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Telemetric recording of neuronal activity

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Abstract

A telemetric system is described which allows the wireless registration of extracellular neuronal activity and vocalization-associated skull vibrations in freely moving, socially living squirrel monkeys (*Saimiri sciureus*). The system consists of a carrier platform with numerous guiding tubes implanted on the skull. Custom-made microdrives are mounted on the platform, allowing the exploration of two electrode tracks at the same time. Commercially available quartz-insulated platinum–tungsten microelectrodes are used. The electrodes can be moved over a distance of 8–10 mm by turning a screw on the microdrive. Vocalization-associated skull vibrations are recorded with a piezo-ceramic element. Skull vibration signal and the signals from the two microelectrodes are fed into separate transmitters having different carrier frequencies. The signals are picked up by an antenna in the animal cage and are sent to three receivers in the central laboratory. Here, the signals are transferred via an analog/digital interface to a personal computer for data analysis and to a video recorder for long-term storage. The total weight of the head mount including carrier platform, microdrive, electrodes, skull vibration sensor, three transmitters, and protection cap is 32 g. The transmitters are powered with two rechargeable lithium batteries, allowing about 8 h of continuous recording. Reliable signal transmission is obtained over a distance of about 2 m. Recording stability allows to follow the activity of specific neurons up to several hours, with no movement artefacts during locomotion.

Keywords: Telemetry; Single-unit recording; Head-mounted transmitter; Intracerebral electrodes; Microdrive; Skull vibration sensor; Vocal communication; Squirrel monkey

1. Introduction

One of the most specific methods currently available in the investigation of behavioral control mechanisms is single-unit recording, that is, the registration of the electrical activity of single neurons during the execution of specific actions. The main advantage of this method over other methods, such as functional magnetic resonance imaging, positron emission tomography, electroencephalography, or magnetoencephalography, is that it combines high spatial resolution with high time resolution in the characterization of behavior-related neural activity. A major problem with the single-unit recording approach, however, is the necessity to make the experimental animal repeat the behavior under study often enough to allow the experimenter a comprehensive data acquisition. The usual approach to this problem is to train the animals in an operant conditioning task with the behavior pattern to be investigated as the operant. While some behavior patterns are conditionable quite readily, others are less. Vocalization, for instance, the behavior of interest in our group, belongs to the latter category. In the rhesus monkey, Yamaguchi and Myers [1] were unable to bring vocalization under stimulus control. Aitken and Wilson [2] succeeded in vocal operant conditioning, but only if instead of food reward a shock-avoidance procedure (Sidman avoidance schedule) was used for reinforcement. Sutton et al. [3], finally, were able to train macaques a vocal operant conditioning task with food as reinforcer. They were unable, however, to control the call type. On the other hand, vocalization is a behavior occurring frequently and in manifold forms in monkeys living in social groups, having the possibility to interact with each other freely. This makes clear that the

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electrophysiological investigation of some behaviors depends upon the availability of a method which allows single-unit recording in freely moving, spontaneously behaving animals. Single-unit recording in freely moving animals has been achieved already more than 20 years ago in rodents [4–6]. In these studies, the experimental animals were connected via a cable and a commutator swivel to the electronic equipment, thus allowing locomotion, exploration, feeding, and other types of behavior within the limits of the cage. Cable-bound recording is a suitable method in animals the locomotion of which is limited essentially to two dimensions and in which behavior is studied in single animals or very small groups of animals. It is not suitable in socially living monkeys, moving in three dimensions and kept in larger groups to allow for abundant social interactions. Also the highly developed manipulative capacities of monkeys, together with their physical strength, prevent the use of cable connections in unrestrained animals. To circumvent these problems, we have developed a telemetric system which permits the wireless registration of single-unit activity and vocalization-associated skull vibrations by the aid of head-mounted miniature radiofrequency transmitters [7]. In the following, this system will be described in detail.

2. Description of method

The system has been designed for the use in squirrel monkeys (*Saimiri sciureus*) and consists of, on the animal side, a headstage with one microdrive for a pair of microelectrodes, a piezo-ceramic sensor monitoring the vocalization-induced skull vibrations, and three radiofrequency transmitters. On the receiving side, it consists of an antenna connected with three receivers, a four-channel video recorder for long-term data storage and a personal computer with an analog/digital interface for data analysis. An additional video camera and microphone serves continuous monitoring of the animals' behavior from outside of the animal room.

