Journal of Neuroscience Methods 176 (2009) 136–143

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

A method for chronic stimulation of cortical organotypic cultures using implanted electrodes

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ARTICLE INFO

Article history:
Received 8 July 2008
Received in revised form 29 August 2008
Accepted 29 August 2008

Keywords:
Chronic stimulation
Structured activity
Cortical network
Plasticity
Organotypic

ABSTRACT

The neural mechanisms underlying some forms of learning and memory require hours or days to be expressed; however it has proven difficult to study these slowly developing forms of plasticity in reduced preparations due to the short-term nature of acute slice preparations and the fact that most culture preparations lack exposure to structured external input, which plays a critical role in normal cortical development and plasticity. To address this limitation, we developed a method for chronic stimulation of organotypic slice cultures using implanted microelectrodes. This method imparts the ability to apply patterned stimulation to cortical tissue for hours or days, and allows intracellular electrophysiological recordings before and after the stimulation. Importantly, the permanent implantation of the electrodes in the tissue assures that the same neuronal pathways are being excited both during the chronic stimulation while the cultures are in the incubator and while recording in the testing phase. This technique establishes a reduced model for studying experience-dependent plasticity.

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1. Introduction

Many forms of learning ultimately rely on changes in network function that occur over days (Buonomano and Merzenich, 1998; Karmarkar and Dan, 2006). For example, most forms of experience-dependent plasticity require days of altered sensory input to be induced (Wang et al., 1995; Kilgard and Merzenich, 1998; Sawtell et al., 2003). While acute slices have proven highly valuable in studying forms of plasticity that can be rapidly induced by discrete events, such as a tetanus, their usefulness in studying slowly developing forms of plasticity has been limited by their short-term viability. Additionally, acute slices do not generally exhibit the complex network dynamics observed in the cortex in vivo and that is believed to contribute to cortical function (Steriade et al., 1993; Petersen et al., 2003). In contrast, both dissociated and organotypic cultures provide a method to observe cellular and synaptic changes over the course of days, and both display propagating neural activity and complex network dynamics (Beggs and Plenz, 2004; Marom and Eytan, 2005; Wagenaar et al., 2005; Johnson and Buonomano, 2007a). Additionally, plasticity studies using both dissociated cultures (Turrigiano et al., 1998; Sutton et al., 2006) and organotypic cultures (Aptowicz et al., 2004; Karmarkar and Buonomano, 2006) have proven valuable in studying slowly developing forms of plasticity, particularly homeostatic changes in synaptic strength or intrinsic excitability induced by global changes in activity levels.

To date, however, the use of both dissociated and organotypic cultures as a model of experience-dependent forms of learning has been limited because cultured networks develop during days or weeks of incubation in an environment lacking any structured activity. Structured or patterned activity is known to play a fundamental instructive role in the development and organization of cortical circuits (Wiesel and Hubel, 1963; Buonomano and Merzenich, 1998; Karmarkar and Dan, 2006); thus, there is a need to develop reduced preparations that can be stimulated with specific input patterns over long periods of time. One promising technique towards this goal is the use of dissociated cells cultured on micro-electrode arrays (MEA), which can be used to chronically apply specific patterns and record activity (Pfeffer et al., 2004; Wagenaar et al., 2005). However, to date establishing robust forms of experience-dependent plasticity using dissociated cultures and MEAs has proven challenging (Wagenaar et al., 2006). Organotypic slices may offer a better reduced model for the study of experience-dependent plasticity since the cortical architecture, connectivity, and electrophysiological properties in these cultures more closely matches in vivo characteristics (Caeser et al., 1989; Annis et al., 1993; Debanne et al., 1995). Thus, we have developed a method to chronically stimulate cortical organotypic slice cultures using implanted microelectrodes. This technique allows for stimulation for hours or days while the slice is in the incubator. Importantly, since the electrodes are in a fixed position, it also insures that

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0165-0270/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jneumeth.2008.08.037

