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Protocol

Recording and marking with silicon multichannel electrodes

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Abstract

This protocol describes an implementation of recording and analysis of evoked potentials in the hippocampal cortex, combined with lesioning using multichannel silicon probes. Multichannel recording offers the advantage of capturing a potential field at one instant in time. The potentials are then subjected to current source density (CSD) analysis, to reveal the layer-by-layer current sources and sinks. Signals from each channel of a silicon probe (maximum 16 channels in this study) were amplified and digitized at up to 40 kHz after sample-and-hold circuits. A modular lesion circuit board could be inserted between the input preamplifiers and the silicon probe, such that any one of the 16 electrodes could be connected to a DC lesion current. By making a lesion at the electrode showing a physiological event of interest, the anatomical location of the event can be precisely identified, as shown for the distal dendritic current sink in CA1 following medial perforant path stimulation. Making two discrete lesions through the silicon probe is useful to indicate the degree of tissue shrinkage during histological procedures. In addition, potential/CSD profiles were stable following small movements of the silicon probe, suggesting that the probe did not cause excessive damage to the brain.

Theme: Disorders of the nervous system

Topic: Epilepsy: basic mechanisms

Keywords: Current source density; Dendritic excitation; Laminar profile; Hippocampus; Silicon probe

1. Type of research

- Electrical activity from a layered structure like the cortex can be optimally recorded by placing extracellular electrodes in different layers of the cortex.
- Simultaneous recordings are made at multichannel electrodes on a linear array, in this case fabricated with precise inter-electrode distances on a silicon probe [1,16].
- Current source density analysis yields the location of current sources and sinks and the time course of the source and sinks.

2. Time required

- Surgery and placement of electrodes, 30–60 min.
- Recordings, 5–120 min.
- Perfusion of animal, 15 min.
- Fixing of brain, 2 days.
- Histology, 30 min.

3. Materials

3.1. Animals

Rats of 250 to 450 g (Charles River, St. Constant, Quebec, Canada).

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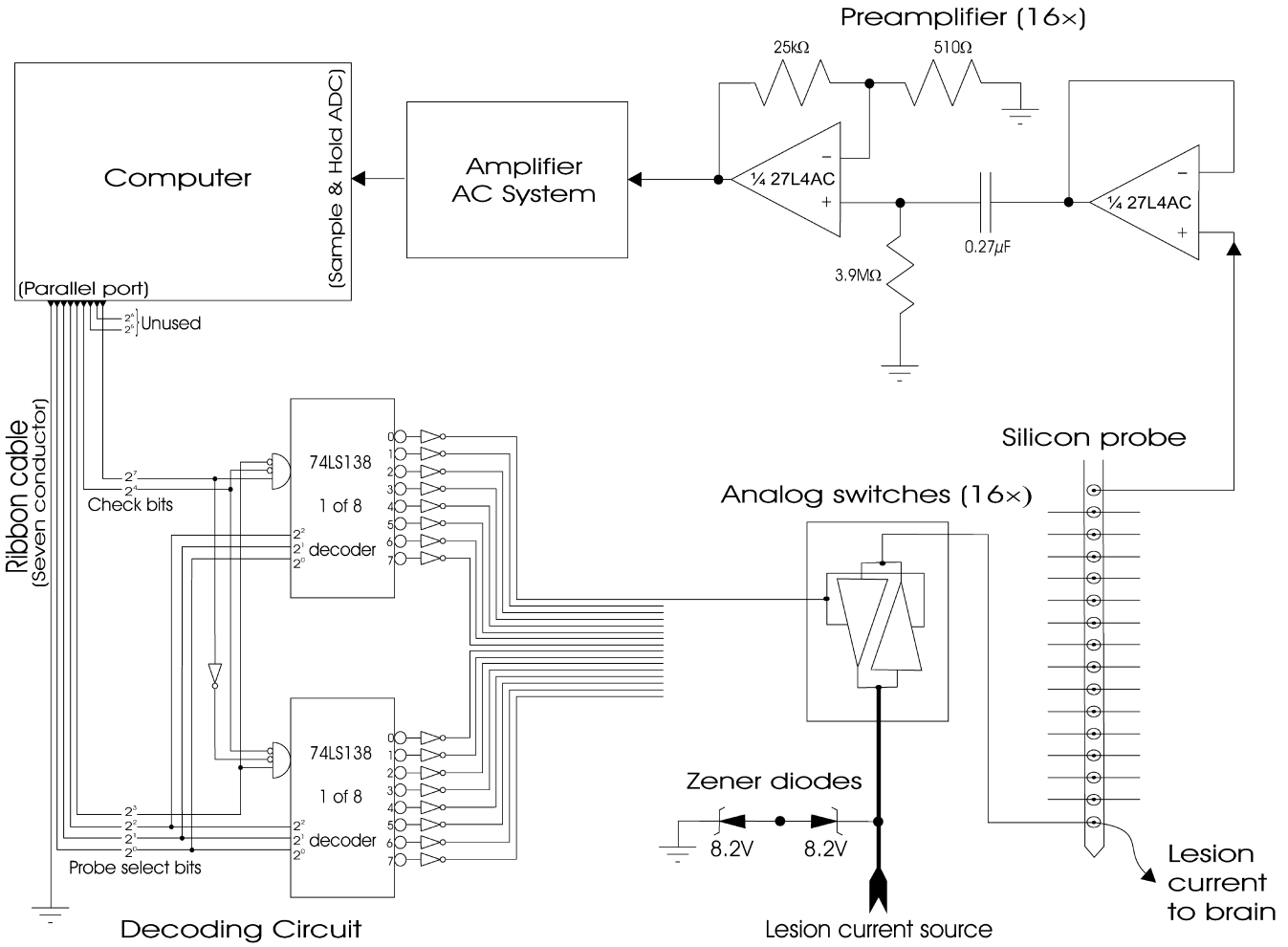