2.1. Headstage

The carrier platform consists of an array of 12×12 stainless steel tubes (outer diameter 0.81 mm, inner diameter 0.51 mm, and length 5 mm), connected with each other by solder and being embedded in a 2×2 cm resin platform. The resin platform itself is embedded in a 3×3 cm Plexiglas platform containing four screwed holes for the fixation of a Plexiglas cap (Fig. 1).

The carrier platform is fixed to the skull in a stereotaxic operation. For this operation, the animal is narcotized (40 mg pentobarbital sodium/kg body weight) and placed into a stereotaxic apparatus. The hair of the head is clipped and the skin is incised in the way shown in Fig. 2A. The skin within the central circular incision is removed, the wound edges are held apart and four T-shaped holes are drilled with a 2.5 mm dental drill into the skull (Fig. 2B). Stainless steel screws (M 2.5, length 5 mm) are inserted into

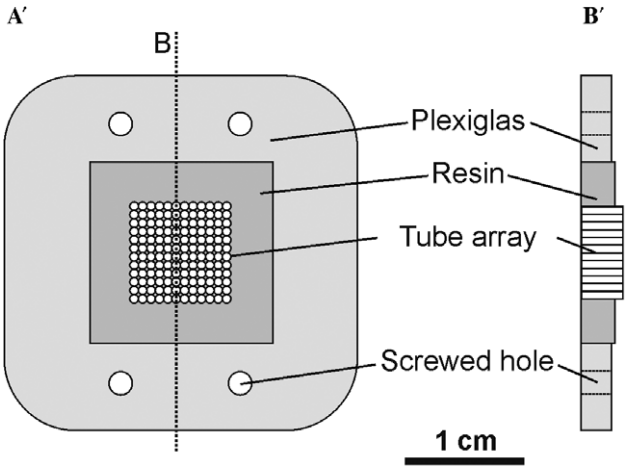

Fig. 1. Carrier platform for implantation on the skull. (A′) View from above. (B′) Cross-section.

the holes with the head being pushed into a position between skull and dura. The screws are fixed with nuts, and the remaining openings are closed with bone wax. The carrier platform is positioned by the aid of a stereotaxic micromanipulator in such a way that specific tubes of the platform are exactly above those brain areas to be explored (according to the stereotaxic brain atlas coordinates). The platform then is fixed to the skull by filling out the complete space between platform and skull with acrylic cement (Paladur; Kulzer, Wehrheim/Germany). Finally, the wound edges are adapted to the head mount and the skin incisions rostral and caudal to the mount are sutured (Fig. 2C).

2.2. Microdrive

For positioning of the microelectrodes, a modified spindle potentiometer (Conrad Elektronik, Hirschau/Germany) is used (Fig. 3). To reduce the size of the microdrive, the potentiometer is milled down to a cross-section of 5×3 mm (height 20 mm), so that only the spindle drive remains. The original slide is removed and replaced by a drop of acrylic cement embracing the worm. At the lower end of the potentiometer case, two stainless steel guiding tubes (outer diameter 0.46 mm, inner diameter 0.26 mm, 12° beveled tip, and length dependent upon the brain structure to be explored) are glued to the case with a distance of 1.15 mm. This distance allows the guiding tubes to enter two diagonally adjacent tubes of the carrier platform. Wires are soldered to the guiding tubes for use as reference electrodes. The microelectrodes are inserted into stabilization tubes (outer diameter 0.24 mm, inner diameter 0.11 mm). Microelectrodes, together with stabilization tubes, are pushed through the guiding tubes backwards and are glued with their ends to the slide with another drop of acrylic, after connection-wires have been soldered to the microelectrodes. We use quartz-insulated platinum–tungsten microelectrodes (Thomas Recording, Marburg/Germany) with a shaft diameter of 80 μm; the impedance at 1 kHz is 1–2 MΩ. For implantation of the electrodes, two holes of 0.5 mm are