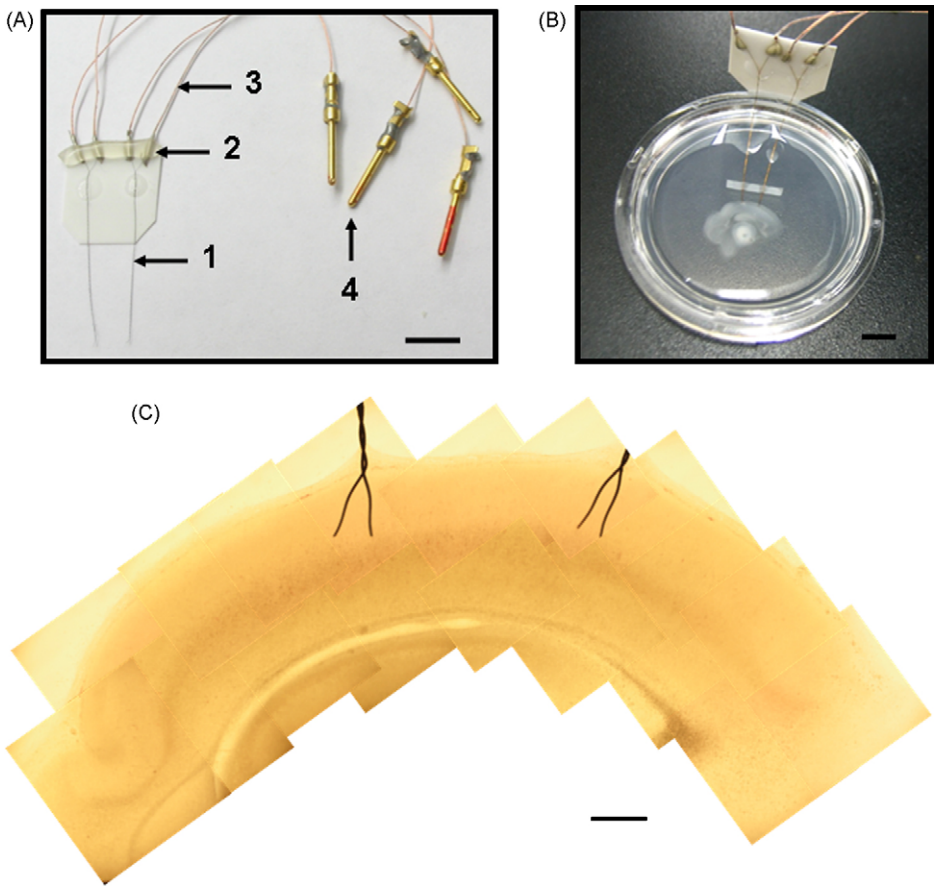

Fig. 1. Chronic microelectrodes implanted in cortical organotypic slice cultures. (A) Two bipolar electrodes (1), composed of coated platinum/iridium microwire, were attached to a plastic mounting and connected to coated copper wire (3) using conductive epoxy (2). Hiden male crimp pins (4) were soldered to the ends of the copper wire to prevent oxidation. See Section 2 for a complete description of microelectrode preparation. Scale bar is 7 mm. (B) The plastic mounting with microelectrodes was attached to the side of a cell culture insert. The microelectrodes were positioned approximately 2 mm apart and a thin strip of parafilm was melted over the wires to hold them in place. Cortical slices were placed over the microelectrodes and positioned so the tips were 400–800 μm from the cortical surface. Scale bar is 4 mm. (C) Montage of an unstained organotypic culture showing placement of the implanted microelectrodes. Scale bar is 500 μm.

the same pathways that received the chronic stimulation are then tested after the tissue has been transferred to an electrophysiology rig for recordings. We have successfully used this method to stimulate for days without any adverse effects to the neuronal networks and we believe that this method represents a promising model for studying experience-dependent plasticity and long-term forms of learning.

