A new type of recording chamber with an easy-to-exchange microdrive array for chronic recordings in macaque monkeys

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Galashan FO, Rempel HC, Meyer A, Gruber-Dujardin E, Kreiter AK, Wegener D. A new type of recording chamber with an easy-to-exchange microdrive array for chronic recordings in macaque monkeys. J Neurophysiol 105: 3092–3105, 2011. First published March 30, 2011; doi:10.1152/jn.00508.2010.—In monkeys, long-term recordings with chronically implanted microelectrodes frequently suffer from a continuously decreasing probability to record single units or even small multiunit clusters. This problem is associated with two technical limitations of the available devices: first, restrictions for electrode movement, and second, absent possibility to exchange electrodes easily on a regular basis. Permitting to adjust the recording site and to use new recording tracks with proper electrodes may avoid these problems and make chronic more similar to acute recordings. Here, we describe a novel type of implant tackling this issue. It consists of a new type of recording chamber combined with an exchangeable multielectrode array that precisely fits into it. The multielectrode array is reversibly fixed to the chamber, and within a minute it can be exchanged against another array equipped with new electrodes at the awake animal. The array allows for bidirectional movement of six electrodes for a distance of up to 12 mm. The recording chamber enables hermetical isolation of the intracranial space, resulting in long-lasting aseptic conditions and reducing dural thickening to a minimum, as confirmed by microbiological and histopathological analysis. The device has a simple design and is both easy to produce and low in cost. Functionality has been tested in primary and secondary visual cortex of three macaque monkeys over a period of up to 15 mo. The results show that even after more than a year, single and multiunit responses can be obtained with high incidence.

implant; multielectrode recording; multielectrode drive; primate; dura thickening

Innovative Methodology

In the brain critically depends on the coordinated activity of large neuronal populations (Engel et al. 2001; Fries 2009; Kreiter 2006). Studying the dynamics of these networks often requires neurophysiologists to obtain data from two or more cortical areas, or from a large number of individual neurons, at the same time. To achieve this, recent studies in awake behaving monkeys successfully utilized epi- or subdural electrode matrices (Rubehn et al. 2009; Tallon-Baudry et al. 2004; Taylor et al. 2005) and chronically implanted intracortical microelectrodes (Bondar et al. 2009; Hatsopoulos et al. 1998; Hoffman and McNaughton 2002; Manyakov et al. 2010; Pooresmaeli et al. 2010; Vargas-Irwin et al. 2010). Whereas sub- and epidural electrodes provide superficial access to local field potentials (LFPs) of large neuronal populations, only intracortical electrodes allow to record spikes from small clusters of neurons or even single units. Recent attempts include microwire arrays (Krüger et al. 2010; Nicolelis et al. 2003; Palmer 1978; Williams et al. 1999) and the “Utah” array (Maynard et al. 1999; Nordhausen et al. 1996; Suner et al. 2005) and the “Michigan” probe (Hoogerwerf and Wise 1994; Vetter et al. 2004) that both use narrowly spaced silicon microelectrodes. All these techniques allow gathering data from many individual neurons simultaneously and have been reported to provide single-unit responses still several months following implantation, occasionally even after 1 or several years (Krüger et al. 2010; Nicolelis et al. 2003; Suner et al. 2005). Because all these techniques use stationary electrodes, a main advantage is that they allow for recording essentially the same cluster of neurons or even the same individual units (Dickey et al. 2009) on a day-by-day basis. Thus chronic recordings with stationary electrodes that stay within a small volume of tissue provide a basis for brain-computer interfaces and the control of neuroprosthetic devices (Carmena et al. 2003; Fraser et al. 2009; Hochberg et al. 2006; Musallam et al. 2004; Santhanam et al. 2006; Serruya et al. 2002; Velliste et al. 2008) and have a high potential for studies related to long-term modulation of neuronal activity as in learning or plasticity (Blake et al. 2006; Dickey et al. 2009; Tolias et al. 2007).

However, many experimental approaches require the acquisition of data from many different units or from units with particular properties and thus make moveable electrodes more favorable than stationary ones. Routinely, in such cases, single and multiunit responses are obtained by inserting one or multiple electrodes acutely at the awake animal each day. Although this procedure provides a superb approach for isolating well-defined single-unit activity, it is particularly time-consuming and has several experimental constraints. First, the number of electrodes that can be handled individually is limited to usually two or three, thus also limiting the number of neurons to be recorded simultaneously. Second, numerous trials for which the monkey has to be rewarded are needed to search and describe target neurons, which limits the number of trials the animal will do in the experimental paradigm. Finally, acute access to the cortex usually implies exposition of the dura to the extracranial space, resulting in an increased risk of bacterial infection and thickening of the dura (Lemon 1984). On the other hand, acute techniques allow bidirectional adjustment of the electrode position, provide access to different electrode tracks, and have almost no restrictions regarding the depth of the target region.
To combine the beneficial aspects of acute recordings (i.e., recordings in which electrodes are inserted into the tissue on a day-by-day basis) with those of chronic recordings (i.e., recordings in which the electrode stays within the tissue for weeks or months) for awake behaving monkeys, implantable devices have been developed that utilize adjustable microelectrodes. For example, Hoffman and McNaughton (2002) and Eliades and Wang (2008) introduced multielectrode arrays that permit electrode movement in small steps but only unidirectional and along a single trajectory. Thus adjustment of the electrodes is possible only until the lowermost position is reached. Bidirectional electrode movement has been provided by a technique of deCharms et al. (1999) who connected a standard electrode microdrive to one of several chronically implanted electrodes. This device was tested in owl monkeys, but the overall design may be adopted for larger monkeys as well. However, connecting an electrode to an external device is time consuming and most likely limited to a single electrode per day, and movement of the electrodes is again restricted to a single electrode trajectory. Using different trajectories is only possible by surgical reimplantation of the electrodes. Cham et al. (2005) introduced a motorized microdrive for bidirectional movement of four electrodes that allowed semicontinuous recordings for up to a few weeks. A disadvantage is the use of a guide tube penetrating the dura, which impairs recordings in superficial cortex.