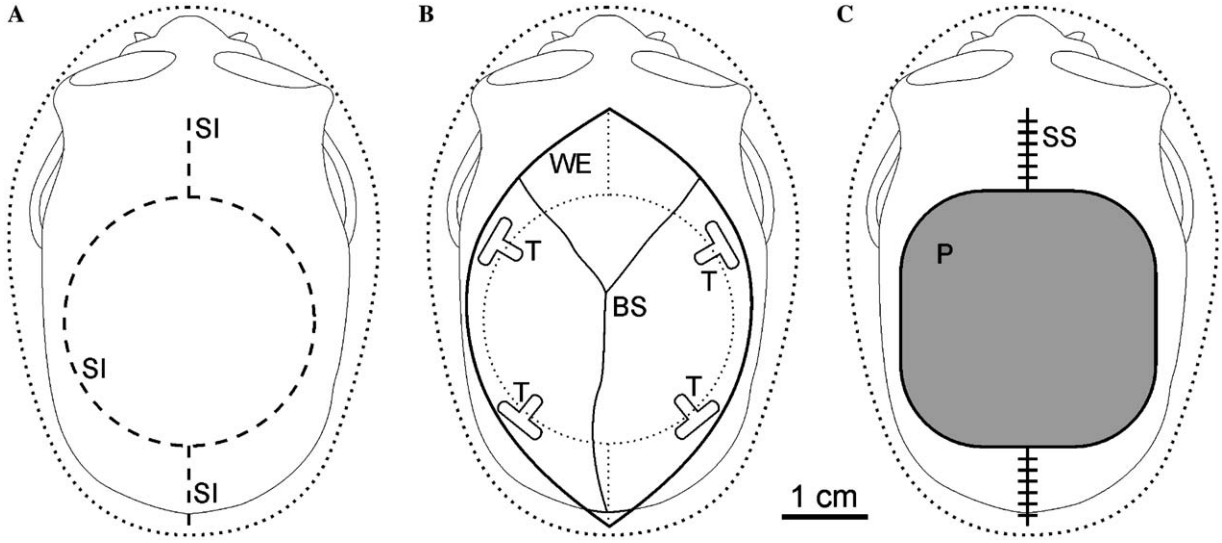

Fig. 2. Stages of platform implantation. (A) Placing of skin incisions. (B) Drilling of T-shaped holes for anchor screw fixation. (C) Platform in place with closed wound. *Abbreviations*: BS, bone suture; P, carrier platform; SI, skin incision; SS, skin suture; T, trepanation; WE, wound edge.

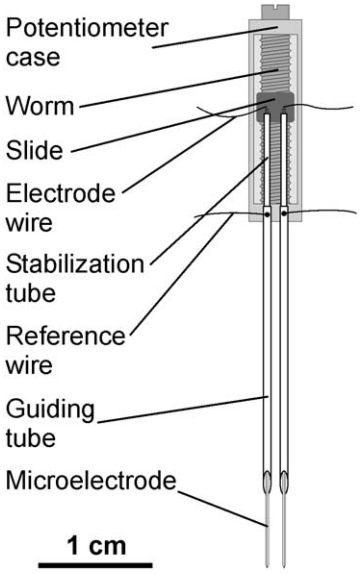

Fig. 3. Microdrive. Detailed description, see text.

drilled through the bone, using two diagonally adjacent platform tubes for guidance. The electrode assembly is directed through the platform tubes and bone openings into the brain. During insertion, the microelectrodes are pulled back into the 0.46 mm guiding tubes to prevent damage of the electrode tips. After insertion, the microdrive is fixed to the carrier platform with a drop of acrylic cement and the electrodes are advanced into the brain by turning the worm of the spindle potentiometer with a screw driver. One full turn corresponds to 320 μm. Due to a large-diameter adapter fixed on top of the spindle, it is possible to move the electrode in very small steps by executing only fractions of a full turn. The electrodes can be moved over a total distance of 8–10 mm. After complete exploration of one pair of electrode tracks, the electrodes are withdrawn, the microdrive is removed from the platform, and a new electrode assembly is inserted at positions yet unexplored. Usually, 5–6 pairs of electrode tracks are explored per animal.

2.3. Skull vibration sensor

To be able to identify the vocalizer out of a group of several vocally interacting animals, we use a skull vibration sensor. The sensor consists of a small piezo-ceramic plate measuring $4 \times 8 \times 0.3$ mm (PXE, Philips) that is soldered at one end onto the electronic board carrying the vocalization transmitter. The other end contains an electric contact with a connection-wire, but otherwise is free to vibrate (Fig. 4). The vocalization-induced skull vibrations are picked up by the sensor and generate voltages large enough to modulate the vocalization transmitter directly. Sensitivity of the vibration sensor can be fine-tuned to avoid over-modulation of the radiofrequency stage during very loud calls. Fine-tuning is carried out by varying the amount of solder at the connection-wire contact.