Fig. 1. Schematic diagram showing lesion circuitry (left) and recording circuit (right). The lesion current source (bottom) access to the electrodes on the silicon probe is normally blocked by the high impedance analog switches. The switches are opened by a decoding circuit that is based on a 4-bit selection scheme. Lesion voltage is limited by the Zener diodes. Each recording channel is connected to a preamplifier as shown, before a main amplifier and computer analog-to-digital converter (ADC) with sample-and-hold circuits. For more details, see text.

3.2. Special equipment

- Multichannel electrodes. Silicon probes (University of Michigan).
- Recording equipment. Custom-made preamplifiers (Fig. 1); 16-channel amplifier (AC Instrumentation, Seattle, WA); WIN-30DS (United Electronics, Watertown, MA); Microsoft Windows based microcomputer.
- Lesion equipment. Lesion circuitry (Fig. 1); Grass 88 stimulator and PSIU6 photoisolation unit (AstroMed, MA).

4. Detailed procedure

4.1. Surgical procedures

The rat was anesthetized with urethane (1.5 mg/kg i.p.) and placed in a stereotaxic frame. Burr holes were drilled in the skull for placement of (1) the recording probe in CA1/dentate gyrus at P3.6–4.5, L2.4–3 (with respect to bregma), (2) stimulating electrodes at (i) alveus in CA1 at 0.5–1.5 mm posterior and slightly lateral to the recording site, (ii) stratum radiatum of CA3b to activate the apical dendritic synapses of CA1, and (iii) the medial perforant path at P4.4, L4, ~3.3 mm ventral to the skull. Stimulation rate was $<$0.1 Hz.

Silicon probes were provided by the NIH NCRR Center for Neural Communication Technology at the University of Michigan. The technology used to fabricate these probes permits precise control over features such as site size and spacing leading to a high degree of reproducibility and great flexibility in their design [1]. The typical probe used had 16 recording sites spaced 50 μm apart on a vertical shank (‘16-channel probe’; catalogue name 5mm50). The width of the shank of the latter probe increased from 33 to 200 μm, measuring 123 μm at the farthest recording site from the tip; the shank thickness was 15 μm. Occasionally,

another '2×6 channel probe' was used; this probe had two shanks separated by 150 μm and six recording sites at 25-μm intervals on each shank. The latter shank increased from 23 to 100 μm, measuring 63 μm at the recording site farthest from the tip. In some experiments, the 2×6-channel probe was moved up or down by 100 μm, or the 16-channel probe by two steps each of 25 μm, in order to obtain more coverage in depth. The stability of the response was determined by superposition of records at the same absolute depth after one or two moves. The silicon probe, packaged on a PC board with a 2×8 male integrated circuit (IC) plug, fits into a matching plug containing 16 custom-made preamplifiers. A preamplifier (Fig. 1) removed the DC (high pass filter with corner frequency at 1 Hz) and amplified each signal by 50 times before the main amplifier (variable gain 2 to 100 times). The main amplifier also had 16 channels, each with adjustable low and high pass filters. The 16 amplified signals were then fed into a 12-bit Analog to Digital Converter (Analog Devices RTI-2100-DS with 0.75 MHz throughput). The 16 signals were held by sample-and-hold circuits until they were sampled, typically at 20–40 kHz per channel. Single or average ($N=4$) evoked potential sweeps were stored by a custom program.

