Neuron, Vol. 18, 529–537, April, 1997, Copyright ©1997 by Cell Press

Reconstructing the Engram: Simultaneous, Multisite, Many Single Neuron Recordings

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Summary

Little is known about the physiological principles that govern large-scale neuronal interactions in the mammalian brain. Here, we describe an electrophysiological paradigm capable of simultaneously recording the extracellular activity of large populations of single neurons, distributed across multiple cortical and subcortical structures in behaving and anesthetized animals. Up to 100 neurons were simultaneously recorded after 48 microwires were implanted in the brain stem, thalamus, and somatosensory cortex of rats. Overall, 86% of the implanted microwires yielded single neurons, and an average of 2.3 neurons were discriminated per microwire. Our population recordings remained stable for weeks, demonstrating that this method can be employed to investigate the dynamic and distributed neuronal ensemble interactions that underlie processes such as sensory perception, motor control, and sensorimotor learning in freely behaving animals.

Introduction

It is well known that even the simplest of behaviors depends on the concurrent activation of large populations of neurons, distributed at different levels of the neuroaxis. Yet, most of the contemporary neurophysiological theories still focus on the individual properties of single neurons without much consideration given for the potential role played by the emergent properties of large neuronal ensembles. In part, this is a direct consequence of the most common electrophysiological approach used to investigate brain function. First introduced by Adrian (1926), the single electrode recording technique aims at sampling the activity of one neuron at a time. As a result, the experimental investigation focuses on the response properties of single neurons and how an individual neuron may encode a given sensory percept or generate a particular behavior (Barlow, 1995). Despite its fundamental contribution to modern neuroscience, the single neuron recording technique severely limits the investigation of the concurrent time-dependent interactions between large neuronal populations (Vaadia et al., 1995; de Charms and Merzenich, 1996), since large neuronal ensembles can be studied only by obtaining sequential unitary samples (Georgopoulos et al., 1986). Therefore, the lack of adequate electrophysiological techniques for neuronal ensemble recordings has contributed to the difficulty in characterizing the distributed and dynamic interactions between large populations of neurons involved in processing and storing information (Sherrington, 1906; Hebb, 1949; Lashley, 1950; Erickson, 1968; Freeman, 1975; Fuster, 1995). Here, we describe the implementation of a technique for simultaneous, multisite, many single neuron recordings and argue that it can be applied to the investigation of large-scale processing of information by populations of a few hundred neurons, located at multiple levels of the neuroaxis of behaving animals.

Results

A total of 22 rats were used in the experiments described here. In these animals, a total of 883 neurons were isolated by electrode configurations like the ones illustrated in Figure 1A. In these configurations, the ideal microwire spacing for cortical and subcortical implants was at 100–250 μm (Figures 1B and 1C); a maximum of 48 microwires were implanted per animal. This arrangement allowed simultaneous recordings from neurons in the trigeminal ganglion (Vg), principal (PrV) and spinal (SpV) nuclei of the trigeminal brain stem complex, the ventral posterior medial (VPM) nucleus of the thalamus, and the primary somatosensory (SI) cortex (Figure 1D). Long-term recordings were obtained by using microwires with blunt tips (Figure 1B), which proved to be much more suitable for long-term chronic recordings than electrodes with fine tips. Our multielectrode probes proved to be very effective since an average of 2.3 ± 0.4 neurons (mean ± SEM) could be isolated per microwire, and, for each array of 16 microwires, an average of 13.9 ± 2.1 electrodes (or 86% of the implanted microwires) yielded at least one discriminable single unit. As an example of a typical recording, Figure 2 illustrates four distinct extracellular action potentials obtained by recording from a single microwire implanted in the rat SI cortex. Subsequent analysis of interspike interval, poststimulus time histograms, and principal component analysis revealed that these waveforms corresponded to four independent cortical neurons. Extracellular action potentials in these recordings averaged 73.7 ± 12.1 mV (range: 50 μV–1.5 mV) , while background μV noise remained at 20 μV, indicating that the average signal-to-noise ratio of our single-unit recordings was around 3.7.

A Many Neuron Acquisition Processor (MNAP, see Experimental Procedures) system (Figure 3) was used for all experiments. The configuration of this system was fundamental for the performance of population recordings, since our strategy was aimed at maximizing the number of units discriminated in real-time, which reduced the time spent on off-line analysis. Analog signals (waveforms) could be captured for validation of discrimination parameters. With this approach, we could perform simultaneous multisite recordings that allowed us to reconstruct the parallel flow of sensory information across several subcortical and cortical relays of the rat trigeminal somatosensory system (Figure 4).