2.4. Transmitters

For the vibration sensor and each of the microelectrodes, a separate transmitter is used. The transmitters are

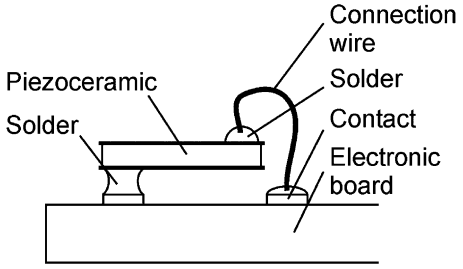

Fig. 4. Skull vibration sensor (modified from [7]).

completely built with surface-mounted technology (SMT) components, assembled on a 20×15 mm printed circuit board in the case of the microelectrode transmitter and on a 20×10 mm board in the case of the vibration transmitter. The latter has a weight of 1 g, the former of 2.1 g; both have a height of 3 mm. Their carrier frequencies vary between 100 and 140 MHz. The transmitters can be purchased from P. Grohrock, München/Germany. Their electronic circuitry has been published in [7]. As shown in Fig. 5, the transmitters are fastened to the inner sides of a Plexiglas protection cap, which itself is fixed to the carrier platform with four plastic screws. The transmitters contain the antenna on the one side of the board (Fig. 5A), while the electronic components are mounted on the other side. For power supply, two rechargeable 3 V lithium batteries (Type 2020, Panasonic) are used. The batteries are fixed to the outside of the protection cap with special holders. The three transmitters can be run with one set of two batteries for about 8 h. The protection cap has a removable cover for access to the transmitters and microdrive; its total size is $30 \times 30 \times 25$ mm.

2.5. Receiving side

The transmitter signals are picked up by two interconnected antennae which are placed in the home cage of the experimental animals. Both antennae are orthogonal in orientation, the one having a length of 2.4 m, the second of 1.2 m. With this configuration, transmitter signals can be received in all parts of a cage having a size of $1.5 \times 1.5 \times 2.5$ m. The antenna signal is amplified (antenna preamplifier PA-21) and sent via a coax-cable to three receivers (Yaesu VR-5000) tuned to the radiofrequencies of the transmitters (Fig. 6). The output of the receivers is sent to a four-channel video recorder (BR-S611E, JVC) for long-term storage and to a personal computer (Pentium IV 2 GHz) via an analog/digital interface (Micro 1401 mkll, CED, Cambridge/UK) for data analysis. Sampling rate is 32 kHz for the neural signal and 48 kHz for the vocal signal. Additional signals reach the video recorder from a video camera installed in the animal room. The signal is displayed on a monitor (TC-1470Y, Panasonic) and serves for continuous observation of the experimental animals from the central laboratory. Furthermore, there is a microphone (ME 64 + K6, Sennheiser) in the animal room, the signal of which is sent via a microphone preamplifier (M-Audio Audio Buddy), an audio amplifier (Neurolog NL 120, Digitimer), and a high-pass filter (Neurolog NL 125, Digitimer; cut-off frequency 300 Hz) to the video recorder, the PC via the above-mentioned analog/digital interface and to a loudspeaker (Canton GL 260) via an audio amplifier (Sony F 335R). The latter allows continuous monitoring of the vocal activity within the animal group from the central laboratory. A second loudspeaker serves to monitor the vocalization-associated skull vibrations transmitted telemetrically from the animal under investigation (Fig. 6).

2.6. Data analysis

Data analysis is carried out using the software Spike 2, Version 5 (CED, Cambridge/UK). Original recordings are displayed in the way shown in Fig. 7. One trace shows the neuronal activity as picked up by one of the two microelectrodes. A second trace shows the skull vibration signal in a spectrographic form, that is, as a frequency-time diagram, synchronous to the first trace. The third trace displays the room microphone signal, again in spectrographic form and time-synchronous to the first trace. By comparing traces 2 and 3, it is possible to decide whether a specific vocalization stems from the experimental animal or a group mate. For the identification of vocalization-correlated neurons, conventional peri-event time histograms are constructed after having submitted the original recording to a spike-sorting procedure, using again Spike 2, Version 5 (CED) as the software.

