Procedure for recording the simultaneous activity of single neurons distributed across cortical areas during sensory discrimination

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We report a procedure for recording the simultaneous activity of single neurons distributed across five cortical areas in behaving monkeys. The procedure consists of a commercially available microdrive adapted to a commercially available neural data collection system. The critical advantage of this procedure is that, in each cortical area, a configuration of seven microelectrodes spaced 250–500 μm can be inserted transdurally and each can be moved independently in the z axis. For each microelectrode, the data collection system can record the activity of up to five neurons together with the local field potential (LFP). With this procedure, we normally monitor the simultaneous activity of 70–100 neurons while trained monkeys discriminate the difference in frequency between two vibrotactile stimuli. Approximately 20–60 of these neurons have response properties previously reported in this task. The neuronal recordings show good signal-to-noise ratio, are remarkably stable along a 1-day session, and allow testing several protocols. Microelectrodes are removed from the brain after a 1-day recording session, but are reinserted again the next day by using the same or different x - y microelectrode array configurations. The fact that microelectrodes can be moved in the z axis during the recording session and that the x - y configuration can be changed from day to day maximizes the probability of studying simultaneous interactions, both local and across distant cortical areas, between neurons associated with the different components of this task.

monkeys | multiple microelectrodes | neuronal ensembles | temporal interactions | brain circuits

A fundamental problem in neuroscience is to understand how the activities of neurons distributed across cortical and subcortical brain areas are coordinated in time during behavior. Ideally, this problem could be addressed by recording the neuronal activities with microelectrode arrays chronically implanted in several cortical and subcortical areas of the brain (1–3). Importantly, this procedure has allowed decoding information from neuronal ensembles associated with behavior (4–7). Although this procedure has been a remarkable achievement, such microelectrodes are permanently fixed in a cortical area, and therefore sample the activity of a fixed neuronal population. Also, chronically implanted microelectrodes may or may not record neural activity. However, even assuming that there is no tissue damage and that circuit architecture is preserved, in many circumstances it is desirable that the microelectrodes can be moved (4). Thus, it would be an important improvement if each microelectrode of the microelectrode array could be moved independently. In fact, this technical improvement has been already achieved, allowing the recording of the simultaneous activity of several single neurons in one cortical area during behavior (2, 8). However, in this case the microelectrode movement is limited to the z axis and, therefore, recordings are restricted to a narrow, fixed patch of cortex. This limitation prevents the simultaneous recording of single neurons across

multiple cortical areas associated with a behavioral task. This limitation is even more problematic if we want to study the temporal interactions between neurons that reside in distant cortical and subcortical circuits associated with a behavioral task. Thus, new procedures are needed.

Here, we report a new setup, which consists of a microelectrode drive (Eckhorn microdrive; see ref. 9) adapted to a neural data collection system (Cerebus 128 Channel Data Acquisition System; see ref. 10), for recording simultaneous activity from many single neurons distributed across five cortical areas. We illustrate this technique with recordings obtained while trained monkeys discriminate the difference in frequency between vibrotactile stimuli (11). In every 1-day recording session, an array of seven microelectrodes is inserted transdurally in each selected cortical area. Each microelectrode can be moved independently in the z axis, and the x - y array configuration can be changed from day to day. For each microelectrode, the neural data collection system allows the discrimination of one to five neurons together with the LFP. Because this procedure is quite reliable, in a short period we have compiled a large database of many groups of neurons recorded simultaneously in five cortical areas during the vibrotactile discrimination task (see Table 1). The procedure was also validated by the fact that the response properties of the simultaneously recorded neurons studied were similar to those previously observed by using single microelectrode arrays (12–14). During vibrotactile discrimination, the activity of the recorded neurons reflected the sensory encoding, working memory, and decision processes of the task. Therefore, the procedure opens the possibility of analyzing the temporal interactions between neurons of local and distant cortical circuits involved in this task.

Results

For recording of the simultaneous activity of single neurons in five cortical areas (Figs. 1 and 2), six dedicated persons are required: one for controlling each microelectrode drive and one for monitoring the behavioral protocol and the task performance of the animal. The job of each dedicated person is to select and discriminate the spike waves recorded from each microelectrode in their respective array (Fig. 2; see *Methods*). The spike trains from all neurons and the LFPs are displayed on computer screens (Fig. 2). During task performance, such spike rasters and LFPs can be ordered in various ways, depending on

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Fig. 2. Schematic representation of the connections between the five microdrives and the neural data collection system. See description in *Methods*. ES, electrode selection; IM, impedance test.