To circumvent these limitations, a device is required that allows for easy bidirectional and independent movement of all electrodes, including the possibility to retract and reinsert them fully into the cortex to access new tracks. At the same time, the device should provide long-term maintenance of aseptic conditions, reduce dural thickening to a minimum, and ensure lasting dura penetrability for fine microelectrodes to avoid the use of transdural guide tubes.

We here present a technical solution that fulfills these requirements. It makes use of a new type of recording chamber to which a multielectrode array can be reversibly attached. The electrodes can be moved along a trajectory of 12 mm and easily replaced by new electrodes once the old ones fail to work properly with no need for anesthesia or surgery, and new electrode tracks can be easily accessed. The recording chamber allows for hermetical isolation of the extracranial space and thus significantly reduces the risk of infection from the environment. As an effect of this isolation, dura thickening is largely prevented and allows for easy electrode penetration even many months following the craniotomy, making measures like dura scraping or use of mitosis blocker (Spinks et al. 2003; Wilson et al. 2005) unnecessary. Moreover, the device is easy to produce and low in cost and therefore ideally suited for use as a standard technique in numerous animals. We tested the device in three macaque monkeys over a period of up to 15 mo.

This article provides a comprehensive description of the technical details and presents our results regarding electrophysiological functionality as well as prevention of both infection of the recording chamber and excessive dural thickening.

MATERIALS AND METHODS

An important objective regarding the design of the device was to keep it as simple as possible. A rendered view of the device is shown in Fig. 1A, and a photograph of it as implanted in the animal is provided in Fig. 2A. The device consists of a custom-made, chronically implanted hollow bone screw and a microdrive array that can be reversibly attached to it with a small fastener. The bone screw serves as the recording chamber, and the microdrive array fits exactly into that chamber. In the following sections, we give a detailed description of the device. However, we like to emphasize that the particular design can be adapted in many ways to meet specific experimental requirements.

Recording chamber. The hollow bone screw (Fig. 1, A and B, #1) is turned into the cranial bone until it just touches the dura mater. It is made from titanium and has an asymmetric thread profile to achieve optimal incorporation into the bone. The outer diameter of the screw is 7.5 mm without thread and 8.5 mm including it. It has a central hole of 5-mm diameter and a length of 18 mm. The width of the central hole is determined by the specific design of the microdrive array to be used, and the length of the screw is directly related to the overall distance over which the electrodes can be moved. Regardless of specific dimensions, only the lower part of the screw must be situated within the bone, and the upper part protrudes a few millimeters. In this way, the site of trepanation is completely isolated from the surrounding tissues, preventing ingrowth of granulation tissue, and, more importantly, significantly decreases the risk of infection emerging from possible gaps between the monkey’s implant and the skull surface. To minimize the required space, the bone screw has no head. Therefore, to simplify implantation of the screw during surgery, the upper opening of the screw has a hexagonal slot of 3-mm depth for turning it into the bone by an Allen wrench (Fig. 1B, inset).

Design of the microdrive array. The microdrive array consists of a basis and six microdrives mounted to it. The basis is made from titanium and has a platform (Fig. 1C, #6) for anchoring the individual drives and a hollow pin (Fig. 1C, #7) that fits exactly into the tunnel of the bone screw. The central hole of the pin has a diameter of 3 mm and contains a cluster of six G23 tubes circularly arranged around a central G23 tube for guiding the electrodes (Fig. 1C, bottom inset; Fig. 1E, #15). The lower openings of the pin and the cluster tubes are closed by a thin layer of silicone (Biopor AB 25 shore; Dreve Otoplastik, Unna, Germany). Additional coating of the pin with highly viscous silicone paste (Baysilone; GE Bayer Silicones, Leverkusen, Germany) before insertion into the bone screw provides long-lasting sterile closure of the recording chamber.

The microdrives that move each electrode are modifications of the screw drive described by Nichols et al. (1998). Briefly, each drive consists of a stainless steel screw (M1.6×20; Hummer & Rieß, Nürnberg, Germany) to which a carrier (4-mm height) is attached that holds the electrode (Fig. 1D, #8). A whole turn of the screw moves the electrode 350 µm in either up or down direction over a maximal distance of 12 mm. At the lower 4 mm of the screw, the thread is removed, and a small notch is turned. Six of these drives are mounted into exactly matching holes (Fig. 1E, #12) at the top of the basis. The drives are fixed by acrylic cement, which is applied through small holes at the side of the basis (Fig. 1C, middle inset). The acrylic encloses the notch at the foot of the screw and thereby provides a simple, robust, low-friction, and zero-clearance bearing. The screw drives are stabilized against lateral bending by a frame situated on three bars mounted to the basis (Fig. 1E, #16, 17).

Each microelectrode is guided down to the tissue using a system of small tubes (Fig. 1, D and E, #9, 10, 15): two tubes with an inner diameter of 350 µm (made out of standard G23 syringe needles) are glued to the carrier of each screw drive (Fig. 1D, #9); the upper part holds the electrode, and the lower part holds another thin tube of 26-mm length, which encloses the microelectrode and protects it from bending (Fig. 1D, #10; hypodermic stainless steel tubing; outer diameter, 330 µm; inner diameter, 165 µm; Science Products, Hofheim, Germany). The electrode protrudes this thin guide tube by ~12 mm, which is the maximal distance the electrode can be moved within the tissue. Additionally, a cluster of tubes is
situatuated within the hollow pin of the basis (Fig. 1E, #15). In the most retracted position, each thin guide tube reaches for ~3 mm into one of the cluster tubes within the hollow pin (Fig. 1C, bottom inset) while the electrode tips are close to the end of the cluster tubes at the foot of the basis. Note that the distance between the end of the thin guide tubes and the electrode tips has to be at least the distance the carrier can move along the screw drive, because otherwise the thin guide tubes could penetrate the dura when moving down the electrode. All tubes are glued using epoxy resin glue with the exception of the cluster tubes, which are glued by epoxy resin lacquer (Epoxyline 6001; Altana Electrical Insulation, Wesel, Germany). The cluster itself is fixed inside the hollow pin of the basis by Biopor AB 25 silicone.