Theoretically, the potential field in the brain is generated in one instant in time [13] and thus simultaneous recording of this potential field by a multichannel electrode is important. One-dimensional CSD(z, t) as a function of depth z and time t was calculated by a second-order differencing formula [6,9,12]:

$$\mathrm{CSD}(z,t) = \sigma[2\Psi(z,t) - \Psi(z + N\times\Delta z, t) - \Psi(z - N\times\Delta z, t)]/(N\times\Delta z)^2$$

where $\Psi(z, t)$ is the potential at depth z and time t, and Δz is the spacing between adjacent electrodes on the silicon probe, i.e. 25 μm for the 2×6 channel probe and 50 μm for the 16-channel probe. Conductivity σ was assumed to be constant across depth, and the CSDs are reported in units of $\mathrm{V/mm^2}$.

4.2. Lesion procedures

After the experiment, the site of each stimulating electrode was lesioned by passing 0.5–1 mA current for 1–2 s duration. The current was derived from a photoisolation unit PSIU6 that was connected to a Grass stimulator. The magnitude of the current was read directly from the Grass stimulator. Accuracy of the current reading was stated to be within 10%, which was confirmed by subsequent calibration by reading the DC voltage drop across a fixed resistor.

A special lesion circuit board was built for making lesions through an electrode on the 16-channel silicon probe. It was designed to be modular such that it would be inserted (or removed) between the preamplifier input stage and the silicon probe. The size of the circuit was restricted because of space constraints and to minimize recording noise. This necessitated the use of surface mount technology (SMT) components. The finished board measured 2.5 cm square. The electronic switching capability provided by the lesion circuit board was necessary to avoid having to connect manually for passing a lesion current. The circuit allowed recording from all 16 channels and subsequent selection of one or more of the 16 sites for lesioning. The DC lesion current (normally 30–50 μA for 1–3 s) was derived from a Grass PSIU6 unit driven by a Grass stimulator (S88 or S44). Initially, each electrode was isolated from the lesion current source by an open analog switch of >10 GΩ. With a software command, a selected analog switch was closed, and a DC current could then be passed through this electrode site. The lesion voltage was limited to ±8.9 V by Zener diodes. The parallel port of the computer was used to generate signals that operate the 16 analog switches by a 4-bit addressing system (Fig. 1). The 4-bit signal, together with two check bits, allows selection of only one of the 16 electrodes at a time, and minimizes the possibility of enabling multiple channels by inadvertent human or hardware error. Four 4066 CMOS chips serve as the analog switches.

After lesioning, the rat brain was removed after intracardial perfusion with phosphate buffered saline and 4% formalin, and allowed to be immersed in formalin for 2 days. It was then sliced into 40-μm-thick coronal sections using a frozen microtome. The lesioned sites and the recording track were identified in coronal sections stained with thionin. Digital photographic images were taken by a Nikon CoolPix 990 camera on a Nikon E600FN microscope, before and after staining.

5. Results

Recordings from the 16-channel probe are shown in Fig. 2. The medial perforant path (MPP) was stimulated and simultaneously-acquired signals from the 16 channels were averaged (four sweeps) to give average evoked potentials (AEPs; Fig. 2A). CSD analysis showed that the earliest event in the dentate gyrus was a sink (area with vertical lines in Fig. 2B and C) with onset of ~2 ms from the MPP stimulus (solid circle). This sink corresponds to the excitation at the middle of the dendrites of the dentate granule cells. The earliest event in CA1 was a sink of ~4 ms onset (area with horizontal lines in Fig. 2B and C), with its maximum assigned as 0 μm. The CA1 sink corresponds to the excitation of the distal apical dendrites by MPP, and it was accompanied by small sources at the proximal apical dendrites (−350 and −350 μm). The CSD responses to a second stimulus pulse (Fig. 2C), 50 ms delayed from the first pulse (Fig. 2B), showed paired-pulse facilitation (increase) of the CA1 distal dendritic sink (arrow in Fig. 2C) but not of the sink at the dentate gyrus (arrow head,