2. Materials and methods

2.1. Stimulating electrodes

Bipolar electrodes for chronic stimulation were made using formvar-coated 90% platinum/10% iridium microwire (0.001″ diameter; California Fine Wire, cat. #100-167). A length of wire (approximately 10 cm) was folded in half and wound together. The ends were separated and the coating was removed from approximately 2 mm of each end of the wire. Two of these wires were affixed with superglue to a 1 cm by 1.4 cm piece of flat plastic mounting cut from a weigh dish (see Fig. 1A for placement). After drying, a length of coated copper wire (Cooner Wire, cat. #CZ1174-clear) was connected to each uncoated end of microwire using conductive epoxy (Allied Electronics, cat. #974-004). The epoxy was allowed to harden and a small amount of hot glue was placed over the epoxy to further secure the wires to the plastic mounting. The free end of each copper wire was soldered to a gold Hiden crimp male pin (Marvac Electronics, cat. #DHD-PIN/M-HR-P1K). This pin protects the copper wire from oxidation while it remains in the humid environment of the incubator and provides a convenient attachment for the test wire which is connected to the stimulus isolator unit during stimulation. After testing for connectivity, the long wound end of the wire was cut to a length of 12–13 mm beyond the end of the plastic mounting. The coating was removed from the final approximately 200 μm of microwire by ignition with a butane torch lighter, and the uncoated ends were separated slightly to prevent short-circuiting. Removing the coating from the final 200 μm of these ends creates a larger surface area in order to decrease current density during stimulation, preventing damage to the tissue (McCreery et al., 1990). Without this step, lesions were observed in the tissue after stimulation (see Fig. 2). Hot glue was used to attach one end of the plastic mounting to the side of a culture plate insert (Millipore, cat. #PICMORG-50), allowing the long, wound ends of the electrodes to rest on top of the insert (see Fig. 1B). The microwire electrodes were positioned approximately 2 mm apart and to prevent movement of the electrodes, a small strip of parafilm was placed over the microwires and melted onto the insert. The inserts with attached electrodes were washed in ethanol and dried under ultraviolet light for sterilization before placement of the tissue.

2.2. Organotypic slice preparation

Organotypic slices were prepared using the interface method (Stoppini et al., 1991; Johnson and Buonomano, 2007a). Before tis-

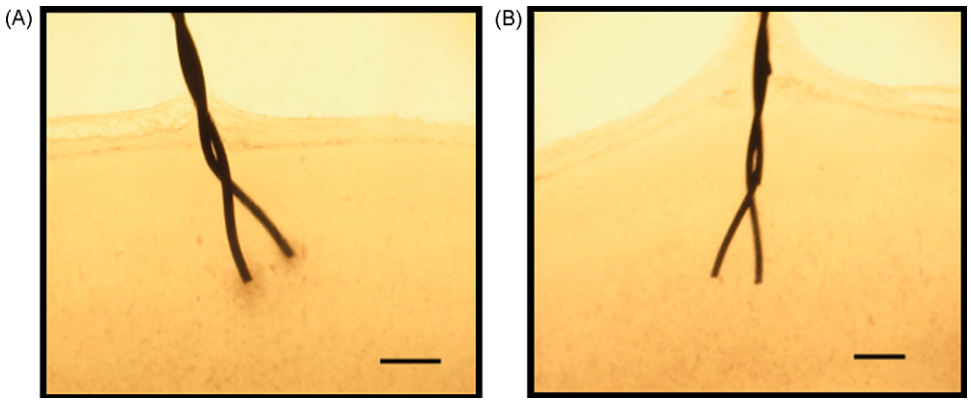

Fig. 2. Comparison of chronically stimulated tissue in areas surrounding electrodes with exposed and unexposed tips. (A) Our first stimulating electrodes were made of microwire which was completely coated to the tip. After 2 days of chronic stimulation composed of a burst of 4 pulses (at 100 Hz) at 200 μA, we observed dark lesions in the tissue surrounding the electrode tips. Bar = 200 μm. (B) When the coating was removed from the final ~200 μm of the electrode tips, lesions were not observed after the same intensity and duration of stimulation. Bar = 200 μm.

sue preparation, each insert, with attached microelectrodes, was placed in a well of a 6-well culture dish with 1 mL of culture media, and placed in an incubator for no less than 20 min in order to equilibrate the temperature and CO_2 levels. Seven-day-old Sprague–Dawley rats were anesthetized with isoflurane and decapitated. The brain was removed and placed in chilled cutting media. Coronal slices (400 μm thickness) containing primary somatosensory cortex, as well as hippocampus and other structures, were cut using a vibratome. The slices were placed on the cell culture inserts with the attached electrodes and positioned using a small sable paintbrush so that the electrodes were under the slice and the tips were 400–800 μm from the cortical surface. Culture media was changed 1 and 24 h after cutting and every 2–3 days thereafter. Cutting media consisted of EMEM (MediaTech cat. #15-010) plus 3 mM $MgCl_2$, 10 mM glucose, 25 mM Hepes, and 10 mM Trisbase. Culture media consisted of EMEM plus 4 mM glutamine, 0.6 mM $CaCl_2$, 1.85 mM $MgSO_4$, 30 mM glucose, 30 mM Hepes, 0.5 mM ascorbic acid, 20% horse serum, 10 units/L penicillin, and 10 μg/L streptomycin. Slices were incubated in 5% CO_2 at 35 °C for 10–28 days before beginning stimulation.