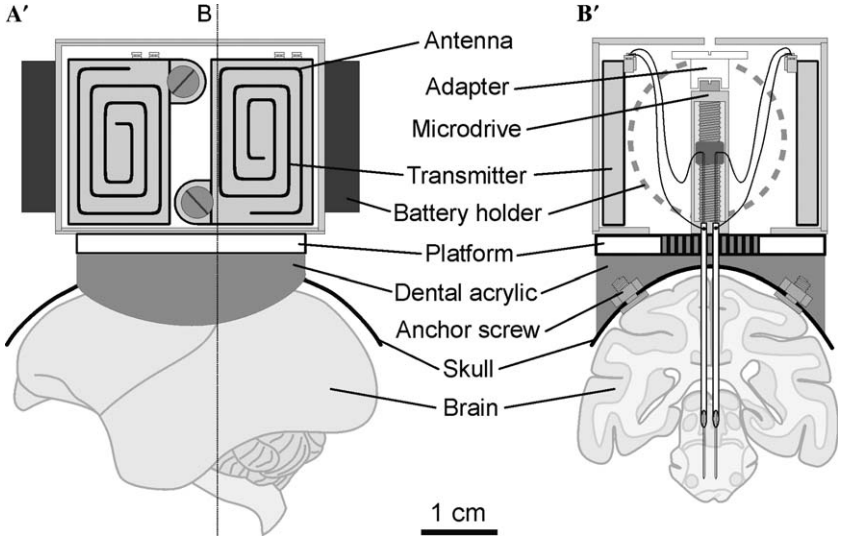

Fig. 5. Head mount. (A′) Side view. (B′) Cross-section.

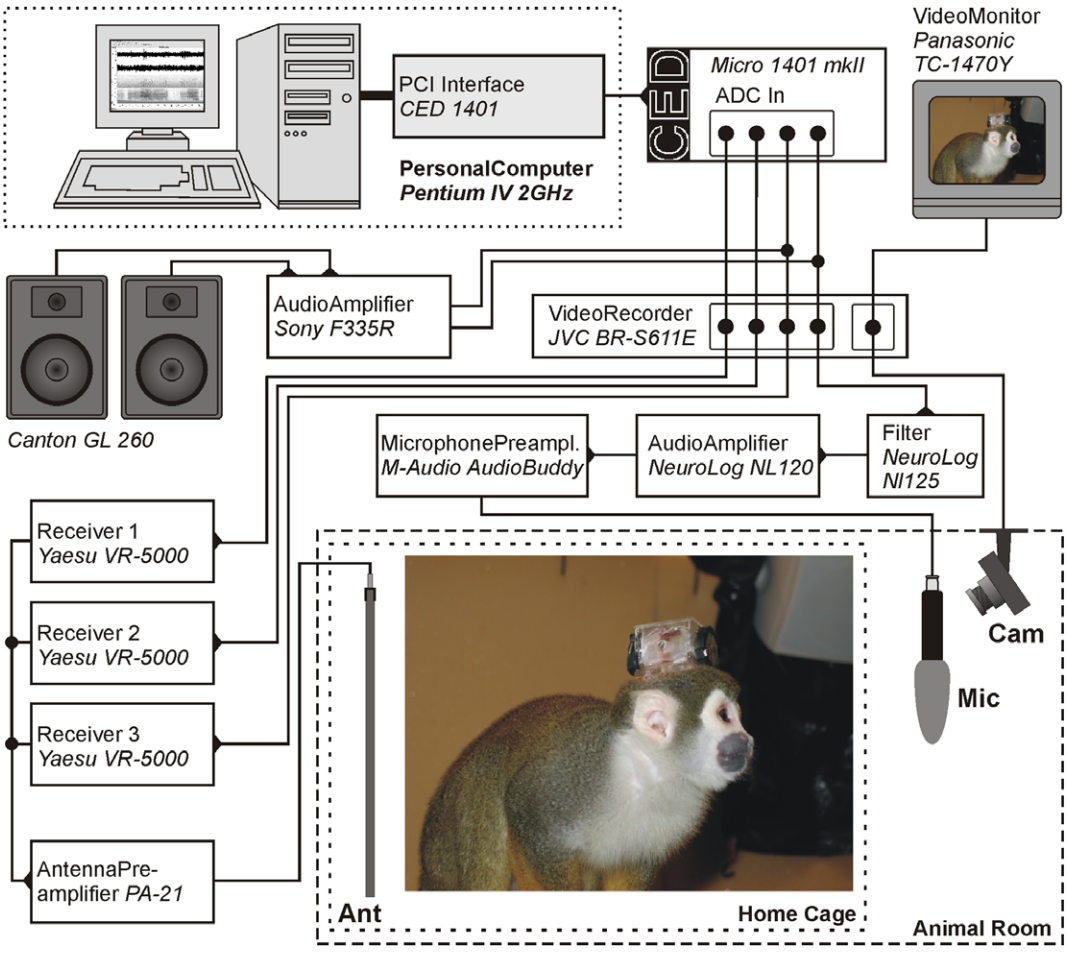

Fig. 6. Wiring diagram of the data-analyzing setup.