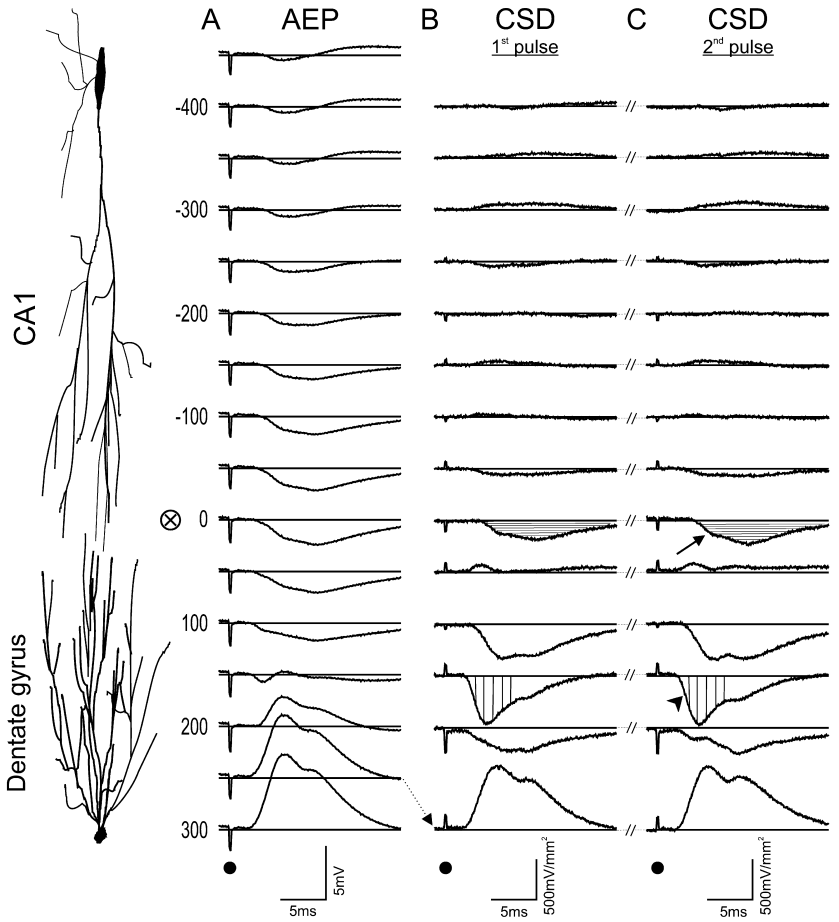

Fig. 2. Laminar profiles of average evoked potentials (A) and CSDs (B and C) acquired by a 16-channel silicon probe placed in CA1 and dentate gyrus, following middle perforant path stimulation (60 μA, 0.2 ms stimulus intensity). Schematic CA1 pyramidal cell and dentate granule cell shown at left. Lesion was made at 0 μm, the site labeled with an open circle with a cross. Positive depth is in the ventral direction. Histology of probe track and lesion is shown in Fig. 3A (rat PBP131). (A) Average evoked potential (AEP; average of four sweeps) profile at 50-μm intervals. (B) One-dimensional CSDs, derived using $N = 1$ ($\Delta z = 50$ μm) in CSD equation in text, derived from AEPs (first-pulse responses only) shown in A. (C) CSDs derived from the second-pulse responses (AEPs not shown); the second pulse was 50 ms after the first pulse. Note paired-pulse facilitation of the distal dendritic sink in CA1 (arrow with shaft at 0 μm of part C) but not of the sink in the dentate gyrus (arrow head at 150 μm, part C).

Fig. 2C). The electrode showing the maximum of the CA1 distal dendritic sink (0 μm in Fig. 2) was connected to a DC current source for 1 s. Subsequent histology revealed a lesion in stratum lacunosum-moleculare of CA1, 0–50 μm away from the hippocampal fissure (Fig. 3A).

Some researchers have expressed concern on the possible damage caused by the silicon probe on the brain. While tissue disruption inevitably occurred, the recordings were sufficiently stable that recordings were possible after small movements of the probe. In three rats recorded with a 2×6 probe (25-μm intervals between two vertical electrodes), the probe was moved vertically down (ventral) by 100 μm. The sets of AEP profiles before and after moving are shown in Fig. 4. In the region of overlap, the AEPs were comparable, suggesting that potentials were stable before and after movement of the recording probe. Fig. 4 illustrates an orthodromic population spike following excitation of the apical dendrites of CA1 pyramidal cells. The negative peaks of the population spike, linked by dotted lines, show the earliest onset latency at the proximal apical dendrites (Fig. 4). CSD analysis (not shown) confirmed that the orthodromic population spike sink originated at the proximal apical dendrites, 150–200 μm from the cell body layer (labeled 0), and then propagated to the cell bodies and the basal dendrites [7].

In another experiment (three rats), the 16-channel probe recorded from the dentate gyrus and CA3c (in the hilus of the dentate gyrus) following CA3b stimulation. After recording AEPs from an original position (position 1), the probe was moved down in two steps, each of 25 μm, to position 2 and then to position 3 (Fig. 5). The choice of a step of 25 μm was because this is half the spatial interval between channels, and the evoked potential at a fixed (absolute) depth could be compared after two steps of 25 μm each. An adequate spatial sampling interval was determined to be 50–75 μm in the cerebellum [6], but finer sampling may yield additional information [9]. The good overlay of the early responses (<5 ms latency) in absolute