2.3. Chronic stimulation

All electrical stimulation via the microelectrodes consisted of charge-balanced, biphasic current pulses, composed of a 100 μs positive pulse followed by a 100 μs delay and a 100 μs negative pulse. Biphasic stimulation was used because it has previously been shown to be more effective in eliciting action potentials and less damaging to the tissue and electrodes than monophasic current (Wagenaar et al., 2004; Merrill et al., 2005). Stimulation pulse patterns were generated using a Master-8 (A.M.P. Instruments) or custom written MATLAB software controlling an analog-output board (National Instruments, PCI-6723), and delivered via stimulus isolator units (A.M.P. Instruments). Chronic stimulation was administered while the cultures were in the incubator.

2.4. Electrophysiology

In order to keep the electrodes attached to the slice during recording, a custom recording chamber designed to hold the cell culture insert was build with SylGard (Dow Corning). Recordings were made from regular-spiking, supragranular pyramidal neurons (Dong and Buonomano, 2005) located less than 500 μm from the cortical surface using IR-DIC visualization. Experiments were carried out in artificial cerebrospinal fluid (ACSF) composed of 125 mM NaCl, 5.1 mM KCl, 2.6 mM $MgSO_4$, 26.1 mM $NaHCO_3$, 1 mM NaH_2PO_4, 25 mM glucose, and 2.6 mM $CaCl_2$. The internal solution for whole-cell recordings contained 100 mM K-gluconate, 20 mM KCl, 4 mM ATP-Mg, 10 mM phospho-creatine, 0.3 mM GTP-Na, 10 mM HEPES, and was adjusted to pH 7.3 and 300 mOsm. Recordings were sampled at 10 KHz, digitized using a CED micro1401 board (Cambridge Electronic Design), and saved for off-line analysis. All analyses were performed using custom-written software in MATLAB.

2.5. I/O function

Input–output analysis was performed by measuring the slope of the initial EPSP in response to 6–8 different stimulus intensities. The intensity vs slope plots were fit with a sigmoid function (Marder and Buonomano, 2003) and the asymptote was used as a measure of maximal response strength. Plots with incomplete data or that were not well fit with the sigmoid function were excluded from the analysis.

2.6. Morphology

Slices were rinsed twice with 0.1 M phosphate buffered saline (PBS), fixed in 4% paraformaldehyde in PBS for 4 h, and then rinsed in PBS and stored in 0.1% sodium azide in PBS until stained. Slices were stained with either cresyl violet (Roboz Surgical Instr. Co.), chicken neurofilament antibody (Chemicon, cat. #AB5539), or DAPI while the slice remained attached to the culture insert. To quantify the number of cells in the tissue surrounding the electrodes, a confocal microscope was used to visualize the number of cells present in a 175 μm square area around the electrode tips (see Fig. 3C and D), and the average cell count from 12 to 18 layers (0.6 μm thickness) for each slice was used as the measure of cell density.

3. Results

In order to provide hours to days of patterned stimulation to cortical organotypic slice cultures while they were in the incubator, we attached two bipolar electrodes to each cell culture insert, and placed cortical slices on the inserts so that the electrode tips were positioned 400–800 μm from the cortical surface. (See Section 2 and Fig. 1 for a detailed description of the electrode design.) These electrodes were used to stimulate the cultures both chronically while they were in the incubator, as well as during the testing phase while the slices were on the recording rig.