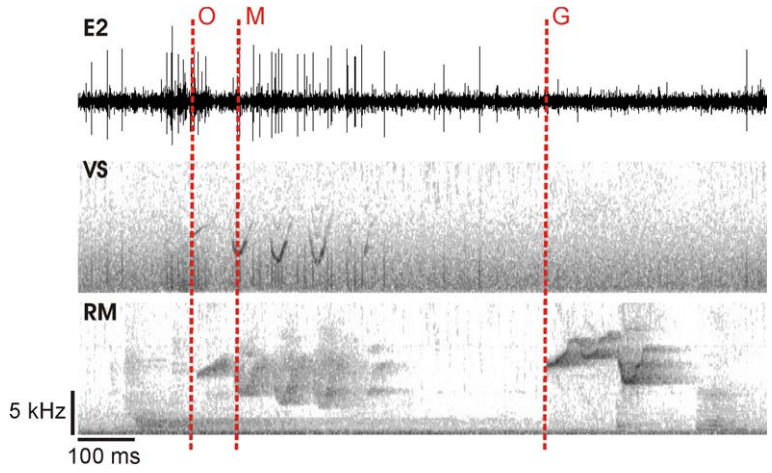

Fig. 7. Example of telemetrically transmitted vocalization-correlated neuronal activity. Upper trace: recording of extracellular neuronal activity from the lateral pontine reticular formation. Middle trace: spectrographic representation of skull vibration signal. Lower trace: room microphone signal. First vocalization is produced by the experimental animal (that is, the animal recorded from); second vocalization stems from a group mate. *Abbreviations*: E2, electrode track; G, onset of group mate vocalization; M, first frequency minimum (used as trigger for peri-event time histograms of rhythmically frequency-modulated calls); O, onset of vocalization of the experimental animal (normal trigger point); RM, room microphone; VS, skull vibration sensor.

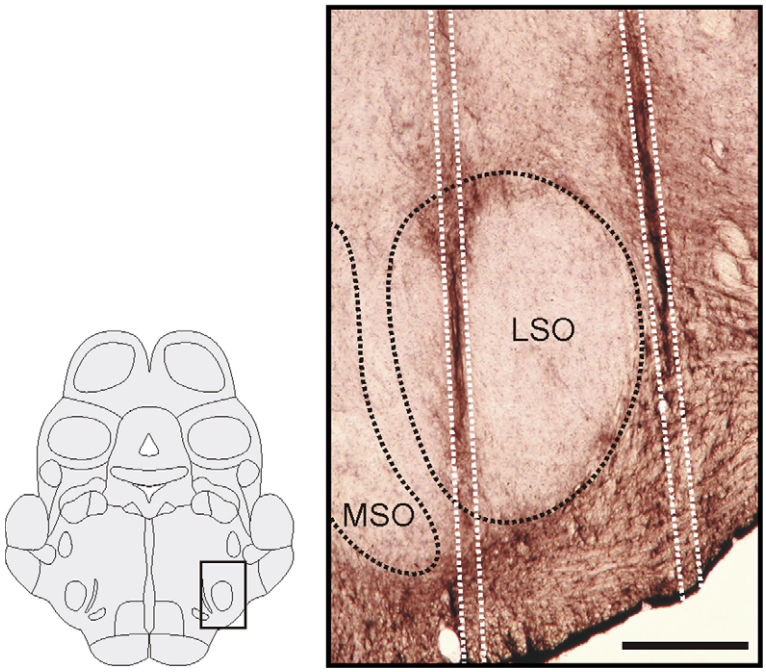

Fig. 8. Left: diagram showing a frontal section at the midbrain-pons transition of a squirrel monkey. Right: glial fibrillary acidic protein staining of the brain area indicated on the left, showing two tracks of electrodes having been removed several months before sacrificing the animal.