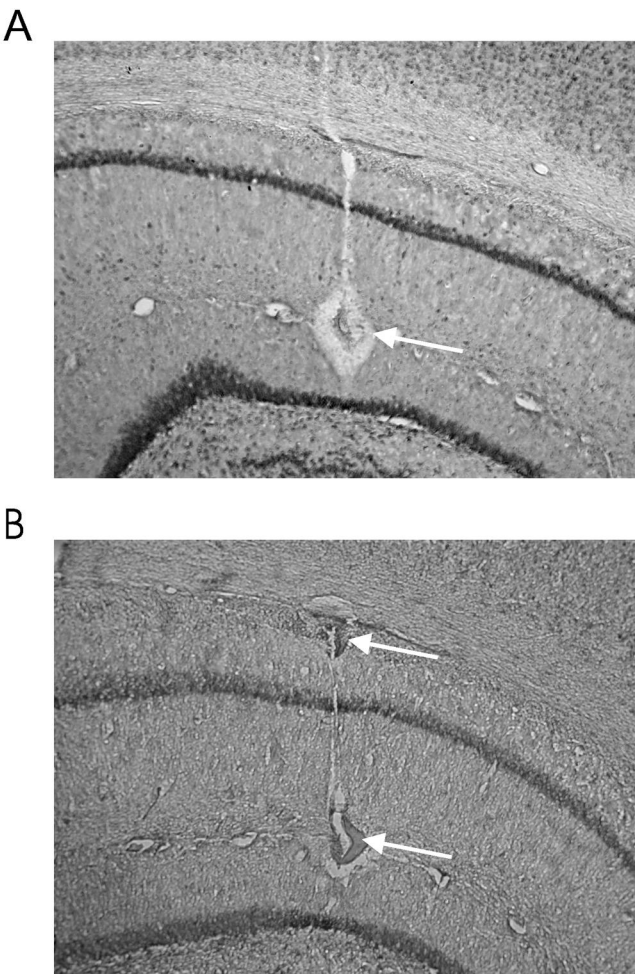

Fig. 3. Histology showing lesion marks through the 16-channel silicon probe. (A) The lesion (arrow) at the site with the maximal CA1 distal dendritic sink (0 μm in Fig. 2) was made by 30 μA DC for 1 s. (B) Two separate lesions at the two outermost electrodes of a 16-channel silicon probe (PBP60). The centers of the electrode sites were separated by 750 μm, while the centers of the lesion in the histological slide was separated by 750 μm as well, but each lesion had an approximate 'radius' of 20 to 25 μm. Each lesion was made by 50 μA DC for 3 s.

depth suggests that the responses (and the hippocampus) were stable before and after the probe movements. However, late responses were more variable. In combination, the two sets of data (position 1 and 2) gave a spatial resolution of 25 μm (Fig. 5A). The larger responses with the later (position 3) than earlier (position 2) argues against damage inflicted by movement of the silicon probe. In terms of physiology, CA3b stimulation evoked in CA3c pyramidal cells a population antidromic spike (shaded gray in Fig. 5) followed by a dendritic excitatory sink that is shown by slanted lines in Fig. 5 [14,15]. CA3b stimulation evoked in the dentate gyrus a proximal dendritic sink (vertical lines) followed by a mid-dendritic layer sink (horizontal lines) attributed to a multisynaptic circuit of CA3–CA1–entorhinal cortex–dentate gyrus [14,15].

In order to investigate the shrinkage of the brain tissue in the histological procedures, a lesion was made, respectively, at the topmost and bottommost of the 16 channels on the probe. The histology of one such experiment is shown in Fig. 3B. The spatial interval between the middle of the two lesions made was found to be 720 and 750 μm in two rats, or the shrinkage was negligible in these tissues as compared to the actual distance of 750 μm between the electrodes. Obviously, shrinkage of brain tissue depends on the particular histological processes used.

6. Discussion

The CSD profiles obtained by simultaneous recordings using a silicon probe were similar to those acquired by mapping using a single microelectrode. A good example is shown following CA3b stimulation (Fig. 5), which evoked a complex sequence of antidromic invasion and dendritic excitation in CA3c (within the hilus), in addition to an early, proximal following by a late, mid-dendritic excitation of the dentate granule cells. These complex spatial and temporal patterns of activity have been reported using single electrode mapping [14,15]. The primary advantage of the silicon probe recordings over single-electrode mapping is that responses are recorded simultaneously without delay across channels; thus, response stationarity over time need not be assumed. The practical advantage of a rapid acquisition of the potential profile (and CSDs) will facilitate studies in which the responses change with time, such as experiments on EEG, synaptic plasticity and pharmacological responses.