By using a sequence of 3-D graphs, Figure 4 offers a

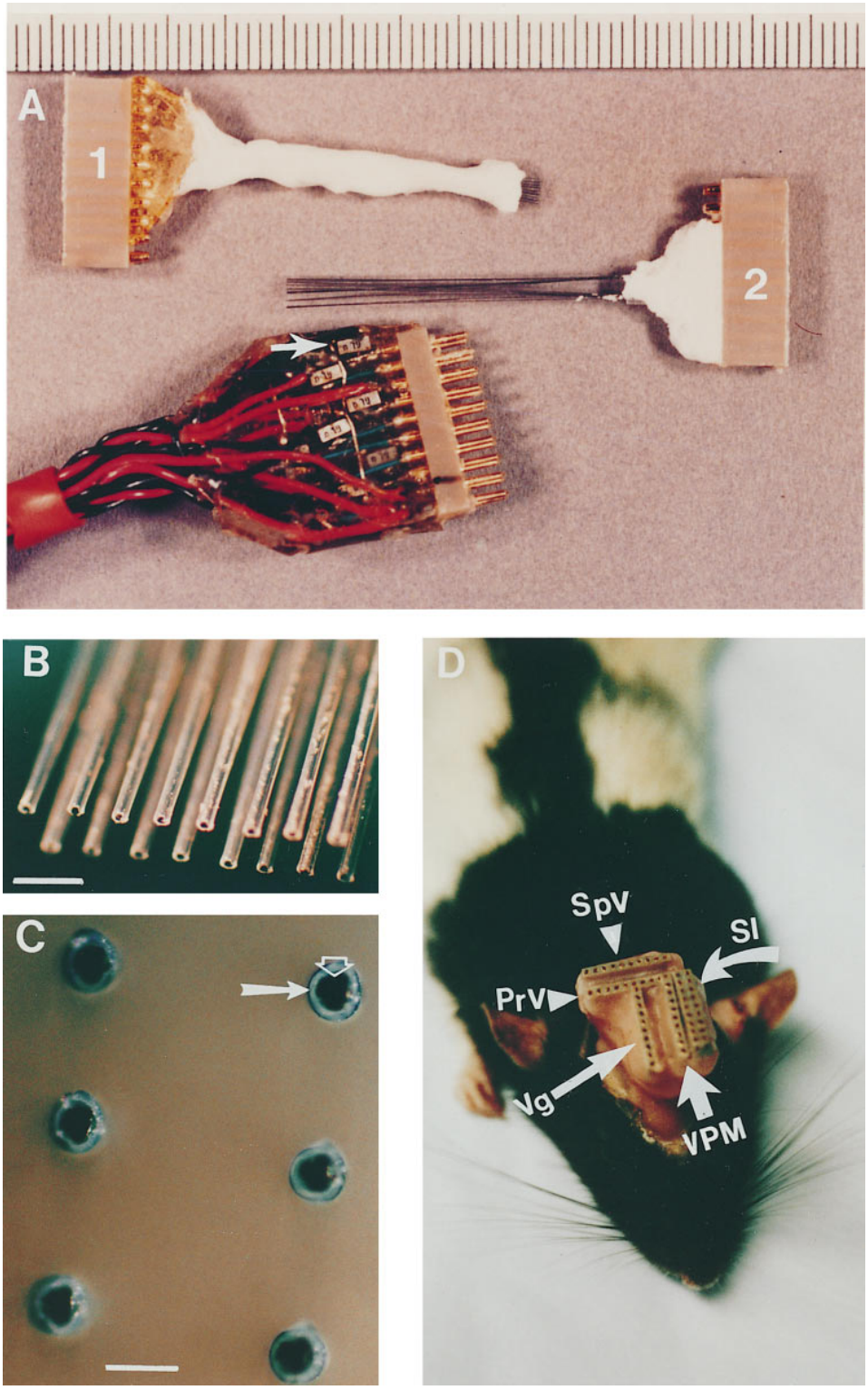


Figure 1. Electrode Configuration for Multilevel Chronic Implants

(A) Microwire arrays (1) and bundles (2) were designed to maximize the sampling across multiple subregions of the cortical and subcortical structures of interest. Arrays (1) were primarily used for cortical and thalamic implants while bundles (2) were used for brain stem implants. Field-effect transistors (arrow) were placed in the head stage to correct a mismatch in impedance and to amplify the extracellular signal.
(B) High power view of a microwire array depicting two rows of eight microwires just before a chronic implant.
(C) Top view of six microwires forming an array. Notice the Teflon coat (solid arrow) surrounding the stainless steel microwire all the way to the blunt tip (open arrow). (D) Example of a multisite implant. Scale bars = (A) 1 mm per division, (B) 250 μm, and (C) 60 μm.

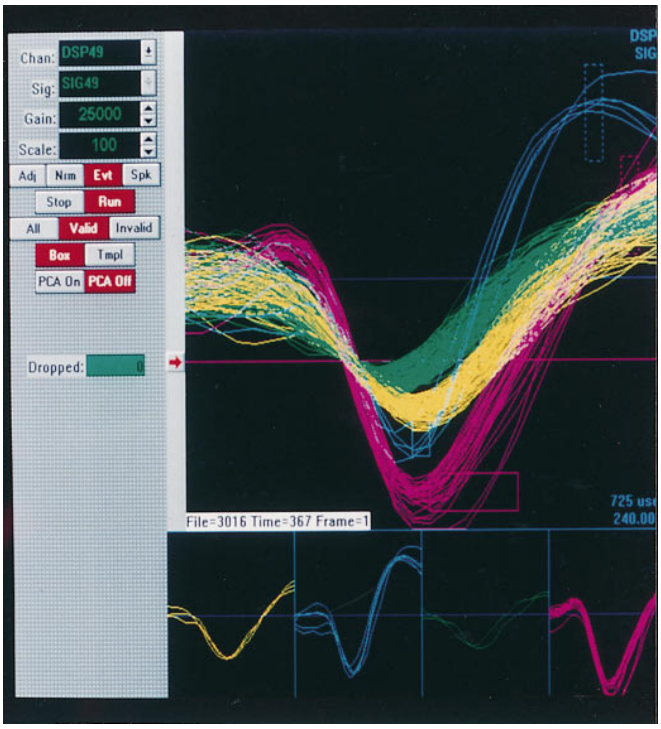


Figure 2. Real-Time Discrimination of Neuronal Action Potentials

Multiple waveforms per microwire were discriminated in our recording paradigm. Each neuron's waveform was identified by using a real-time principal component algorithm combined with a pair of time-voltage boxes.

way to visualize the spatiotemporal spread of sensory information as it ascends from the brain stem to the neocortex and how most of the somatosensory system is still responding long after the onset (time 0) of a discrete tactile stimulus. Analysis of multisite recordings revealed that, whereas a very restricted sensory response is usually observed in the PrV, the sensory responses in the SpV, VPM, and SI cortex cannot be defined as discrete representations of the cutaneous periphery. Figure 5 further supports this finding by demonstrating that stimulation of different single whiskers produced unique spatiotemporal patterns of sensory responses from the same ensemble of 30 neurons in the SI cortex. Inspection of each of these population maps reveals that the same cortical neurons contributed, with different response magnitudes and latencies, to the responses triggered by the stimulation of different whiskers (Figure 5). Only by reconstructing the spatial and temporal domains of the ensemble response can one reveal the continuous, distributed representations that define the location of the tactile stimulus.

