A new planar multielectrode array: recording from a rat auditory cortex

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Abstract

In the study of the spatiotemporal properties of the cortex, a demand often arises for recording local field evoked potentials (LFEP) and neural spikes from a quantity of points at close range from each other. In such a situation a device composed of a lot of electrodes assembled in a single bunch would be suitable. Such circumstances gave us the impetus to create the device described in this paper, namely a new planar electrode array for in vivo multisite extracellular recording. The device is made of plastic and includes platinum electrodes 50 µm in diameter. The array of 64 incorporated microelectrodes is placed on the surface of the cortex of anesthetized rats. Recordings could be made through all electrodes for more than 1 h without damage to the cortex. The inter-polar distance is approximately 100 µm so that each individual electrode can record activity from a separate population of neurons near the cortical surface. The recording system described here is highly useful for visualizing spatiotemporal structure of the cortical activities and for imaging dynamic neuronal assemblies.

Introduction

Based on recent studies using multisite recording from the in vivo brain the multichannel extracellular recording system has been shown to be promising for examining synaptic communication and network organization among and between neurons (Weliky and Katz 1999, Takahashi et al 2000, Shimono et al 2002, Oka et al 1999). Application of the planar microelectrode array to in vivo recordings of spontaneous as well as stimulus-evoked responses from the auditory and somatosensory cortex has been highly successful (deCharms et al 1999, Ohl et al 2000, Johnson and Welsh 2003).

In order to improve the quality and spatiotemporal localization of recorded data, we increased the number of electrodes to 64 (8 × 8 array). The probe was also designed to reliably record evoked potentials and neural spikes from the brain surface. The use of this probe allows us to investigate the spatiotemporal organization of various cortical areas, as the probe is easily moved from one cortical site to another.

The electrode array presented here was connected to the MED64 system (Okada et al 2003). The individual electrodes in the array were sufficiently separated from each other to enable simultaneous recording of distinct neuron pools. A sound stimulus was used in the present study to examine potentials evoked from the surface of the primary auditory cortex of rats and both local unit activity and field potentials were recorded simultaneously through all the electrodes. This device is suitable for spatially and temporally mapping stimulus-evoked activity from surface recordings of any brain region of the small mammals.

Multielectrode leads have been actively used in experimental neuroscience over decades but all existing variations of this method are not without definite disadvantages. If one uses a needle-shaped multielectrode array, the trauma of cortical tissue is reasonably severe and tends to widen during the experiment because of cortex...
pulsation. In the case of blood vessel injury the traumatic process continues even after withdrawal of the multielectrode array. This makes a fast change of array localization during experiment extremely difficult. The existing multielectrode arrays of the surface type do not cause traumatic complications, but as a rule these units are suitable only for recording field potentials and unsuitable for recording of neural spikes. In acute experiments with small animals, change of array localization often is a technical problem owing to the comparatively large size of the array. Generally the electrodes for the surface lead of the local field evoked potentials (LFEP) have low impedance and are unfit for recording of spike activity. The system we described here is free of these deficiencies. It does not traumatize the cortex during an experiment; it is easy to shift to another site; it is suitable for recording of local field potentials as well as neural spikes.

Materials and methods

The present experiments were conducted on adult Sprague Dawley rats (weight: 250–500 g; age: 2–3 months). The animals were given a subcutaneous injection of atropine sulfate (0.04 mg kg⁻¹ body weight, Bayer Corp.) and anesthetized by IM injections of ketamine hydrochloride (Ketalar-50 20 mg kg⁻¹, Parke-Davis) and xylazine hydrochloride (2.0 mg kg⁻¹, Bayer Corp.). Ketamine hydrochloride (10 mg kg⁻¹) was then given as needed (IM: approximately once per hour) to maintain the level of anesthesia during the recording session. The animal was fixed on the stereotaxic frame using ear bars with channels that allowed sound transduction into the middle ears. The cranium above the auditory cortex was removed (Sally and Kelly 1988) using a dental drill, the dura mater was also removed (Nicolesis and Ribeiro 2002, Nicolesis et al 2003, Knerom et al 2000, Kudrimoti et al 1999). At the end of experiment, the animal was sacrificed by an overdose injection of Nembutal (IP; 200 mg kg⁻¹ body weight, Bayer Corp.) (Tsytsarev and Tanaka 2002). All procedures were carried out according to the NIH Guide for the Care and use of Laboratory Animals (National Institutes of Health Publications, No. 80-23, revised 1978). The multielectrode array was constructed with insulated platinum wire electrodes and insulated copper wire (50 µm diameter) spacers. First, eight insulated platinum wires (50 µm diameter, Eiko Kagaku, Tokyo) and seven insulated copper wires (50 µm diameters, Eiko Kagaku, Tokyo) were alternately bound together by epoxy glue to form a single row. The tips of the wires in each row were aligned by cutting the excess portion of the tips. All 15 rows of wires were alternately bound together by epoxy glue to form a single row. The tips of the copper wires were insulated and therefore did not make an impact on the state of the cortex.