In our experience with different types of multichannel electrodes available to biomedical researchers, the silicon probes in this report [1,2,16] are the best for studying depth profiles of electrical activity in the cortex. The dimensions of a 16-channel Prohaska probe [3] were considerably larger than those of a silicon probe, such that tissue disruption inevitably occurred (unpublished observations). The Kuperstein and Eichenbaum [8] probe was not ideal for CSD analysis since the availability of contiguous (adjacent) working channels was not guaranteed.

The mechanical disruption of the brain by a silicon probe was small, and the track of an acutely placed silicon probe may be difficult to detect sometimes. Ylinen et al. [16] and Bragin et al. [2] deliberately left the probe in the brain tissue to be identified during histological sectioning. We believe that it is difficult to keep the probe in place, after removal of the skull and dura, and it is even more difficult to be sure that the probe did not move between recording and histological sectioning. Thus, an alternate method of localizing the electrode is desired, and we used the conventional lesion technique.

We designed a system such that a modular lesion circuit board could be inserted between the input amplifiers and the recording probes. We have implemented the system only for acute recordings, although the same could be done

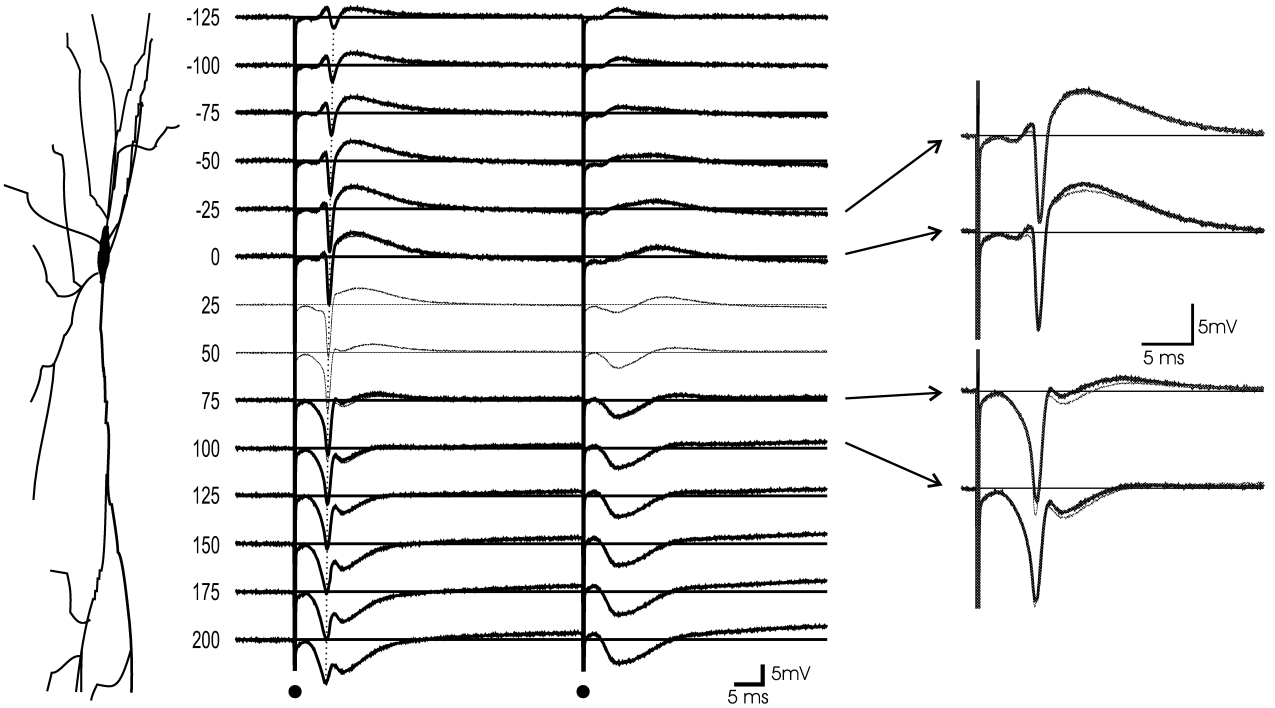

Fig. 4. Stability of average evoked potentials (AEPs) following two sequential vertical (downward) movements of 2×6 probe, each of 100 μm. Responses were evoked by paired-pulse stimuli of 150 μA and 50 ms interpulse interval applied to CA3, with first pulse evoking a population spike and second not. Sets of AEPs were plotted at expected absolute depths, at every 25 μm, with overlapping traces at common depths. The middle set of AEPs is shown as light traces, as compared to dark traces for the upper and lower sets of AEPs. Sites with overlapping AEPs are expanded on the right side, but shown with first-pulse responses only.

for chronic recordings as well. By using software-addressable switches, any of the 16 electrodes can be connected to the DC lesion current. Previously, we showed that a 50-μA DC for 3 s would make an ~50–100 μm diameter lesion of the brain [7]. Even 30 μA for 0.5 s was able to make a lesion at some electrodes (Fig. 3A). We have not systematically studied the reason for this variability in lesion current, but it is likely that the DC impedance of an electrode site may vary, especially after repeated use. Subsequent recordings could be made from an electrode that had passed a lesion current, with no apparent difference from before the lesion.