Perhaps the most important result obtained here was the demonstration that our ensemble recordings can remain stable for long periods. Poststimulus time histograms (Figure 6A) were used to demonstrate that the sensory responses of the same set of cortical neurons remained extremely constant for several hours. Stable recordings like these demonstrated that our paradigm can be used for long-term, real-time monitoring of neuronal ensemble activity in behaving animals. This is a fundamental feature of our paradigm, since it allows one to continuously quantitate the modifications induced by learning on large populations of cortical and subcortical neurons during behavioral tasks.

Using our paradigm, reliable ensemble recordings were obtained from the same animals for several weeks after the implantation surgery. In these experiments, a maximum of 100 neurons were obtained when 48 microwires were implanted. Since different animals received different numbers of microwires (48, 32, or 16), the average number of recorded neurons in our animal sample was 41 $\pm$ 19 (mean $\pm$ SEM). Further quantitative analysis of our results revealed that 9, 13, and 21–30 days postsurgically, the percentage of microwires that yielded recordable single neurons was 86% $\pm$ 11%, 90% $\pm$ 7%, and 85% $\pm$ 11%, respectively. During the same period, the number of neurons isolated per microwire also remained very stable or even increased slightly: at 9 days postsurgically, 2.14 $\pm$ 0.47; at 13 days, 2.39 $\pm$ 0.32; and 2.92 $\pm$ 0.26 neurons per microwire 21–30 days postsurgically. Since most animals were sacrificed after a month of recordings, we have not determined how much longer viable recordings could be maintained in rats. However, we observed that in a few animals, recordings were maintained for 2 months after the initial surgery. Figure 6B provides an example of our long-term recordings in rats by demonstrating that the same set of microwires provided viable extracellular recordings over a period of 5 weeks in the same animal. Moreover, by using the same approach to record from cortical neurons in primates (owl monkeys), we have maintained viable recordings of 50–70 single units for 8–15 months (Nicolelis et al., 1996). Evidently, the possibility of carrying out such long-term recordings, while sampling from the same set of neurons, allows multiple experiments to be repeated in the same animal and multiple paradigms to be tested in the same experimental sample. In addition, it allows us to investigate the impact of behavioral training on the properties of neuronal circuits by continuously studying the physiological