With this system of tubes, all electrodes are carefully guided toward the tissue and protected from mechanical disturbance at the same time. Because the spatial arrangement of the cluster tubes also determines the spatial arrangement of the electrodes, the distance between adjacent electrodes is ~600 μm and ~1,200 μm between opposing electrodes. For each electrode, mechanical fixation is realized by crimping the upper tube of the carrier to it.
A

Fig. 2. Photographs of: A, the fully connected microdrive array as inserted into the bone screw (not visible) on the animal's skull, highlighting the fastener (2) and the lateral deepenings (14) for precisely defined reinsertion of the array after electrode exchange; B, the protecting cap covering the device in-between recording sessions; and C, the layout of the permanently implanted parts consisting of bone screw (1), fastener (2), and a ring for anchoring the protecting cap, a 2 × 3-pin matrix (18) to connect the cables crimped to the electrodes, and a D-subminiature (19) for connection of the preamplifier. The matrix and the D-subminiature are connected by cables embedded in the acrylic cement. Numeration corresponds to the numbers as used in Fig. 1.

Turning the screw of an individual drive moves the carrier in either upward or downward direction, given that the carrier itself is protected against rotation. This is accomplished by close proximity to both directly neighboring carriers and the bars that carry the stabilizing frame. Additionally, even in case one carrier is lowered further down, so that it is not in direct contact anymore to the neighboring carrier, its lateral position is stabilized through the system of guiding tubes. As explained, each carrier is connected to one of the cluster tubes inside the pin of the basis via the thin guide tube that is glued to the lower holding tube of the carrier. Thus the guide tube stabilizes the horizontal position of the carrier and causes movement of the carrier along the vertical axis when turning the screw.

Microelectrodes can be easily replaced by a set of new ones by just loosening the fastener (Fig. 1A, #2) from the microdrive array. The set of new electrodes is inserted by using a second complete microdrive array prepared beforehand. Alternatively, if only a single microdrive array is available, a dummy might be used to close hermetically the bone screw. However, using a second array is easily possible since the whole device is low in cost. Thus replacement of the electrodes can be done within a minute and without the need for additional anesthesia or surgery. To accomplish spatially well-defined reinsertion, the basis of the array has several lateral deepenings separated by 15° (Fig. 1E, #14), to which the screw of the fastener can be fixed (Fig. 1E, #2). The set of new electrodes may be inserted in the same position as the former set to allow continuation of recordings at the same cortical positions or alternatively be slightly turned to give access to a new set of tracks.

The whole microdrive array as described here has a total height of 40 mm, 18 mm of which is situated inside the bone screw. The total weight of the array together with the bone screw is 12.4 g.

Additional parts. During nonrecording periods, the microdrive array must be covered by a protecting cap. The detailed construction of this may be freely chosen to meet other experimental requirements, such as position on the skull, presence of additional recording chambers, and conformance to cap systems established in the laboratory. For our purpose, we used two slightly different types of aluminum caps, one of which is shown in Fig. 2B. This cap has an outer diameter of 32 mm and a height of 30 mm, and the other one has the same height but a diameter of 26 mm. Both types had been constructed in a way providing free access to the microdrive array and the recording chamber when the cap is removed.

Besides the bone screw, the only permanently implanted parts of the device are the fastener to fix the microdrive array, a ring base to which the protecting aluminum cap is attached, and connectors to conduct the signals (Fig. 2C). All of these parts are embedded in acrylic cement. For proper positioning during embedding, the fastener is attached to the basis of the array (or a dummy) with a special device (Fig. 1E, inset).

To convey the electrode signals to the preamplifier, a dual-connector system is used that allows for fast and easy disconnection when exchanging the microdrive array. The electrodes get connected by cables crimped to their upper end using crimp sleeves cut from ferrules (0.5 mm × 8 mm; Conrad Electronics, Hirschau, Germany). The free end of each cable is soldered to a socket (LB0.76; MultiContact, Allschwil, Austria), which in turn is plugged onto one of the corresponding counterparts (LS0.76) arranged in a 2 × 3 matrix inside the aluminum cap (Fig. 2C, #18). By fine cables embedded in acrylic cement, this matrix is connected to a nine-pin D-subminiature socket (Fig. 2C, #19; D-SUB; Series 910; Binder, Neckarsulm, Germany) fixed on the animal's skull, which serves to connect the preamplifier. Three additional cables soldered to the D-SUB offer ground signals for the microdrive array and the animal and connect an indifferent electrode (coiled Pt-Ir wire, 150-μm diameter) placed inside the bone near the trepanation. During nonrecording periods, the D-SUB is secured by a cap made out of a male counterpart.

During surgery, we only placed a dummy inside the recording chamber, whereas the microdrive array was introduced after the monkey recovered. The dummy consisted of the array base (Fig. 1E, light blue) filled with Biopor AB 25 silicone. The foot of the basis was coated with a thin layer of silicone, too.