The advantage of lesion at a selected electrode of the 16-channel probe cannot be overemphasized. The event of interest may occur only at one channel, and the closer the mark (lesion) is to the recorded event, the more certain is the location of the event [4,10]. We showed the lesion at the site of the maximal sink (excitation) evoked by medial perforant path in hippocampal CA1 (Fig. 3A). The latter sink occurred near the hippocampal fissure, among the distal dendrites of the hippocampal CA1 pyramidal cells [5,11].

We showed that the silicon probes used did not disrupt the brain tissue significantly, and stepping may be used to increase the spatial sampling interval or extend the coverage in depth. With a fixed position of the 16-channel probe, only 16 signals spanning 750 μm will be simultaneously recorded. However, the signals at fixed depths were stable before and after upward or downward movements of the probe, as shown for average evoked potentials and CSDs (Figs. 4 and 5). Response stationarity is still an issue, as indicated by the more variable late, multisynaptic responses (Fig. 5). A more elegant solution is to fabricate probes with closer spatial intervals and more channels. Probes with 32 channels at 20–25 μm intervals are being fabricated now. Although the complexity will be increased, the present recording/lesion system could be upgraded to record and lesion from 32 channels.

7. Quick procedure

7.1. Surgery

Rats are anesthetized and placed in a stereotaxic frame. Stimulating electrodes and multichannel silicon probe are placed in the hippocampus.

7.2. Lesion

Analysis to identify current sources and sinks is performed on-line, and a recording channel showing a specific sink or source is connected to the lesion current source. Histology of the brain is then done.