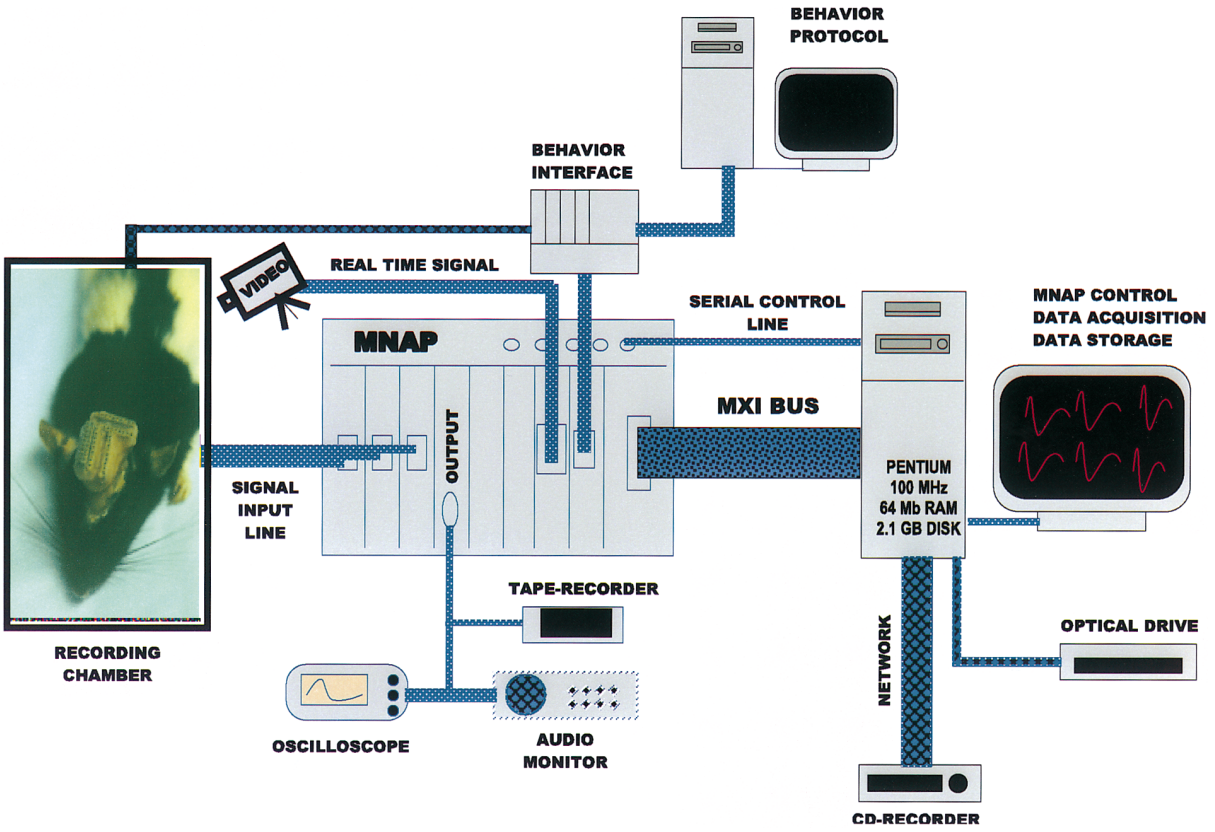


Figure 3. Integration of Recording and Behavior Setups

This schematic diagram describes the organization of our behavior–electrophysiological setup for neuronal ensemble recordings. Notice that a single microcomputer controls the 96-channel MNAP and experimental data storage, transmitted from the MNAP to the personal computer by the fast-speed MXI bus. The MNAP is responsible for data sampling, waveform discrimination, generation of real-time time signals for synchronization of external devices, such as a video camera, and the behavior interface. An output board allows visual and audio monitoring of the analog signals. The data files are transmitted through a network for temporary backup storage in an optical drive and subsequently for long-term storage in CD–ROMs. A second microcomputer edits behavior protocols and controls the behavior setup.

attributes of neuronal populations from the onset of training until the animals reach performance criterion. Finally, it is conceivable that chronic neuronal ensemble recordings will permit one to investigate whether neuronal population activity can be reliably used for controlling prosthetic devices, an area of growing interest in the field of biomedical engineering.

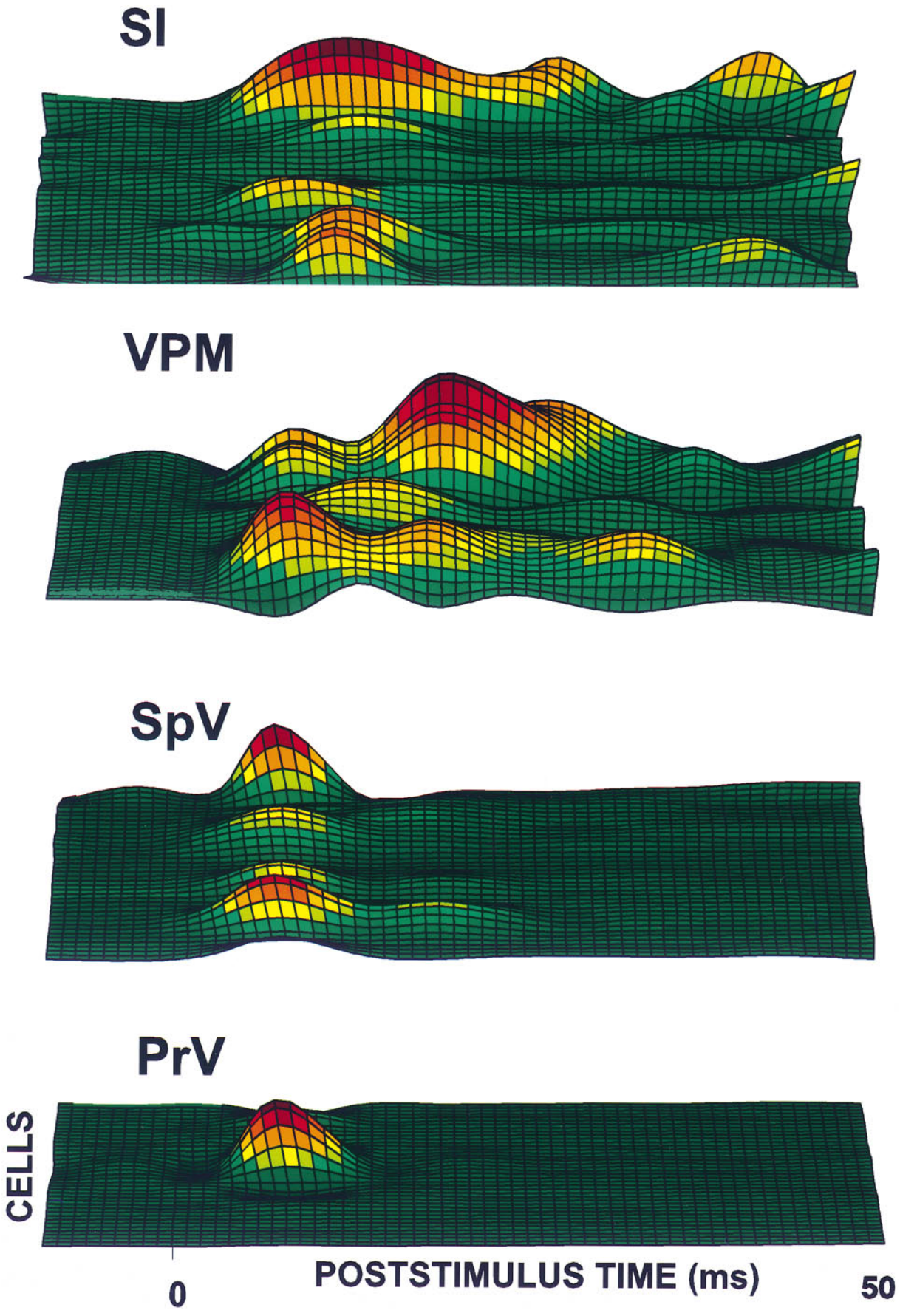
Discussion

Our results illustrate some of the advantages, as well as a few potential applications, of a paradigm for simultaneous, multisite neuronal ensemble recordings in behaving animals. By employing this technique, we obtained very stable recordings of the concurrent extracellular activity of up to 100 single neurons per animal (48 microwires implanted, 86% yield, and an average of 2.3 neurons/microwire), dispersed along the entire rat neuroaxis, with a time resolution of about 250 μs and a total tissue sampling area per site of about 2 mm^2. The combination of these features, particularly the high temporal resolution, the longevity of the recordings, and their distributed nature cannot be matched by any other electrophysiological (e.g., EEG, field potentials, or single-unit recording) or imaging technique (optical recording of intrinsic signals or voltage-sensitive dyes) designed for the study of large-scale brain activity in experimental animals.