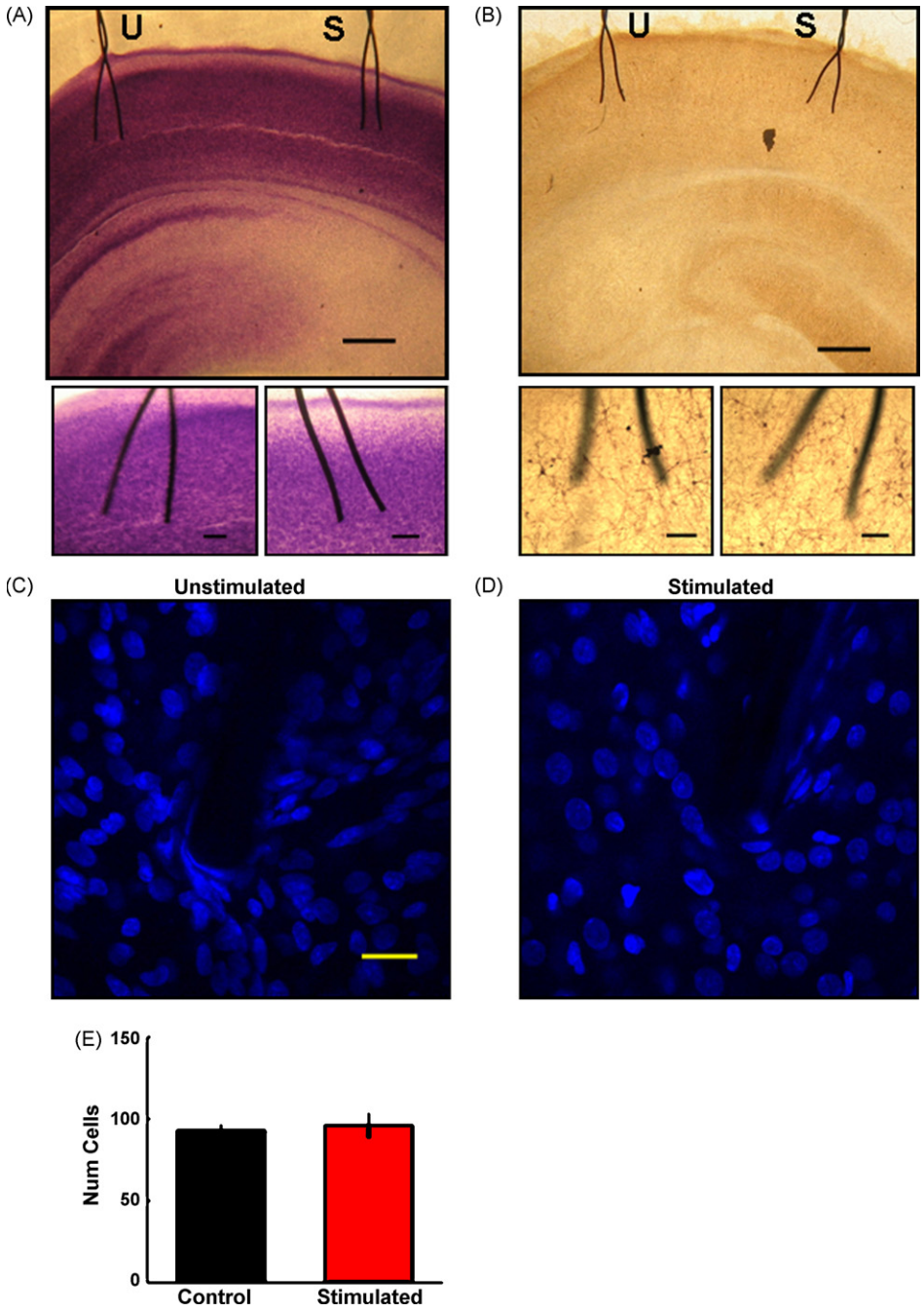

Fig. 3. Normal architecture and density of neuronal processes after chronic stimulation. (A) The slice was stained with cresyl violet to demonstrate density and morphology of cell bodies. No visible differences in cell density were observed between tissue surrounding unstimulated (U, lower left panel) and stimulated (S, lower right panel) electrodes. (B) Neurofilament staining shows an even distribution of neural process surrounding unstimulated (U, lower left panel) and stimulated (S, lower right panel) electrodes. (C and D) Cells were visualized by DAPI staining, and the number of cells in a 175 μm square (0.6 μm thick layer) surrounding the stimulated (D) or control (C) electrodes was counted. (E) Mean number of cells in the area surrounding each electrode was not significantly different (mean = 96 ± 7 and 93 ± 3 for stimulated and control electrodes, respectively, $p = 0.70$). For (A) and (B), large panels bar = 500 μm; small panels bar = 100 μm; for (C) and (D), bar = 25 μm.