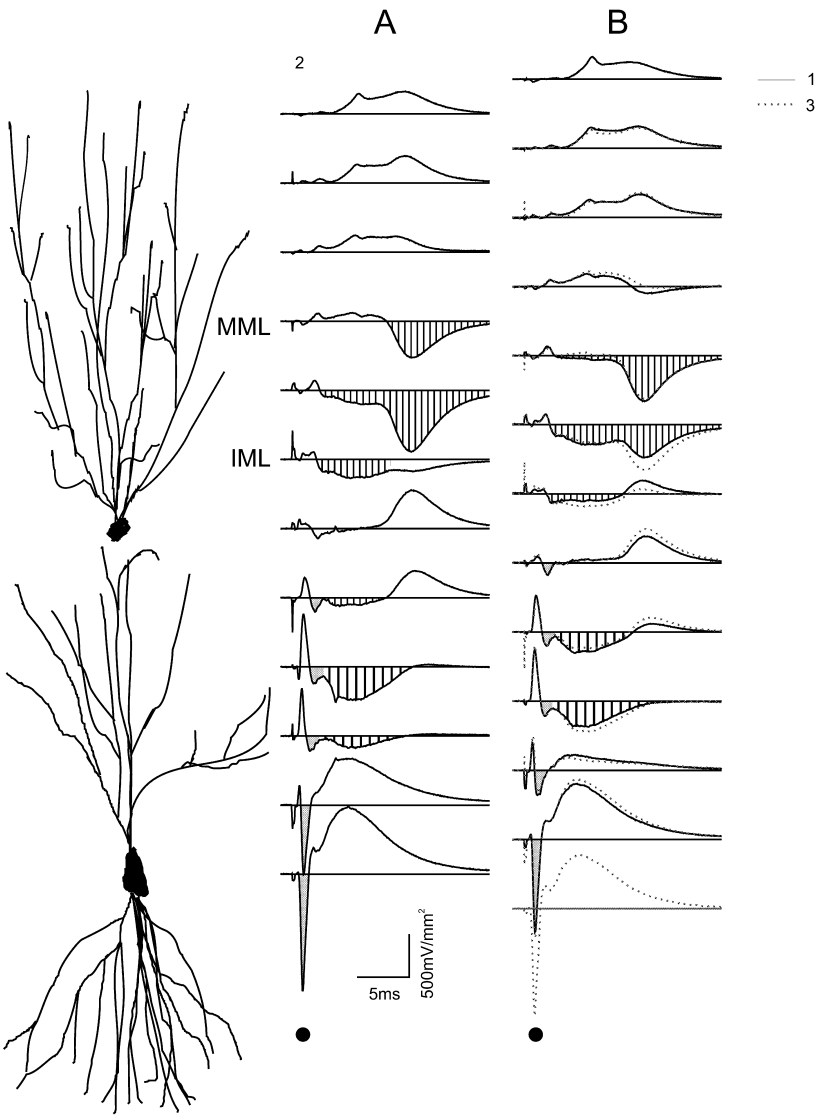

Fig. 5. CSDs from 16-channel probe in the dentate gyrus and hilus following stimulation of CA3b. Schematic dentate granule cell and CA3c cell in the hilus are shown on the left. Probe recorded first in position 1, then moved 25 μm down to position 2, and then another 25 μm down to position 3. (A) The CSDs at position 2, shown at 50-μm intervals. (B) Overlay of CSDs at positions 1 and 3, shown at 50-μm intervals. CA3b stimulation evoked an antidromic spike (sink shaded gray, immediately after the stimulus labeled with a solid circle) followed by dendritic excitation (sink with slanted lines) in CA3c pyramidal cells. CA3b stimulation evoked in the dentate gyrus an inner molecular layer (IML) sink (vertical lines) followed by a late, 20-ms latency middle molecular layer (MML) sink (horizontal lines). The early responses (<5 ms) showed minimal variations in the overlap, while the late responses (arrow) showed more variation.

8. Essential references

[2], [6], [9]

References

[1] S.L. Bement, K.D. Wise, D.J. Anderson, K. Najafi, K.L. Drake, Solid-state electrodes for multichannel multiplexed intracortical neuronal recording, IEEE Trans. Biomed. Eng. 33 (1986) 230–241.

[2] A. Bragin, J. Hetke, C.L. Wilson, D.J. Anderson, J. Engel Jr., G. Buzsaki, Multiple site silicon-based probes for chronic recordings in freely moving rats: implantation, recording and histological verification, J. Neurosci. Methods 98 (2000) 77–82.

[3] G. Buzsaki, E. Grastyan, J. Czopf, L. Kellenyi, O. Prohaska, Changes in neuronal transmission in the rat hippocampus during behavior, Brain Res. 225 (1981) 235–247.

[4] K.J. Canning, L.S. Leung, Current source density analysis does not reveal a direct projection form the perirhinal cortex to septal part of hippocampal CA1 or dentate gyrus, Hippocampus 9 (1999) 599–600.

[5] K.J. Canning, K. Wu, P. Peloquin, F. Kloosterman, L.S. Leung, Physiology of the entorhinal and perirhinal projections to the hippocampus studied by current source density analysis, Ann. NY Acad. Sci. 911 (2000) 55–72.

[6] J.A. Freeman, C. Nicholson, Experimental optimization of current source-density technique for Anuran cerebellum, J. Neurophysiol. 38 (1975) 369–382.

[7] F. Kloosterman, P. Peloquin, L.S. Leung, Orthodromic spike in

hippocampal CA1 originates in the proximal apical or basal dendrites in urethane-anesthetized rats in vivo, J. Neurophysiol. 86 (2001) 2435–2444.

[8] M. Kuperstein, H. Eichenbaum, Unit activity, evoked potentials and slow waves in the rat hippocampus and olfactory bulb recorded with a 24-channel microelectrode, Neuroscience 15 (1985) 703–712.

[9] L.S. Leung, Field potentials in the central nervous system-recording, analysis and modeling, in: A.A. Boulton, G.B. Baker, C.H. Vanderwolf (Eds.), Neurophysiological Techniques. Applications to Neural Systems. NeuroMethods, Vol. 15, Humana, Clifton, NJ, 1990, pp. 277–312.

[10] L.S. Leung, K.J. Canning, 'Reply to Liu and Bilkey's reply', Hippocampus 9 (1999) 603–604.

[11] L.S. Leung, L. Roth, K.J. Canning, Entorhinal inputs to the hippocampal CA1 and dentate gyrus in the rat: a current–source-density study, J. Neurophysiol. 73 (1995) 2392–2403.

[12] U. Mitzdorf, Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena, Physiol. Rev. 65 (1985) 37–100.

[13] R. Plonsey, Bioelectric Phenomenon, McGraw Hill, New York, 1969.

[14] K. Wu, K.J. Canning, L.S. Leung, Functional connections between CA3 and the dentate gyrus and CA1 revealed by current source density analysis, Hippocampus 8 (1998) 217–230.

[15] K. Wu, L.S. Leung, Enhanced but fragile inhibition in the dentate gyrus in vivo in the kainic acid model of temporal lobe epilepsy—a study using current source density analysis, Neuroscience 104 (2001) 379–396.

[16] A. Ylinen, A. Bragin, Z. Nadasdy, G. Jando, I. Szabo, A. Sik, G. Buzsaki, Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms, J. Neurosci. 15 (1995) 30–46.