Another contribution of the technological approach described here is the considerable reduction in computer requirements for carrying out large-scale ensemble recordings. While alternative approaches may

Figure 4. Multilevel Recordings in Behaving Animals

Simultaneous multilevel neuronal ensemble recordings allowed us to quantify the spatiotemporal spread of neuronal activation across many processing levels of the rat trigeminal somatosensory system, following stimulation of a single whisker. At each level of the pathway, 3-D graphs were used to represent patterns of neuronal ensemble activity. In each of these graphs, the x axis represents the poststimulus time (in ms), the y axis represents the number of neurons recorded at each level, and the color-coded gradient in the z axis was used to represent the response magnitude of the neurons (in spikes/s). In this scheme, dark red indicates the highest firing (140–150 spikes/s), and dark green indicates the lowest firing (0–10 spikes/s). SI, somatosensory cortex; VPM, ventral posterior medial nucleus of the thalamus; SpV, spinal nucleus of the trigeminal brain stem complex; and PrV, principal nucleus of the brain stem trigeminal complex.


SI
VPM
SpV
PrV
CELLS
0
POSTSTIMULUS TIME (ms)
50

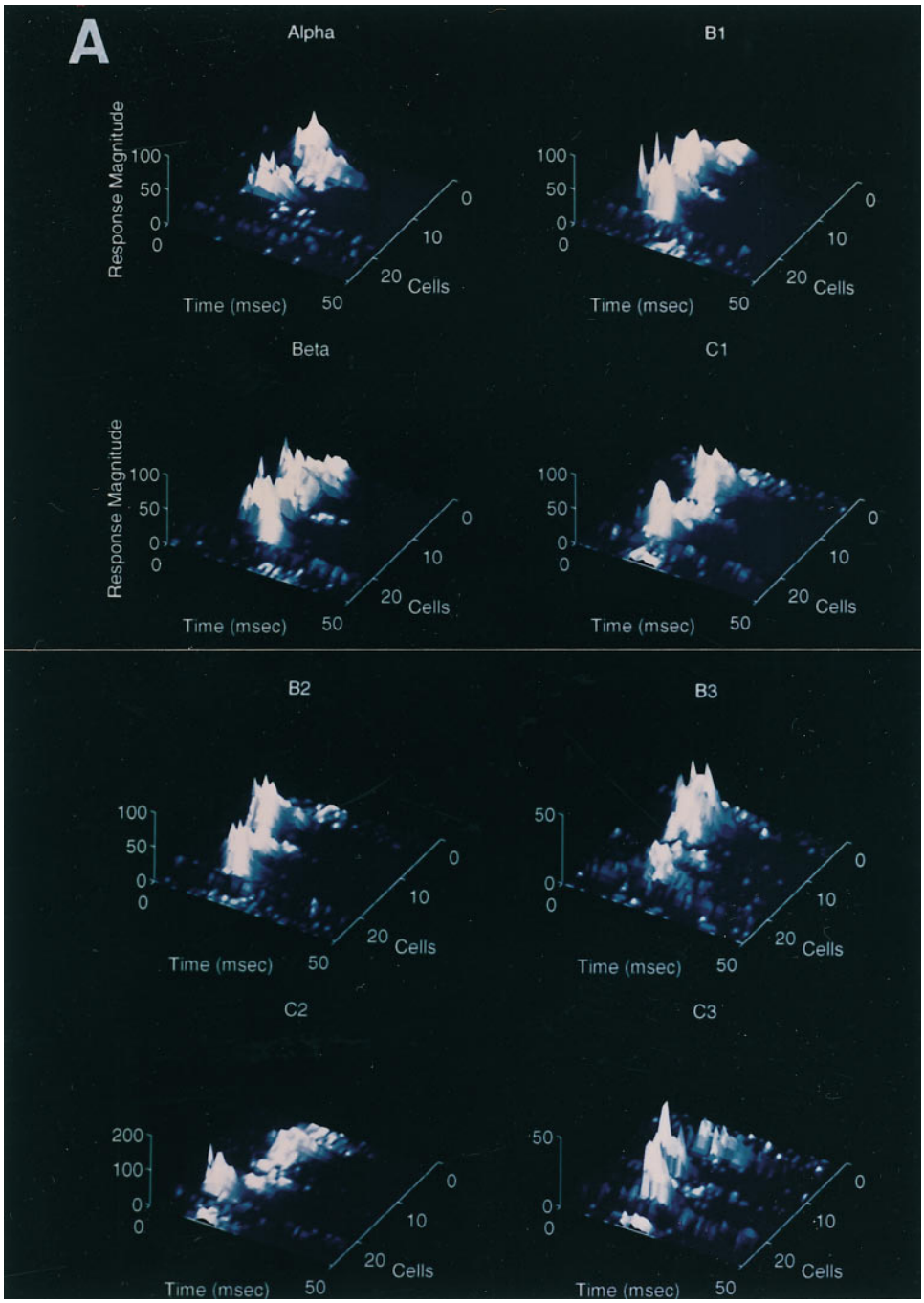


Figure 5. Distributed Responses in the Rat Somatosensory Cortex

In each of the 3-D graphs depicted here, simultaneous neuronal ensemble recordings were employed to quantify the spatiotemporal spread of cortical responses following the stimulation of a single whisker (identified on the top of the graph). The x axis represents poststimulus time, and the z axis represents response magnitude in spikes/s. All neurons were located in the infragranular layers of the primary somatosensory cortex.