Assembly. The hollow bone screw, the basis, the stabilizing frame, and the screw drive carriers have to be manufactured in a workshop. The carriers are made out of polycarbonate plastic (Makrolon) and have a 0.6-mm bore for the screw, a 0.5-mm bore for the G23 tubes, and a horizontal 1-mm slit to disconnect electrically the upper and lower parts of the G23 tube (Fig. 1D). Carriers are expendable items and should be produced in sufficient amounts. For assembly of the carriers, we first fixed a 10-mm G23 tube medially into the 0.6-mm bore of each carrier using ordinary epoxy resin glue. The tube is then cut at the level of the slit using a small cutting disc. Subsequently, the thin guide tube is glued to the lower part of the G23 tube (cf. Fig. 1D). Second, the cluster tubes are prepared by arranging six G23 tubes
around a central tube. The length of the tubes corresponds to the length of the hollow pin of the basis (cf. Fig. 1C), which is 15 mm for the device as described here. All tubes are glued using epoxy lacquer. Hardening of the lacquer is accomplished by heating it for 1 h at 160°C. Subsequently, the cluster of G23 tubes is generously coated with Biopor AB 25 silicone and slid into the hollow pin of the basis. Importantly, the lowest part of the tubes should exactly align with the lowermost part of the pin. After curing, possibly exceeding silicone is removed by a scalpel. Next, all parts are autoclaved with the exception of the carrier-tube assemblies, which are immersed in 3% hydrogen peroxide for 24 h. The further assembly has to be done under aseptic conditions. First, the carriers are arranged by moving each of the thin guide tubes, which are attached to them, into one of the cluster tubes. Next, the supporting frame is mounted to the basis, and the drive screws are put through the corresponding holes in the frame, carefully turned through the 1.25-mm bore of the carrier, and finally mounted to the basis by filling acrylic cement solution into the corresponding lateral slots of the basis. To load the electrodes, the carriers are lowered to their bottommost position (so that the end of the thin guide tubes aligns with the end of the cluster tubes), and electrodes are inserted backwards with their distal end first. Then, the carrier is retracted to its uppermost position and again lowered by minimally half a turn of the drive screws. Under stereomicroscopic control, each electrode is withdrawn until the tip exactly aligns with the end of the corresponding cluster tube and gets fixed by crimping it to the upper tube of the carrier. The carriers are fully retracted again, and the foot of the basis is briefly placed on a thin layer of fresh Biopor AB 25 silicone for closing all tubes with a coat of maximally 0.5 mm. Finally, a fine cable for transmitting the signal is crimped to each electrode, and the crimp sleeves are electrically isolated by a coat of nail polish.

To reuse an array, carriers and guide tubes have to be replaced because they cannot be used again once an electrode is crimped to it. Acrylic cement used for bearing the screw drives is removed by acetone. With some experience, cutting the various tubes to length and assembly of all parts of the array can be accomplished within 4–5 h.

Surgery. To test functionality, a screw chamber was implanted over primary visual cortex of three male macaque monkeys. All experimental and surgical procedures followed the American Physiological Society Guiding Principles in the Care and Use of Animals and the Regulation for the Welfare of Experimental Animals issued by the Federal Government of Germany and were approved by the local authorities.

Surgery was performed under strictly aseptic conditions and followed a protocol previously described in detail (Wegener et al. 2004). For implantation of the hollow bone screw over primary visual cortex, we first made a circular trepanation using a trepan with 7.5-mm outer diameter (Meisinger, Neuss, Germany) perpendicular to the surface of the skull. Through this trepanation, the screw was driven into the bone until its lower end was just touching the dura mater. The inner opening of the screw was closed with a dummy. Finally, the connectors and the ring to anchor the protecting aluminum cap were implanted.

Following recovery from surgery, the dummy was exchanged against the fully assembled microdrive array, and electrodes were connected and then advanced until entering nervous tissue.

Visual stimulation and data acquisition for testing functionality. We recorded signals from 10 sets of penetrations in 3 monkeys, each using a newly equipped microdrive array with 6 tungsten microelectrodes (3 MΩ, 100-μm shank diameter; rounded fine tips with standard profile; Frederic Haer, Bowdoin, ME). Electrodes remained in the tissue for up to 18 wk.

We regularly tested visual responses of chronically recorded neurons over a period of 15 mo in *monkey S*. To map receptive fields (RFs), we used an automated mapping procedure consisting of rapid presentation of circular dots (Fig. 3A). Dots were shown in random order at 1 out of 435 different positions within the lower visual hemifield, each for 150 ms, followed by a blank period of 50 ms before the next dot appeared. To avoid repetition effects, presentation of each dot was followed by a sequence of at least 6 dots being at least 3 stimulus positions apart. To estimate orientation selectivity, a subregion containing the RF was mapped with flashed bars of 4 different orientations (Fig. 3B). Bar presentation followed the same temporal sequence as used for the dots except that each bar was presented for 250 ms and was followed by a sequence of 3 bars being at least 3 stimulus positions apart. Dots and bars were scaled according to eccentricity. The size of the stimuli was computed by the function $D = 0.04 \times E + 0.25$ (where $D$ is dot diameter/bar length and $E$ the eccentricity), resembling the size of V1 classic RFs, as measured by minimum discharge approaches (Hawel and Wiesel 1974; Smith et al. 2001). Width of the bars was 10% of their length.

During stimulus presentation, the monkey sat in a primate chair 80 cm in front of a 22-in. Cathode ray tube (CRT) monitor (100-Hz frame rate, $1,280 \times 1,024$ pixels) and was engaged in a dimming task requiring fixation. Eye movements were monitored using a custom-made video eye-tracking system. Direction of gaze was restricted to a circular fixation window with a radius of 0.9° centered on the fixation point. Eye movements exceeding this window resulted in termination.
of the trial. Successful performance of a trial was rewarded with a drop of water.