Seven other rows containing the eight platinum recording electrodes were constructed in an identical manner. Another set of seven spacing rows were made similarly; these spacing rows were constructed with 15 insulated copper wires. The recording unit was assembled by placing a spacing row (i.e., 15 copper wires) between each of the eight rows containing the platinum recording electrodes. The tips of the wires in each row were aligned by cutting the excess portion of the tips. All 15 rows of wires were fixed together using epoxy glue. The recording surface of 225 wires, including the 8 × 8 planar arrays of platinum recording electrodes together with 161 inert copper spacers, is shown in figure 1(A).
array of wires was covered by polyethylene tubing and then inserted into 0.26 mm (outer diameter) stainless steel tubing. The probe surface was extended approximately 7 mm from the tip, and the tubing assembly was fixed in place using epoxy glue. After allowing the glue to completely polymerize, the surface of the planar electrode array was ground flat using diamond sandpaper. The outer ends of the platinum wires were connected to a 68-pin connector (Eiko Kagaku, Tokyo).

The entire rod-shaped multielectrode array probe, consisting of the $8 \times 8$ planar arrays of platinum recording electrodes, the tubing assembly and 68-pin connector, is shown in figure 1(B). Figure 1(C) illustrates the contact area between the planar multielectrode array, which contains 64 platinum electrodes and the surface of the rat auditory cortex. The planar array has a working surface area of about 0.6 mm$^2$. The device is reusable; it was used for more than 20 experiments without any problem.

The multielectrode probe was mounted on a three-dimensional manipulator (Narishige, MWO-202, Tokyo, Japan) and positioned close to the cortical surface, just above the primary auditory cortex (AI) as detailed elsewhere (Takahashi et al 2005a, 2005b). A gold plate (10 mm$^2$) was embedded under the skin and used as a reference electrode. As illustrated in figure 1(C), the probe was slowly lowered toward the cortical surface until electrical contact was established. The multielectrode probe was connected to the Panasonic SH-MED64.

Evoked potentials, as well as stimulus-driven unit activity, were recorded following the presentation of click acoustic stimuli of 0.05 ms pulse length, 50 dB sound pressure level, that were generated using Wave Factory 1941 (NFI Electronic Instruments, Yokohama, Japan) and delivered directly to the ear by connecting the speakers to the channel of each ear bar. Clicks were presented once every 10 s. Data were stored in MATLAB (MatWorks, Inc.) format for offline analysis. A ‘click’ is a short sound impulse in a wide frequency diapason having a supraliminal loudness. Owing to these properties, a click results in the generation of a response to the sound stimulation of most of the sound sensible neurons of an audio cortex.

The local field potential (LFP) record is the sum of all neural activity within a volume of tissue. A signal is recorded using an extracellular microelectrode, placed sufficiently far from individual local neurons to prevent any particular cell dominating the signal. In our experiments, the signal is then low-pass filtered $\sim 300$ Hz, to obtain the LFP. The positioning of the electrode allows the activity of a large number of neurons to contribute to the signal. The unfiltered signal reflects the sum of action potentials from cells within approximately 50–1000 $\mu$m from the tip of the electrode (Rutten et al 2001). The low-pass filter removes the spike component of the signal and passes the lower frequency signal, the LFP. An electrode introduced into the brain or placed on the brain surface of a living animal can detect electrical activity that is generated by the neurons adjacent to the electrode tip. If the electrode is a microelectrode, with a tip size of about few micrometers, the electrode usually detects the activity of several neurons. Recording in this way is generally called multiunit recording, or recording of spike activity. Microelectrodes used for extracellular single-unit recordings are usually either glass micropipettes filled with a weak electrolyte solution similar in composition to extracellular fluid, or very fine wires that are insulated except at their extreme tip.

For recording LFP, the artifact caused by cortical pulsation was significantly eliminated by averaging; thus evoked field potentials presented in this manuscript represent averages from 20 trials. Nevertheless, mechanical cortex pulsation is a problem of great concern. One way to decrease the pulsation is to fix the multielectrode device in the XYZ-manipulator not rigidly but with the help of soft rubber gaskets. In that case a working tip floats with the cortex surface without any damage. In another method, the device is fixed rigidly and the cortex opening is made as small as possible. When the multielectrode device is aimed at the area under consideration a few drops of high density silicon oil are applied on the surface. That way is advantageous for young animals, especially with the use of deep anesthesia when the pulsation amplitude is moderate. For evoked spike activities, recordings were performed within 50 ms before and 50 ms after the stimulus onset; both raw data and averaged data from many trials are presented. When measuring the field potential evoked by a sound click signal, by using a low-pass filter, the frequency band was 1 Hz to 500 Hz. For neural spikes we used a range of 100 Hz to 10 kHz.