require a large number of microcomputers to be synchronized (Wilson and McNaughton, 1994), in our paradigm, a single personal computer is used to control the entire electrophysiological setup and store neuronal spiking data, while a second microcomputer, interfaced to the MNAP, controls the behavior setup.

As required for any paradigm designed for the study of neuronal ensembles, our recordings allow large samples of neurons to be monitored per animal. The use of microwire arrays was fundamental to achieve this goal. While neuronal ensemble recordings have been obtained acutely in the past (Gerstein and Clark, 1964;

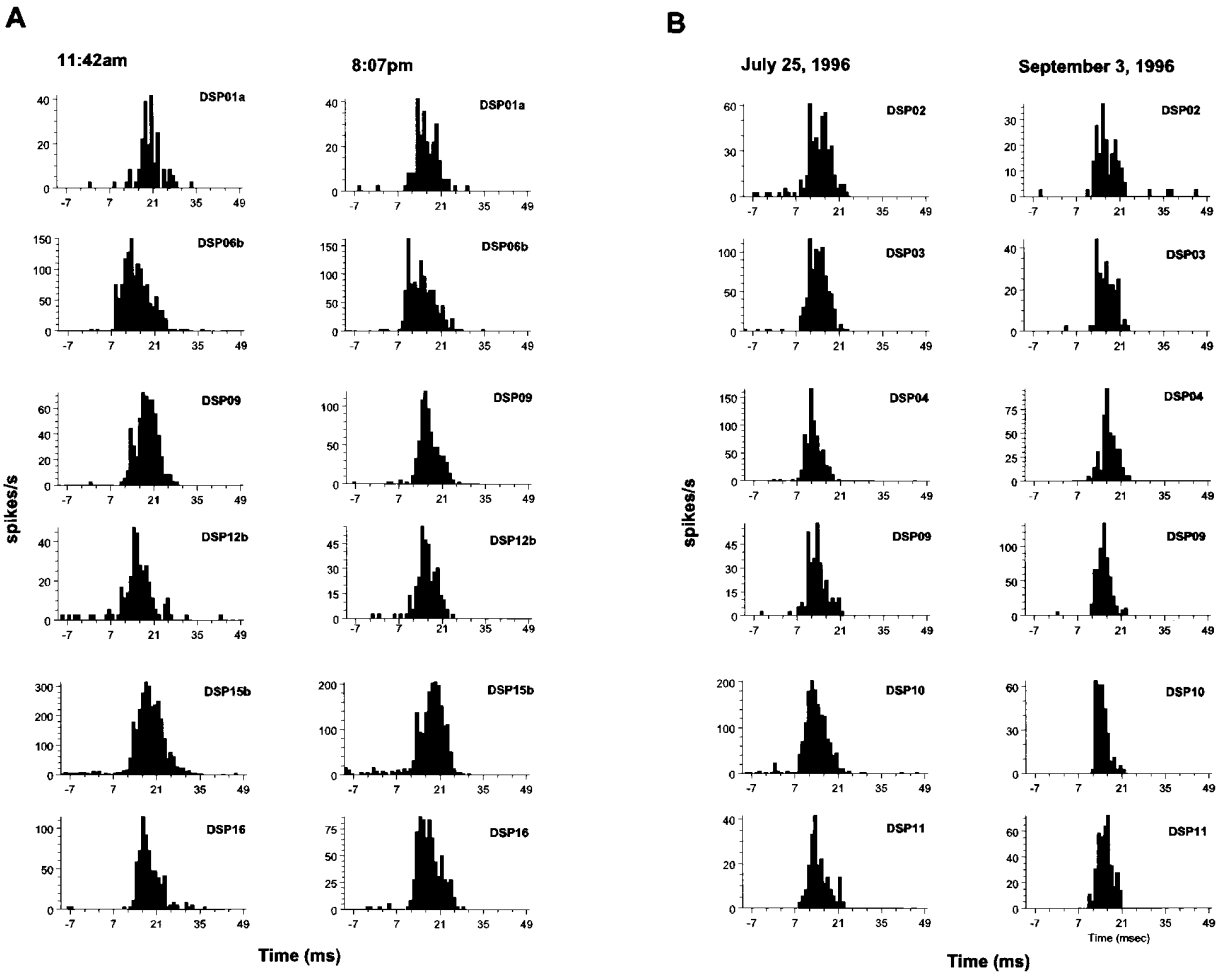


Figure 6. Recording Stability and Longevity

(A) Poststimulus time histograms depict the sensory responses of a set of six cortical neurons at two distinct times.
(B) Microwires provided viable recordings for several weeks.