interest are our recordings in S1 and S2. S1 is composed of two contiguous areas: 3b and 1. Area 3b is in the bank of the postcentral gyrus, and anterior to area 1. S2 is located ≈ 6 mm below the cortical surface, between the anterior end of the intraparietal sulcus and the lateral sulcus. Therefore, areas 3b and S2 are located deep with respect to the cortical surface. Thus, an important question is whether microelectrodes can be reliably inserted into areas 3b and S2. The answer is yes, with an error of ≈ 100 – 200 μm , when the recording is repeated at the same target location (the neurons had similar cutaneous receptive fields at the same height relative to the cortical surface). This procedure allowed testing several recording protocols in S1 and S2 (while activity in DPC, MPC, and M1 was simultaneously recorded). In these experiments, we selected neurons from S1 (areas 3b and 1) with the same cutaneous submodality [quickly adapting (QA) or slowly adapting (SA) responses], and selected S2 neurons with large cutaneous receptive fields confined to the fingers of the stimulated hand. Stimuli were delivered to the skin and neuronal responses were analyzed as functions of stimulus frequency. In principle, the procedure is ideal for studying the simultaneous activities of neurons that share cutaneous receptive fields and that respond during the vibrotactile discrimination task. Fig. 3A shows nine neurons recorded simultaneously: five in area 3b and four in area 1. These traces were obtained with three microelectrodes in area 3b (S1-E1 to S1-E3 of Fig. 3A), two of which recorded two neurons from the electrode tips (S1-E2 and S1-E3 of Fig. 3A), and four microelectrodes in area 1 (S1-E4 to S1-E7 of Fig. 3A), each recording one single neuron. All of these neurons from areas 3b and 1 had small cutaneous receptive fields on the distal segment of the third digit, had QA properties, and modulated their firing rates during stimulus presentation as reported before (12).

As indicated above, simultaneous neuronal recordings allow for the analysis of temporal interactions during task perfor-

mance. To illustrate this interaction, we quantified the synchrony of the neurons in Fig. 3A by analyzing their correlations (15, 16). The example shown in Fig. 3B is from a pair of neurons recorded simultaneously in area 3b (S1-E1, black asterisk in Fig. 3A) and area 1 (S1-E4, red asterisk in Fig. 3A). Clearly, these two neurons show a temporal dependency during stimulus presentation, which manifests as peaks above chance at 3 and 7 ms in the correlogram. This suggests that the 3b neuron drives the area 1 neuron, because the latter tends to fire 3 and 7 ms after the area 3b neuron fires. Fig. 3C shows an example of temporal interaction within a local circuit. It shows the correlogram from two neurons recorded through the same microelectrode in S2 (black and red crosses of S2-E9 in Fig. 3A). The correlogram peak indicates that the neuron corresponding to the red ticks typically responded ≈ 3 ms after the neuron with the black ticks (Fig. 3C).

A greater challenge is to analyze the temporal interactions between neurons belonging to distant cortical circuits. An example of such analysis is shown in Fig. 3D. In this case, the pair of neurons consisted of one from S1 and another from S2. Fig. 3D shows their responses during stimulus presentation and the corresponding correlogram. It can be seen that the neuron from S2 produced a spike 11 ms after a spike from the S1 neuron more often than expected by chance.

In addition to using spikes from individual neurons, we can also analyze correlations between the LFPs recorded with the same microelectrodes in these cortical areas. The example in Fig. 4 illustrates the kinds of results that can be obtained when data from multiple cortical areas are collected simultaneously. The resulting matrix of correlations shows the distributed nature of the sensory information encoded in these areas.

Discussion

Previously, we described the responses of single neurons from several cortical areas associated with the different components of the vibrotactile discrimination task (12–14). But how neurons from these cortical areas join efforts to solve this task is not known. This is a difficult problem, mostly because there are no reliable recording methods to obtain the simultaneous activities of large populations of neurons from both local and distant circuits during behavior. Initially, we tried implanting chronic microelectrode arrays (5, 6, 17) at fixed positions in five cortical areas associated with the vibrotactile discrimination task. However, we found that many of the recorded neurons were not associated with the task. For this reason, we adapted the Eckhorn method (9), in which the microelectrodes cannot only be moved in the z axis but can also be inserted at different points in the selected cortical area. An important property of this recording method is that microelectrodes can be reliably inserted both superficially and in deep structures such as S2, which is ≈ 6 -mm deep, and even in subcortical structures such as the somatosensory thalamus. Also, the data collection system is good enough for our purposes, because it allows for up to five single units per microelectrode (although usually one to two units per electrode) together with the LFP. In brief, the most important result of this study is that, from day to day, we were able to reliably record the simultaneous activities of neurons from several cortical areas associated with the vibrotactile discrimination task. The procedure is flexible enough that it allows us to search for relevant responses associated with our task, because the microelectrodes can be moved in the z axis, and because they can be reinserted into the recording chamber by using the same or different x - y microelectrode array configurations. However, a limiting power of this approach would be that many laboratories might not have six dedicated persons to daily recording sessions, and automated procedures should be considered as a future addition to this technique (18). Despite this limitation, our approach seems well suited for investigating the fine functional connectivity between cortical areas. The detailed