**Results**

Figure 2 shows various neuronal activities recorded at nine adjacent channels of the planar array from a single animal. Figure 2(A) shows auditory-evoked potentials recorded after a sound click. Each response is the average of 20 sweeps. The maximum value of the recorded evoked potentials is approximately 100 $\mu$V. Figures 2(B) and (C) illustrate unit activity recorded at the same electrodes shown in figure 2(A). Figure 2(B) illustrates the click-evoked firing observed in single trials; the traces shown illustrate the firing of individual neurons 10–20 ms after click onset (time 0). Single- and multi-unit neuronal spikes were consistently observed about 20 ms after the stimulus in 31 channels (including channels 27, 35, 44). As a rule, we recorded the neural activity over one to five channels (up to a maximum of approximately 10 per cent of the number of channels). The closeness of a neuron to the working tip of an electrode is a requirement for successful recording. Figure 2(C) shows the low level of spontaneous unit activity recorded at the same channels. These data clearly show that an increase in unit firing is associated with the click and, as will be presented below, that unit activity is highly correlated with the early peak negativity associated with the click.

The data presented above demonstrate that the planar array probe records both locally evoked field potentials and spiking activity of neurons located close to the cortical surface. Figure 3 demonstrates the spatiotemporal distribution of click-evoked local field potentials. Each panel shows the amplitude of each of the electrodes in the $8 \times 8$ array; the size of each filled circle correlates with the voltage recorded at each
Neuronal activities recorded at nine adjacent channels. The channel number is indicated at the low left corner of each panel.

(A) Evoked responses elicited by an auditory stimulus (sound click). The stimulus (sound click) was presented at the beginning of the recording. Traces are the averaged evoked potentials elicited over 50 trials. (B) Stimulus-evoked spike activity (multiunit recording) recorded at the same channels as the ones used in (A).

Spatial and temporal distribution of the amplitude of the evoked potentials in the auditory cortex. The average amplitude (negative deflection) of the potential is shown at each site for each 10 ms period after click (stimulus) onset. The negative-going amplitude at each time point is coded for each electrode by the diameter of the circle (see bottom right legend for the amplitude–radius coding). These evoked potentials, like the cumulative histograms shown in figure 4 below, were made by averaging 20 consecutive recordings to click stimuli.

Discussion

The development of multielectrode arrays that enable high-resolution recording of neuronal activity in vivo (Bezzi et al 2002, Pesaran et al 2002, Rutten et al 2001) has contributed significantly to progress in this field. In particular, surface recordings using the planar microelectrode array in vivo has successfully been used to demonstrate the spatial and
temporal organization of cortical columns in the auditory and somatosensory cortex of awake rats (Diamond 2000). The present experiments demonstrate the feasibility of utilizing planar electrode arrays to easily obtain spatial and temporal profiles of cortical activity at multiple sites. Other techniques have been used to obtain data of cortical activities, such as voltage-sensitive dye and intrinsic optical imaging (Tsutsui and Tanaka 2002), but the present method demonstrates much better temporal resolution. An important advantage of using this newly developed planar multielectrode array is that very little cortical damage is produced.

The planar multielectrode array described in this manuscript allows investigators to examine rapidly the spatiotemporal features of spontaneous and evoked responses across of the cortex. A 0.75 mm × 0.75 mm (0.5625 mm²) square patch of cortex can be evaluated with 64 electrodes at each recording site (figure 1(C)), and it takes only minutes to move from one location to an adjacent site. In addition, the small probe, by design, makes a considerable part of the cortical surface accessible to observation under minimal surgical intervention.

This planar multielectrode array can be used to record spatially localized field potentials as well as the spontaneous and stimulus-evoked action potentials of neurons located close to the cortical surface (figure 2). This is because each electrode has a diameter of 50 μm and has rather small impedance (40–160 kOhm). The latency period of neuronal responses peaked 30 to 35 ms after the stimulus, which is typical of rat auditory cortex neurons (Takahashi et al 2004).

Among the main parameters used for judging recording performance are the signal-to-noise ratio and the stability of recording over long periods of time. Qualitatively observed, the signal-to-noise ratio of our recorded data was lower than that of the data recorded with glass or metal needle-type electrodes in conventional extracellular recording (Takahashi et al 2005a, 2005b). This is due to the low resistance of each electrode as well as the subtle instability of contacting the electrodes to the cortical surface.

Stable contact of the probe to the cortical surface is a key issue in achieving long-term recordings. We were able to record neuronal activities for at least 30 min and routinely longer than 1 h. Finally, the results of the present study demonstrate that this newly developed multielectrode array is useful for visualizing spatiotemporal patterns of evoked potentials in response to auditory stimuli. The tonotopic organization of the auditory cortex is well documented and studies have demonstrated that its spatial and temporal features can be recorded epidurally. As shown in figure 3, the maximal short-latency evoked response was highly focused to a few electrodes at 20–30 ms after the click.

These results demonstrate the use of the described electrode array for construction of electrophysiological maps of the cortex with a high degree of spatial and temporal resolution. The device presented here has the potential to serve as a powerful tool for spatiotemporal investigation of multiple cortical areas in acute in vivo experiments.

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