Data analysis. The preamplified signal of each electrode was filtered into a low- (<300 Hz) and a high-frequency band (0.7–5 kHz) and recorded with a sampling rate of 1 and 25 kHz, respectively. Spikes were detected offline by thresholding the signal. Isolation of single units was achieved by a semiautomatic spike-sorting procedure (Harris et al. 2000) using waveform parameters such as principal components (Abeles and Goldstein Jr. 1977; Glaser and Marks 1968). For plotting spikes, we computed the interquartile range (Shoemaker 1999) of spike waveforms. The interquartile range is an estimator of variability and was calculated by analyzing 256 quantiles of the distribution of waveforms in 32 bins, with spike waveforms interpolated by a factor of 15. This procedure provides a map indicating the relative probability of a spike waveform computed from the waveforms of all spikes, with value 0 representing the median of the distribution. For a related approach, see also Eliades and Wang (2008). RF maps were obtained by computing the baseline-corrected spike density functions in response to individual stimuli. LFPs of each trial and electrode were wavelet-transformed following a core routine proposed by Torrence and Compo (1998) utilizing complex Morlet wavelets with Gaussian shape both in time and frequency. Power spectral density (PSD) was computed by taking the square of the absolute value of this transformation divided by the Nyquist frequency (500 Hz). For a detailed description, see Taylor et al. (2005).

To test for stimulus-evoked modulations of the LFP, we defined a time-frequency window in the range of 40–160 Hz (γ-band) for the time of stimulus presentation. For each individual trial, we performed baseline-correction and normalization by subtracting and dividing through spontaneous activity recorded during the first 100 ms of each trial. Subsequently, we computed the mean of the PSD within this window and then took the median of all means on a trial-by-trial basis. To determine size and location of both single-unit and LFP RFs, the mean response to each stimulus location was spatially filtered by taking the average response from overlapping stimuli weighted by their relative overlapping area and then interpolating filtered power and mean rate, respectively, by using Gaussian radial basis functions (Moody and Darken 1989).

Histology. About 15 mo after the first electrode insertion, monkey S was euthanized for histopathological examination of the dura mater. Fixation of the tissue was accomplished by transcardial perfusion with 4% paraformaldehyde. Postmortem, the acrylic implant and the recording chamber were removed from the skull, and a 2 × 2-cm piece of dura together with connected bone tissue was taken from the site of trepanation. For comparison, another piece of dura was taken from a region over the frontal lobe.

Following fixation in 10% phosphate-buffered formaldehyde for at least 24 h, small stripes of dura mater protruding from bony edges of each sample were automatically paraffin-embedded, sectioned at 3 μm, and stained with hematoxylin and eosin (H&E; Varistain Gemini; Thermo Fisher Scientific) for light microscopy. Tissue samples comprising cranial bone attached to dura mater were placed in Osteosoft decalcification solution (Merck, Darmstadt, Germany) for 25 days, postfixed in 10% phosphate-buffered formaldehyde, and processed for light microscopy as described above. All sections were cleared with xylene and coverslipped with Eukitt mounting medium (Kindler, Freiburg, Germany). Histopathological examination of all sections was carried out using an Olympus microscope (model BX51; Olympus Optical). Photomicrographs were taken by a standard charge-coupled device (CCD) camera (ColorView I, Soft Imaging System; Olympus), and digital measurements of dura mater thickness were made by CellB image analysis software (Soft Imaging System; Olympus). Mean thickness was determined by measurements at 8–24 different sites of dura mater within up to 3 sections.

RESULTS

The design of the recording chamber and the microdrive was motivated by the main objective to provide long-term chronic recordings with flexible recording depth, accessibility to new recording tracks, and the possibility to replace easily electrodes. To achieve this, the device had to fulfill three main requirements: first, long-term hermetrical isolation of the intracranial space; second, bidirectional electrode movement; and third, quick and easy replacement of electrodes without the need for any additional surgery or anesthesia. Moreover, we wanted the design to be as simple as possible and low in cost to allow fast and cheap manufacturing for routine use in the laboratory. We first describe the results of our testing period regarding sterile closure of the recording chamber, and then we demonstrate the overall electrophysiological functionality of the system.

Isolation of the intracranial space. We implanted the device in 3 monkeys. Monkey S was tested for ~15 mo, and monkeys P and F both were tested for ~5 mo. Altogether, we used 10 sets of electrodes: 6 sets were inserted into monkey S, and 2 sets each into monkeys P and F. During the time the microdrive array was in place, we routinely checked the area covered by the aluminum cap for liquid that might have leaked out of the recording chamber but never observed anything. The only measure we took for maintaining aseptic conditions at the trepanation was thoroughly rinsing the recording chamber with 7.5% solution of Braunol (Braun, Melsungen, Germany) each time the electrodes were replaced. Thus the recording chamber of monkey S was rinsed not more than 6 times during a period of 15 mo, and the chamber of the other 2 animals was rinsed 2 times during 5 mo. All animals were perfectly healthy during the course of the experiments, and in all cases no signs of infection or inflammation could be found inside the recording chambers.

On nine occasions, we took a sample from the interior of the chamber before rinsing, using a cotton swab. All samples were sent to a microbiological laboratory for expert analysis. Figure 4A indicates the times the samples were taken and plots the overall timeline of the testing period for each of the monkeys. In monkeys S and F, neither germs nor fungi could be grown from normal or enriched aerobic and anaerobic cultures, respectively. From the first sample of monkey P, minor amounts of Staphylococcus intermedius and Acinetobacter lwoffii were grown from the enriched anaerobic culture, but the next two samples, taken ~6 wk later, were free of contamination.

Histopathological examination of the dura mater from monkey S revealed focal mild, nonsuppurative chronic reparation and reorganization processes of normal degree, characterized by mild proliferation of fibroblasts with capillary ingrowth (granulation tissue) accompanied by few lymphoplasmacytic infiltrates, as a result of tissue damage associated with disclosure of cranial bone and dura mater penetration, restricted to the site of trepanation (Fig. 4C). Importantly, the histopathological changes are not suggestive of any infectious etiology (e.g., bacteria or fungi).