Fig. 3. Simultaneous recording from single neurons across cortical areas. (A) Raster plots from 72 neurons from five cortical areas during vibrotactile discrimination. These recordings were made by using a seven-microelectrode drive in each area (in M1, only five microelectrodes were inserted during this session). For each microelectrode, one to five units could be isolated; these groups of units are indicated at the left of the figure. Each row of consecutive ticks represents the activity of one neuron. The x axis represents time throughout one trial of the vibrotactile discrimination task: f1, first stimulus frequency and f2, second stimulus frequency. (B) Cross-correlation analysis of two neurons recorded simultaneously in S1, one in area 3b (black ticks) and the other in area 1 (red ticks) of S1 (black and red asterisks in A). Each pair of rows in B (black and red) corresponds to a single trial. The responses are shown during six trials of f1 stimulus presentation at a frequency of 22 Hz. Stimuli were applied to the distal segment of the third finger of the right hand. The corresponding covariogram is shown below the raster plot. Counts above the broken line indicate intervals during which the two neurons had significant temporal dependency (15). In this example, neuron 2 fires spikes more often than expected by chance ≈ 3 and 7 ms after a spike of neuron 1. (C) Similar analysis for a pair of neurons recorded simultaneously in S2 by using the same microelectrode. One of the two neurons (red plus in A) discharged reliably 3 ms after the other neuron (black plus in A). (D) Cross-correlation analysis of two neurons recorded simultaneously, one in S1 (red circle) and the other in S2 (red circle). The S2 neuron responded 11 ms after the S1 neuron.

functional connectivity can also be studied by identifying by antidromic activation the neurons that project to a particular target (19). Thus, this procedure seems quite appropriate for investigating not only the interactions between cortical areas, but also between cortical and subcortical structures in behaving monkeys.

Methods

Discrimination Task. The discrimination task used here has been described before (11). Monkeys were handled according to institutional standards that meet those of the National Institutes of Health and Society for Neuroscience.

Microelectrode Drives and Preparations for Recording. The microdrive and microelectrodes used here have been described in detail (Thomas Recording; see ref. 9). Essentially, the microdrive carries seven stainless-steel tubes (304- μ m outside diameter and 150- μ m inside diameter) aligned with a changeable circular thimble (head implant, as reported in ref. 20) (Fig. 1B). The tubes can be arranged in any x-y pattern, constrained by the 304- μ m diameter. The changeable head implants (7-mm diameter) contain linear arrangements of 304 or 500 μ m located centrally or eccentrically (0–2,000 μ m) within the circular head implant (Fig. 1C). These head implants can be changed from day to day for mapping the response properties of the exposed cortical area. One microelectrode (1–1.5 M Ω ; see ref. 9) is inside each of the tubes, and the tips end of the microelectrodes are ≈ 1 mm back from the end of the tubes. This position avoids damaging the tips of the microelectrodes. The distal end of the head implant is covered with a thin rubber band to prevent fluid into the carrier tubes. The head implant is positioned inside a small chamber (7.2 mm of internal diameter) previously implanted after a small craniotomy to expose

the dura above the targeted cortical area. The microdrive is lowered until the O-ring bears on the top of the chamber, in such a way that the end of the head implant rests just over the dural surface, therefore closing the chamber. Each microelectrode is moved at a speed of 20 μ m/s, crosses the thin rubber band and dura, and stops once it reaches the top of the cortex. This maneuver is made under computer control and continuous impedance testing of each microelectrode (Fig. 2). Once all microelectrodes are on the top of cortex, the recording session begins by gently lowering (5–20 μ m/s) the electrodes into each cortical area to isolate the activity of single neurons.

Data Recording. Each seven-microelectrode drive is connected to a device that allows the selection of one of three functions for each microelectrode: neural recording, impedance testing, and electrical microstimulation (Fig. 2). Microelectrode signals are sent to preamplifiers through a master connection box and from them to the neural processing system by means of optic fiber (Cerebus 128 Channel Data Acquisition System, Cyberkinetics Neurotechnology Systems; see ref. 10). The master connection box directs each preamplifier output to the Cerebus. This box solves the problem of the different connectors for the Eckhorn drive and the Cerebus [Bayonet Neill Concelman Connectors (BNCs) used as outputs from Thomas Recording to SFMC-118.Samtec as inputs by Cyberkinetics Neurotechnology Systems]. The neuronal data are then organized and stored trial-by-trial in a PC computer. A dedicated person operates each microdrive and monitors data through programs available in the neural data processing system by means of local network (Switch Ethernet 100/1,000 MHz). Spike amplification, filtering, and selection are established through an independent PC computer for each microelectrode drive (pcS1, pcS2, pcMPC, pcDPC, and pcM1) by using the Cerebus software. Spike amplitudes were between 40–400 μ V, well suited for spike discrimination (normally 1 or 1–3 spikes). By using Visual C++ and Matlab applications, neuronal data

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