reviewed by Kruger, 1983), most studies aimed at obtaining chronic recordings of single units have employed microwire bundles (Kubie, 1984; Shin and Chapin, 1990). The main reason for this choice is the stability and reliable yield of single neurons that can be obtained with microwires. Here, data obtained with microwire arrays further demonstrate that in regions of moderate cell density, such as the cortex, thalamus, and trigeminal brain stem complex, one does not need to employ more complex electrode configurations, such as tetrodes (Wilson and McNaugton, 1994; Gray et al., 1995), for successfully carrying out neuronal ensemble recordings. The difficulty in manufacturing tetrodes, and the fact that no group has so far provided quantitative data to demonstrate that these electrodes maintain high neuronal yield (15–20 neurons per tetrode) for many weeks, further justify the use of arrays of isolated microwires as the most efficient solution for carrying out chronic neuronal ensemble recordings. New multichannel electrode designs have recently appeared in the literature (Hoogerwerf and Wise, 1994; Nordhausen et al., 1994), but there is still scant evidence to support their use in long-term chronic recordings. In fact, we and others have recently demonstrated that by using microwire arrays, reliable long-term neuronal ensemble recordings can be obtained in many mammalian species currently used in neurophysiology. Those include adult and young ferrets (Nicolelis et al., unpublished data), owl monkeys (Nicolelis et al., 1996), and rhesus monkeys (Perepelkin and Schwartz, 1996).

Many neuroscientists (Sherrington, 1906; Hebb, 1949; Lashley, 1950; Lilly, 1958; Erickson, 1968; Somjen, 1972; Freeman, 1975) foresaw that to understand how sensory information is processed, converted into percepts, stored into memory, and then used to generate behaviors, one needed to comprehend the physiological principles that underlie large-scale interactions among widely distributed and interconnected populations of neurons. The available experimental evidence has supported this view by indicating that most fundamental brain functions involve highly dynamic and distributed neuronal interactions (Freeman, 1983; Georgopoulos et al., 1986, 1993; Nicolelis and Chapin, 1994; Wilson and McNaugton, 1994; Fuster, 1995; Nicolelis et al., 1995;

Welsh et al., 1995; Deadwyler et al., 1996). Therefore, the results described here further support the notion that, as neuronal ensemble recordings are incorporated into the arsenal of current neurophysiological techniques and explored to their full potential, we will have, at last, a realistic chance of reconstructing the neuronal engram, a task which, as put by Somjen (1972), has eluded us all.

Experimental Procedures

Microelectrode Design

Different configurations of microelectrode matrices were specially designed (NB Laboratories, Dennison, TX) to allow simultaneous recordings of the extracellular activity of large numbers of single neurons, distributed across up to five distinct neuronal structures that define the rat somatosensory system. In all experiments described here, stainless steel, Teflon-coated microwires (50 μm in diameter, California Fine wire) were used to build different microelectrode matrices, each of which was designed to maximize the number of neurons sampled throughout the spatial domain of different cortical and subcortical structures. For instance, microelectrode arrays, containing two parallel rows of eight microwires each, were used for chronic implants in the rat primary (SI) somatosensory cortex. In these arrays, the distance between the microwire rows varied from 0.5–1 mm, and the distance between pairs of microwires in a row varied from 100–250 μm (Figure 1). Another commonly used configuration was the microelectrode bundle, containing either 8 or 16 microwires staggered at different lengths, which were primarily used to record from neurons in deep structures, such as the ventral posterior medial nucleus of the thalamus and the different subnuclei of the trigeminal brain stem complex. Arrays and bundles were built by first soldering the microwires to a 20-pin plastic connector, then covering the connections with a thin layer of epoxy. Two ground wires were also soldered to free pins in the plastic connector. Finally, polyethylene glycol was used to mold the arrays and bundles and provide them with enough stiffness to be implanted in the brain.

Surgical Procedure

Microwire arrays and bundles were implanted during stereotaxic surgeries. For these procedures, rats were first anesthetized with a single injection of pentobarbital sodium (50 mg/kg, I.P.) and then transferred to a stereotaxic apparatus. Supplementary doses (one third of the original) of pentobarbital were given when necessary. The head was shaved and then cleaned with a betadine solution. A single midsagittal incision of the skin was used to access the surface of the skull. Following retraction of soft tissue, the periosteum of the dorsal surface of the skull was completely removed, and the bone surface was scrubbed with saline until no sign of blood was left. The surface of the skull was then dried, and a series of small craniotomies were produced with a high speed dental drill at the stereotaxic coordinates required for the implantation of electrode arrays in the Vg, PrV, SpV, VPM, and SI cortex. A second series of four to six craniotomies were open so that stainless steel screws could be firmly attached to the skull. These screws were used for securing probes and for grounding purposes. Just before the implantation, microwires forming bundles were cut to the ideal length, using a sharp pair of scissors, and then soaked in a supersaturated solution of sucrose. Special care was taken not to allow the sucrose to cover the microwire tips. After drying, the arrays and bundles were mounted in the holder of a hydraulic micropositioner (Kopf, Tujunga, CA) and subsequently slowly driven (~100 μm/min) into the brain. Although the 16-microwire arrays could be driven through the dura most of the time, in some instances, a small slit was made in the dura overlying the SI cortex to facilitate the implant. Single and multiunit activity were monitored continuously throughout the surgery to help locate the position of each electrode. During these recordings, the receptive field of each unit was qualitatively characterized until the arrays were positioned in the structure of interest. After reaching the final target, the ground wires of each connector were wrapped around one or two of the metal screws previously implanted in the skull. The craniotomy was then sealed with a layer of agar (4% in saline). Once the agar solidified, dental cement was applied until it reached the top border of the plastic connector. This procedure was repeated until all arrays and bundles had been implanted. The end result of one of these implants is illustrated in Figure 1D. To finish the procedure, the skin was loosely sealed around the probes and the animal was transferred to a recovery cage. The analgesic Buprenorphine (0.1–0.5 mg/kg S.C. every 8 hr) was administered for 2–3 days while the animal recovered from the surgery. Electrophysiological recordings started 5–7 days after the surgery. Experiments were carried out in a recording chamber using anesthetized and freely behaving animals.