These data indicate that the device is capable to isolate hermetically the intracranial space and to ensure aseptic conditions at the dural surface, resulting in only mild tissue response to the presence of the electrodes. Because chronic inflammatory stimuli are a major cause for dural thickening and hamper dura penetration by fine microelectrodes (Baker et
The limitation of inflammatory processes to a minimal degree should considerably reduce dural thickening. As an indirect measure, following retraction from the tissue, we carefully inspected 58 out of the 60 electrodes used in the 3 monkeys under the microscope. None of the electrodes showed any obvious sign of mechanical damage with the exception of 2 electrodes that had a bend at the outermost tip and did not provide a reliable signal. This indicates that dural thickening was reduced to a degree not affecting proper electrode penetration.

The dura underneath the trepanation exhibits a focal hemorrhage, most probably due to the insertion of an electrode just before perfusion of the animal to mark the trepanation center, and mild to moderate fibrosis. Additionally, an area probably just below the wall of the screw chamber shows focal thinning with no signs of cellular infiltration or proliferation. Table 1 summarizes measurements of dura thickness at the site of trepanation, 0.5 cm adjacent to the trepanation, and from a control piece of dura taken from above the frontal lobe. For each part, measurements were made at 8–24 sites in altogether 5 histological sections. Average thickness of the dura underneath the trepanation was 729 μm at the center and 195 μm at the margin compared with 467 μm of the control dura. Dura thickness measured at 0.5-cm distance from the trepanation was slightly increased (mean 1,123 μm; Fig. 4D) compared with the site of trepanation and the control site.
Electrophysiological testing. In monkey S, the array was tested for a period of 15 mo using automated mapping procedures to reveal visually driven responses from V1 and V2. The other two monkeys were engaged in different experiments and were only used to record ongoing activity from primary visual cortex. These two animals delivered electrophysiological data for ~3 mo each.

In monkey S, 4 sets of electrodes remained in the tissue for ~9 wk and another set for more than 18 wk. Electrodes of the last array tested stayed in the tissue for 3 wk. Reinsertion was done using the same array orientation 4 times, and an orientation turned by 15° another 2 times. Figure 5A shows 10 traces of the continuous signal recorded from 1 electrode following presentation of a dot at the same position. Thresholding the signal and offline analysis of waveform parameters allowed isolation of a single unit and estimation of the corresponding RF (Fig. 5, B and C). The neuron showed a well-defined responsive region of ~1° diameter that corresponds to the known size of V1 RFs as obtained in awake monkeys by careful spatial mapping of isolated single units (Snodderly and Gur 1995; Wurtz 1967). Stimulation outside the RF had no effect. The top of Fig. 5D gives additional examples of single neurons that have been recorded at different times during the period we tested the device, and the bottom provides examples from the 15th mo following 1st electrode insertion, obtained with the last array tested. Figure 5E shows additional spike waveforms recorded from the other 2 monkeys.

Of the 6 electrode sets tested in monkey S, the 1st 2 sets were used to get familiar with the technique and to record depth profiles (where electrodes occasionally also stayed within white matter for several consecutive days). The other 4 sets were used to record mainly from gray matter. With these sets, we performed 97 recording sessions. Two out of 24 electrodes were used to record mainly from gray matter. With these sets, we obtained V2 responses. By further lowering down the electrode even further provided RF maps from 2 multiunits with shifted RF center and larger size. RF size and location and the succession of responsive and unresponsive regions are in accordance with a track from V1 to V2 (Gattass et al. 1981). Figure 6E gives an example of multiple neurons recorded simultaneously, each with another electrode. As expected for nearby electrodes in V1, the maps show distinct spatial locations of the RFs with a clear area of overlap. The 6th electrode was located deeper in the tissue and did not provide spiking activity but nevertheless showed a weak modulation at the retinotopic location where we obtained V2 responses. By further lowering down the electrode, this recording site delivered clear LFP RF maps on the following days (data not shown).

Even though we used standard tungsten microelectrodes with no additional coating to improve biocompatibility, we did not observe any obvious change in the overall recording quality. For example, the ratio of RF maps obtained from single and multiunit activity was 73% in electrode set #3, 51% in set #4, and 74% in set #5. The last set was tested between days 410 and 456 and provided RF maps in 83% of the recordings. Note that all data used for the examples given in Fig. 6, C–E, were recorded more than 1 yr after the 1st electrode insertion.

To test whether responses of chronically recorded units also exhibit feature-specific characteristics, we occasionally con-
ducted another automated mapping procedure by presenting bars of four different orientations in rapid succession, where each bar was shown for 250 ms. Not every unit with a well-defined spatial RF map also revealed orientation-specific responses using this stimulation protocol, but in many cases neurons showed a clear orientation preference. Figure 7A gives an example of a single unit recorded with electrode set #1 that most strongly responded to horizontally oriented bars and only weakly to vertical bars. Orientations of 45° and 135°, respectively, elicited intermediate responses. Figure 7B shows the spike density functions of orientation-sensitive responses from another two single units and one small multiunit following presentation of differently oriented bars at the RF centers. The neuronal activity patterns are consistent with responses as known from acute recordings, with a transient increase in firing rate, a diminished sustained activity, and a somewhat decayed return toward spontaneous activity after stimulus offset. Following subtraction of spontaneous activity, the orientation indices $\left(\frac{R_{\text{max}} - R_{\text{min}}}{R_{\text{max}} + R_{\text{min}}}; R = \text{response per stimulus condition}\right)$ of the four units shown in the figure were 0.32, 0.41, 0.48, and 0.36. This is well in the range of V1 orientation tuning as reported earlier (Gur et al. 2005; Schiller et al. 1976). Figure 7C gives an example of a recording from which we were not able to extract any distinguishable spike patterns. Still, computation of the γ-band response revealed a well-defined responsive region of about $2 \times 2^\circ$ visual angle showing a precise localization within the visual field over all stimulus conditions and clear dependence of PSD amplitudes on stimulus orientation. Thus besides providing spatially highly selective RF maps, chronically recorded neurons also revealed feature-specific response patterns.

