986). Matrix size was 128 pixel and the field of view was 100 mm in all directions.

From the 3D data orthogonal sections of the heads in frontal, sagittal and horizontal orientation were obtained using the external water marks as reference points. Based on these sections, cortical topography for

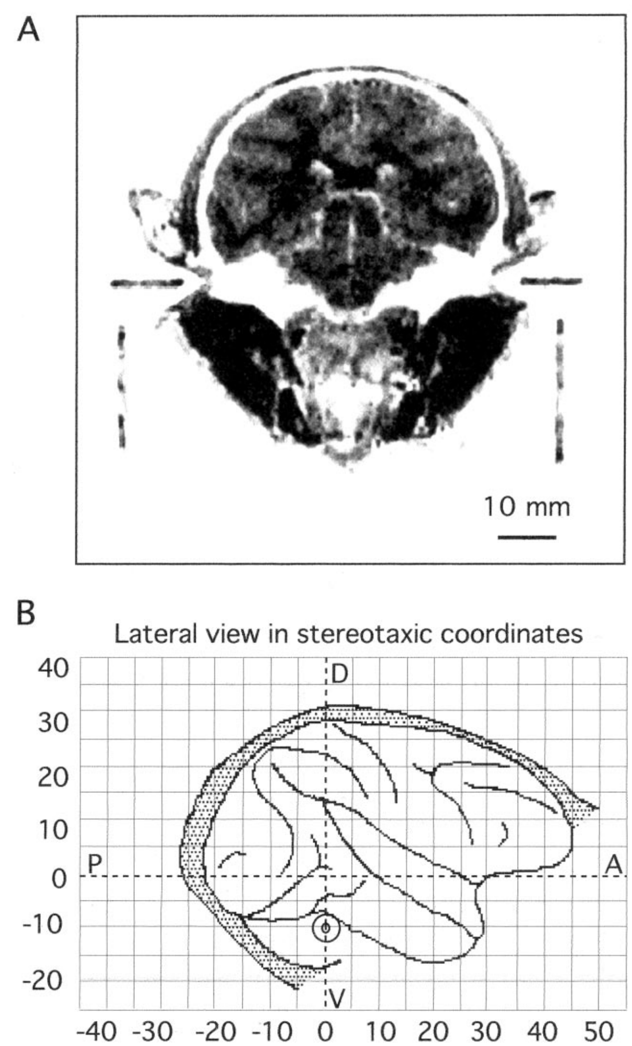

Fig. 5. (A) MRI scan of a monkey's head and (B) reconstructed cortical topography. The negative in (A) shows a frontal section at anterior–posterior coordinate 0 with vertical and horizontal water marks.

each animal was reconstructed in stereotaxic coordinates (Fig. 5B). These maps were used for further location of the recording sites in stereotaxic coordinates.

5. Discussion and conclusion

We present a method for head fixation and chronic recordings in awake monkeys, that has been used in a modified version for recordings from two animals in a visual task. Because the skull of living, in particular growing animals, is not absolutely rigid, a continuous pressure of the fixing posts onto the skull may produce slight deformation of the bone. It is therefore suggested to maintain the permanent pressure small and to release the pressure of posts slightly during recording-free intervals. For stability of the bone, one should also take care that animals receive a sufficient amount of calcium and vitamin D in their food or are regularly exposed to ultraviolet light.

Our experiments started when the animals were about 2 years old, and in 2 years of recording, their heads increased significantly in size. At intervals of 1–2 months we re-adjusted posts and frame, and did not notice obvious skull deformations from our system. Since the animals are still alive, a direct anatomical investigation has not yet been made.

Although we have used this system with small monkeys (*Macaca fascicularis*) with relatively thin bones, it should also work fine with species with a stronger skull (e.g. Rhesus monkeys) if the posts and frame are proportionally enlarged. Optimal locations for the posts and an adequate size of the frame should, however, be evaluated before installation.

The system leaves practically all the surface of the brain accessible for recording. Although the posts themselves would obstruct the direct access of electrodes to the small areas underneath, one of the advantages of this system is that the location of posts could be changed if necessary.

Using this technique, one may easily install several recording tubes and record simultaneously from different areas in the brain. In our experiments, we started with recordings in area V4 in one hemisphere and later installed another tube over the opposite side to perform simultaneous recordings from V4 neurons in both hemispheres.

After 2 years of recording, we decided to have a longer break in experiments with these animals. Fixing arcs were disconnected, posts and recording tubes were taken away and the trained animals were left alive. Some weeks later, these animals were indistinguishable from others that never underwent surgery for installment of a head fixation system.

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