2.7. Histology

Verification of the recording sites is carried out histologically at the end of the experiments. The animals are perfused transcardially with physiological saline, followed by 4% paraformaldehyde after a lethal dosis of pentobarbital sodium (160 mg/kg body weight). The brains are removed and cut on a cryotome at 40 μm. The sections are stained for glial fibrillary acidic protein according to Benevento and McCleary [8]. This staining allows to identify the electrode tracks even many months after withdrawal of the electrodes (Fig. 8). Every second section is counterstained with cresyl violet (Nissl stain) to enable brain structure identification.

3. Concluding remarks

The telemetry system described here has been used successfully in several studies [7,9–11]. Animals lived for more than one year without loosening of the platform. Single neurons can be held for hours, and even rapid locomotion does not cause movement artefacts. A drawback of the system is that the electrode position cannot be changed telemetrically. Recording from a new position thus needs capturing the animal and turning the microdrive screw manually. As this causes stress to the experimental animal as well as to the remaining group, we do not change the position of the electrodes more than twice per day. This makes the collection of data from a higher number of neurons quite time-consuming.

Telemetry systems for the transmission of single-unit activity have been described also by other authors [12–17]. Some of them use bundles of microwires as recording electrodes [12,13,17]. This has the advantage over stiff electrodes that recording from one and the same neuron is possible over longer periods (sometimes even over several weeks). The disadvantage is that after implantation, electrode position cannot be changed anymore. In the other studies, stiff electrodes are used with manually operated microdrives. Only two of the systems have been used in primates. The one system [16, see also Sun et al., this issue] has been developed specifically for eye gaze-tracing studies in macaques and combines a microelectrode recording device with two video cameras mounted on the head. The whole system has a weight of 800 g. As this weight is too high to be carried completely on the head, part of the system is packed in a jacket worn by the animal. The other system [17] contains a 16-channel amplifier connected with a bundle of microwires used as recording electrodes. The amplifier is connected with a microcomputer which allows the telemetric selection of specific electrodes. The system has a weight of 235 g. Until now, the system has been tested only in chair-restrained monkeys, however.

References

[1] S. Yamaguchi, R.E. Myers, Brain Res. 37 (1972) 109–114.
[2] P.G. Aitken, W.A. Wilson, Brain Lang. 8 (1979) 227–240.
[3] D. Sutton, C. Larson, E.M. Taylor, R.C. Lindeman, Brain Res. 52 (1973) 225–231.
[4] S.A. Deadwyler, J. Biela, G. Rose, M. West, G. Lynch, Electroenceph. Clin. Neurophysiol. 47 (1979) 752–754.
[5] G. Fontani, Physiol. Behav. 26 (1981) 331–333.
[6] J. Kubie, Physiol. Behav. 32 (1984) 115–118.

[7] P. Grohrock, U. Häusler, U. Jürgens, J. Neurosci. Meth. 76 (1997) 7–13.
[8] L.A. Benevento, L.B. McCleary, J. Neurosci. Meth. 41 (1992) 199–204.
[9] F. Düsterhöft, U. Häusler, U. Jürgens, Neuroreport 11 (2000) 2031–2034.
[10] F. Düsterhöft, U. Häusler, U. Jürgens, Neuroscience 123 (2004) 53–60.
[11] R. Tammer, L. Ehrenreich, U. Jürgens, Behav. Brain Res. 151 (2004) 331–336.
[12] H.R. Skutt, R.G. Beschle, D.G. Moulton, W.P. Koella, Electroenceph. Clin. Neurophysiol. 22 (1967) 275–277.
[13] H. Eichenbaum, D. Pettijohn, A.M. Deluca, S.L. Chorover, Physiol. Behav. 18 (1977) 1175–1178.
[14] C. Pinkwart, H.W. Borchers, J. Neurosci. Meth. 20 (1987) 341–352.
[15] A. Nieder, J. Neurosci. Meth. 101 (2000) 157–164.
[16] Y.L. Lei, N.L. Sun, F.A.W. Wilson, X.S. Wang, N.H. Chen, J.Z. Yang, Y.P. Peng, J.H. Wang, S.H. Tian, M.H. Wang, Y.D. Miao, W.N. Zhu, H. Qi, Y.Y. Ma, J. Neurosci. Meth. 135 (2004) 35–41.
[17] I. Obeid, M.A.L. Nicolelis, P.D. Wolf, J. Neurosci. Meth. 133 (2004) 33–38.