Fig. 5. Examples of chronically recorded single units. A: individual signal traces of 1 electrode of monkey S in response to a 150-ms dot presentation at the position indicated by the large bracket. Time 0 indicates stimulus onset. B: waveform of an isolated single unit from the trace shown in A, based on waveform analysis and computation of the p-interquantile range (see MATERIALS AND METHODS). C: corresponding RF map of the single unit shown in B. The map represents the mean firing rate of the neuron in response to rapid stimulation with individual dots. The gray square indicates center position of the fixation point. D: spike waveform distributions of single units recorded from monkey S at different days following the craniotomy. The bottom row shows waveforms of 3 neurons that were recorded at the end of the testing period, indicating that single-unit activity could reliably be obtained even after more than 1 yr of chronic recordings. E: additional spike waveforms recorded in monkeys P and F during ongoing activity. The rightmost plots shows neurons recorded at the end of each animal’s testing period.

Innovative Methodology

EXCHANGEABLE MICRODRIVE ARRAY FOR CHRONIC RECORDINGS
DISCUSSION

Extracellular recordings in primates face a number of methodological challenges, and specific scientific questions often require development of specific technical approaches. However, there are two requirements that are important to any experimental approach: first, ensuring aseptic conditions and sustained accessibility of recording sites; and second, delivering data for periods as long as possible. We here introduced a new recording device that offers a significant improvement in the tissue for several months. Next, we will discuss the particular design of the device in light of our results and compare it with current experimental approaches.

Screw chamber. Screwing the recording chamber directly into the bone has two particular advantages: first, it mechanically separates the site of trepanation from the surrounding tissue, and thus reduces ingrowth and proliferation of granulation tissue to a minimum. The formation of granulation tissue can be considered as a natural response to tissue damage in terms of wound healing mechanisms. However, if excessive, it dramatically impairs recording ability and thus needs to be reduced by particular and continuous countermeasures (Baker et al. 1999; Lemon 1984; Spinks et al. 2003). Second, combining the screw chamber with a microdrive array that exactly fits into it allows for hermetical isolation of the trepanation and prevents invasion of infectious agents from the surroundings of the chamber. With this design, aseptic conditions within the recording chamber were continuously ensured, routine measures regarding maintenance of aseptic conditions became unnecessary, and dura thickening was reduced to a minimum.
array adds another important contribution, as suggested by the is likely that the mechanical barrier provided by the microdrive revealed by histopathology of dura and bone tissue. Finally, it vice situated directly on top of the dura were only mild as the craniotomy, penetrating electrodes, and the recording de-

stiums would usually promote dural growth (Lemon 1984). since continuous inflammatory processes due to infectious prquence of the aseptic conditions within the recording chamber, preparation. At first, this can be interpreted as a direct conse-

used and the measurements we obtained from the histological penetrability of the dura with the very fine microelectrodes we considered this an important improvement of the currently avail-

able recording chambers for awake behaving monkeys. For a different type of chamber that also provides improved isolation of the intracranial space, although still needing routine me-

sures regarding maintenance, see Gray et al. (2007).

The second aspect of the new device is prevention of considerable dural thickening as indicated by the continuous penetrability of the dura with the very fine microelectrodes we used and the measurements we obtained from the histological preparation. At first, this can be interpreted as a direct conse-

quence of the aseptic conditions within the recording chamber, since continuous inflammatory processes due to infectious stimuli would usually promote dural growth (Lemon 1984). Second, reparation and reorganization processes in response to the craniotomy, penetrating electrodes, and the recording de-

vice situated directly on top of the dura were only mild as revealed by histopathology of dura and bone tissue. Finally, it is likely that the mechanical barrier provided by the microdrive array adds another important contribution, as suggested by the fact that average dura thickness at the trepanation site is even lower than 0.5 cm away from trepanation. This would be in line with the findings of Spitler and Gothard (2008), who used silicone elastomers to seal hermetically the trepanation and reported reduced or absent dural growth, too. Similar to the recording chamber and microdrive array in our design, such elastomers provide a mechanical barrier on top of the dura, probably supporting prevention of thickening. In contrast, just using layers of silicone is not sufficient to prevent growth of granulation tissue and dural thickening (Gray et al. 2007; Wilson et al. 2005).

Taken together, our findings indicate that hermetical isolation of the intracranial space and a mechanical barrier provide the basis for prevention of excessive dural thickening and make measures like scraping of the dura (Lemon 1984) or use of antimitotic compounds (Baker et al. 1999; Spinks et al. 2003) unnecessary.