Instrumentation for Real-Time Ensemble Recordings

Simultaneous recordings of large samples of single neurons were carried out by an MNAP (Spectrum Scientific, Dallas; see Figure 3). In the most complete configuration, the MNAP allows simultaneous sampling from 96 microwires and discrimination of up to four individual action potential waveforms per wire, for a maximum of 384 recorded neurons. In this configuration, head stages (NBLABS, Dennison, TX), containing 16 field-effect transistors (FETs, Motorola MMBF5459) arranged in two rows of eight at the end of TVC-insulated cables, are used to connect the 20-channel plastic connectors cemented in the animal's head to the MNAP preamplifiers (Figure 1D). In all recordings, the FETs were set as voltage followers with unit gain. The MNAP preamplifiers contain differential OP-Amps (gain 100; bandpass 100 Hz–16 kHz). Their output signals are transmitted, through ribbon cables, to 96 A/C-coupled differential amplifiers on 6 input boards, each containing 16 amplifiers. Once in the input boards, the analog signals pass through the first level of amplification (jumperable gain of 1, 10, or 20), are filtered (bandpass 400 Hz–8 kHz), and reach the final stage of amplification (programmable multiplier stage, ranging from 1–30). These boards also include one 12-bit analog-to-digital (A/D) converter per channel, which simultaneously digitizes the waveforms defining extracellular action potentials at 40 kHz. After A/D conversion, the signals are routed to DSP boards, each of which contain four digital signal processors (DSP, Motorola 5602) running at 40 MHz (instruction read at 20 MHz). Each DSP handles data from eight input channels and contains 32 K 24 bits of SRAM and 4 K 16-bit words of dual port SRAM memory. A timing board is responsible for distributing timing and synchronization signals to the entire MNAP. It also provides a digital time output that is used to synchronize external devices or to drive a video timer for a professional VCR used to store video records of the animal's behavior. The DSP boards also provide inputs for sampling digital pulses generated from behavioral cages. A single host Pentium microcomputer (100–200 MHz, with 64 MB of RAM and 2.1 GB of disk space) running a C++ software (SSCP, Spectrum Scientific, Dallas) in the Windows 3.11 operating system (Microsoft, Seattle) controls the MNAP over a serial line. Spike discrimination programs are downloaded from the PC host to the DSPs. Single spikes are discriminated by combining a modified version (Nicolelis and Chapin, 1994) of a principal component algorithm (Abeles and Goldstein, 1977), running in real-time, and one pair of time-voltage windows per unit. The size and position of the time-voltage boxes are defined by the experimenter to isolate the waveforms that belonged to a given unit. Spikes are only accepted as valid when they pass through both boxes. During the experiment, the time of occurrence of each of the valid spikes for all 96 channels is transferred to the hard disk of the PC host through a parallel bus (MXI-Bus, National Instruments, CA), which can transfer 2 MB of data/s. Digitized samples of the spike waveforms are also recorded periodically and stored for off-line analysis using a visualization program (SpikeWorks, Spectrum Scientific, Dallas). Optical drives (1.2–2.4 GB cartridges, Pinnacle Inc., CA) are used for temporary backup of the data files. These files are then transferred through an ethernet-based network to a computer server containing a CD-Recorder (PinnacleMicro, CA), which produces permanent records of the data in CD–ROMs. The MNAP also supplies options for analog backup using 8–16 channel tape recorders. In all experiments, low threshold tactile stimulation (step pulses of 100 ms in duration, delivered at 1–10 Hz) is provided by a computer-monitored Grass S8800 stimulator, which drives a vibromechanical probe. The digital outputs of

the stimulator were also transmitted to the MNAP to be stored with the spike data.

Population Data Analysis

Neural ensemble data were processed off-line using a series of graphics, statistical, and numerical analysis packages. The first level of analysis involved the production of single cell poststimulus time and cumulative histograms, which were used for defining the spatiotemporal receptive fields of single neurons. All of these preliminary steps of data analysis were carried out by using a spike train analysis software (Stranger, Biographics, Winston-Salem, NC) specially designed to handle large neuronal ensemble data sets. Spatiotemporal reconstructions of single neuronal receptive fields and population maps were carried out by using a graphics interface software, developed in C++, combined to MATLAB (Mathworks, MA). Finally, statistical packages like CSS–Statistica (Statsoft, Tulsa, OK) and SPSS (SPSS Inc., Chicago) were used for applying multivariate statistical methods to the analysis of neuronal ensemble data.

Acknowledgments

We thank Suzette Casal, Brett Carswell, and Kevin Tri Nguyen for their invaluable technical support; Larry Andrews (NBLABS, Dennison, TX) for manufacturing our microwire arrays, bundles, and head sets; Harvey Wiggins (Spectrum Scientific, Dallas) for the design and continuous support of our MNAP systems; Alexander Kirilov (Biographics, Winston-Salem, NC) for the support of Stranger; and Erika Fanselow for photographic work and comments on the manuscript. We are particularly indebted to John K. Chapin, a pioneer in the field of neuronal ensemble recordings, for his continuous suggestions and advice. M. A. L. N. also thanks professor Gyorgy M. Bohm for being a continuous source of motivation. This work was supported by grants from the National Institute of Dental Research (DE-111121–01), the Whitehall Foundation, the McDonnell Pew Foundation, the Klingenstein Foundation, the Duke-Sandoz program, and a Whitehead Scholar Award to M. A. L. N.

Received November 5, 1996; revised February 25, 1997.

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