**Electrophysiological properties.** A challenging problem in chronic recordings is long-term stability of electrodes and recording sites, i.e., obtaining single- and multiunit activity even several months or years following implantation (Hatsopoulos and Donoghue 2009; Polikov et al. 2005; Zhong and Bellamkonda 2008). Within cortical tissue, chronically implanted electrodes are likely to induce foreign body reactions like local inflammation processes (Biran et al. 2005; McConnell et al. 2009), encapsulation of the recording surface (Schmidt et al. 1976; Szarowski et al. 2003; Turner et al. 1999), and formation of glial scars (Griffith and Humphrey 2006), thereby increasing electrode impedance (Grill and Mortimer 1994; Newbold et al. 2004; Williams et al. 2007) and the distance between electrode tips and nearby neurons (Liu et al. 1999). Strategies to minimize these tissue reactions include use of biocompatible materials such as polymers (Musallam et al. 2007; Rousche et al. 2001; Schmidt et al. 1988; Suner et al. 2005) and ceramics (Moxon et al. 2004; Singh et al. 2003) for electrode insulation as well as coating electrodes with anti-inflammatory agents (Kim and Martin 2006; Zhong and Bellamkonda 2005, 2007). Another problem with chronically

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**Fig. 7. Chronically recorded, orientation-tuned V1 responses.** A: RF maps of a single neuron in response to a briefly flashed bar of 0, 45, 90, and 135° orientation. Orientation of the bar is represented by the frame color of the RF maps and used to indicate orientation throughout the figure. The scale bars in the top left plot depict 1° of visual angle in vertical and horizontal direction. B: spike density functions of additional units showing the response pattern to different orientations over time. Gray-shaded areas represent time of stimulation. C: LFP RF maps for different stimulus orientations as obtained by the normalized (Norm.) PSD within the frequency range of 40–160 Hz.
implanted electrodes is mechanical damage caused during dura penetration or within the cortical tissue. For example, Schmidt et al. (1988) reported cracks in the Parylene-C insulation of chronically implanted electrodes that developed at some time during implantation, resulting in reduced electrode selectivity.

Our device offers a simple solution to these problems and permits long-term chronic recordings by, first, moving electrodes within the tissue and, second, replacing them once in a while. Moving chronically implanted microelectrodes has been used previously in small animals (Fee and Leonardo 2001; Haiss et al. 2010; Lansink et al. 2007; Muthuswamy et al. 2005; Sato et al. 2007; Swadlow et al. 2005; Wilson and McNaughton 1993; Yamamoto and Wilson 2008) as well as in nonhuman primates (Cham et al. 2005; deCharms et al. 1999; Eliades and Wang 2008; Hoffman and McNaughton 2002; Jackson and Fetz 2007) and offers the possibility to pass the glial sheath surrounding the tip of the microelectrode. Replacing the electrodes provides the possibility to continue the experiment with fully intact electrodes once the old ones indicate mechanical damage, broken electrical connection, or reduced impedance. Additionally, insertion of new electrodes gives access to new recording tracks.

To the best of our knowledge, the device we here introduced is the first to combine sterile closure of the trepanation, bidirectional moveable electrodes, and quick and easy electrode replacement. It provided a high ratio of single and multunit responses even at the end of the testing period after ~15 mo and most likely would have done so for another few months or more. These results have been achieved by using standard tungsten microelectrodes and no agents to prevent glial reactions. For those studies that do not depend on stationary electrodes or on a very high number of simultaneously recorded electrodes, the new device is a valid alternative for chronic recordings in awake, nonhuman primates.

However, as pointed out earlier, we have achieved to combine the benefits of chronic with those of acute approaches. Therefore, the question may arise whether the new device is also an alternative to day-by-day insertion of microelectrodes as used in the majority of neurophysiological studies. A very important advantage of acute approaches is the possibility to adjust electrodes in the range of micrometers, thus allowing superb isolation of single units. Our design of the device does not reach this precision. However, if the experimental question does not critically depend on optimally isolated single-unit responses, recording of multunit activity is a good alternative (Supèr and Roelfsema 2005). In this case, because of the possibility to leave the electrodes inside the tissue, the time saved to place the electrodes, and long-lasting sterility within the recording chamber, our technique might also be considered as an alternative to conventional acute approaches.

Summary and possible improvements. The main advancement of the chronic device introduced here is a new type of recording chamber. The chamber is screwed directly into the bone until it slightly touches the dura and fully isolates the intracranial space. The second advancement is the development of a microdrive array that can be attached reversibly to the recording chamber. With these technical innovations, we achieved three important experimental proceedings: 1) virtually perfect maintenance of aseptic conditions over long time periods with no necessity for further care taking; 2) isolation of the dura mater from the extracranial space, allowing for lasting penetrability with fragile microelectrodes without scraping or applying pharmaceutical substances; and 3) possible and easy substitution of chronically used electrodes within just a minute and at the awake animal, allowing for optimal signal quality over prolonged recording periods. Additionally, the surgical procedure to implant the screw chamber is very simple, without particular risk, and can be done in short time. Thus the system fulfills the 3R goals (Russell and Burch 1959) first by enabling to gather more data from an individual animal and second by reducing the risk of infectious processes and experimental failure.

Importantly, these innovations are not restricted to the specific design of the microelectrode array we introduced here. For example, screwing the recording chamber directly into the bone is a general principle that does not depend on a certain diameter of the chamber or on a specific design of the microdrive array to be used with it. Using such a recording chamber might also be considered for acute approaches. In this case, the chamber could be closed by an exactly fitting, silicone-coated plug. Increasing the chamber diameter is possible as well, although to some extent it might be limited by the curvature of the skull. However, an important constraint might be the use of titanium for manufacture, as titanium has been proved to be incorporated by the bone particularly well (Pohler 2000).

For the design of the microelectrode array, we aimed to keep things as simple as possible. However, one easily can think about several upgrades. First, the number of electrodes can be increased by a larger diameter of the basis. Second, instead of manually adjusting electrode position in z-direction, the screw drives might be connected to computer-controlled miniature motors, which would allow for electrode positioning with a resolution of a few micrometers. Third, holding and contacting the electrodes might be done in a reversible manner by a more advanced construction of the carrier-tubes combination, which would permit electrode replacement without disassembling the microdrive array. However, a particular strength of our version is its simplicity and low costs, which allow using it as a standard laboratory technique even with low budget.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
Innovative Methodology

EXCHANGEABLE MICRODRIVE ARRAY FOR CHRONIC RECORDINGS

REFERENCES