3.1. *Effects of chronic stimulation on tissue*

In vivo use of chronically implanted electrodes, including therapeutic use in humans, has shown that chronic stimulation can produce local lesions of the neural tissue and become progressively less effective (Shepherd et al., 1991; Henderson et al., 2001; Merrill et al., 2005). To minimize this effect we used charge-balanced, biphasic current pulses, which has previously been shown to be most effective in eliciting action potentials (allowing lower current amplitudes to be used), produce less tissue damage, and prevent corrosion of the electrodes (Wagenaar et al., 2004; Merrill et al., 2005). To determine whether chronic stimulation produced any detectable lesions or damage in the slice tissue, stimulation was administered via one electrode for 2 days while the slice remained in the incubator. Stimulation consisted of 1 pulse or a burst of 4 pulses (at 100 Hz) every 10 s at 150–200 μA. This intensity range was chosen because it was previously found to be at or above the intensity necessary to produce the maximal synaptic response (Johnson and Buonomano, 2007a). In our initial attempts at chronic stimulation, we found that in some slices, the tissue surrounding

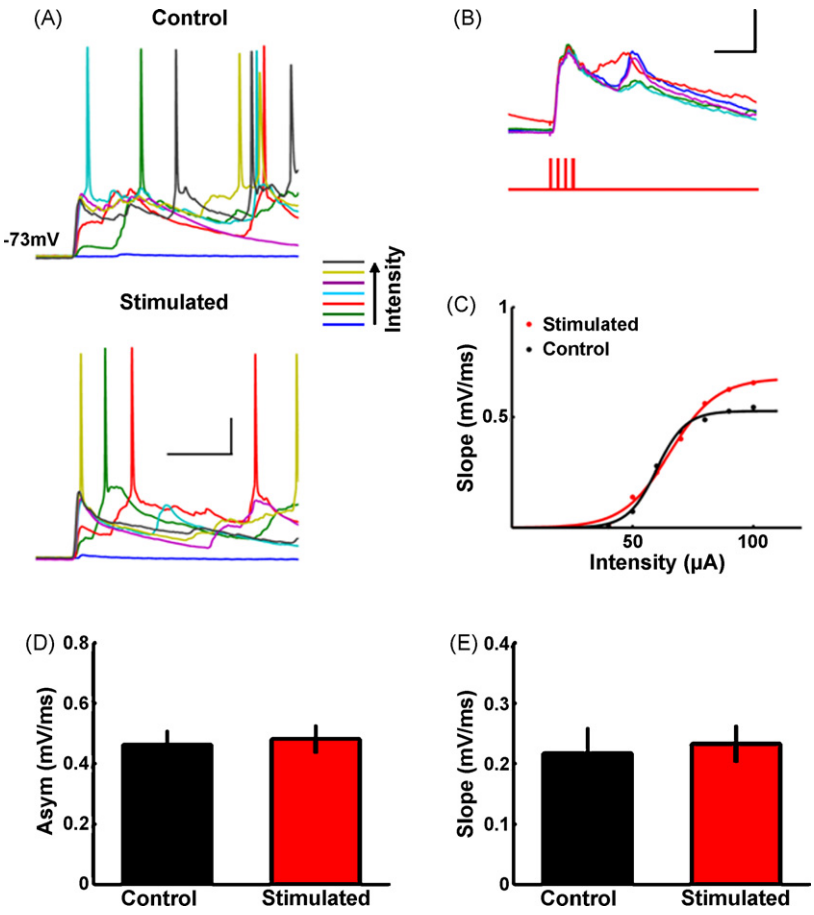

Fig. 4. Evoked PSPs from chronically stimulated and unstimulated electrodes. (A) Example of evoked PSPS in response to increasing current intensities (40–100 μA) via the control or stimulated electrode. Calibration bars = 100 ms and 20 mV. (B) Example of PSP responses evoked from the pulse pattern used for chronic stimulation for 2–4 days. Stimulation consisted of a burst of 4 pulses at 100 Hz, presented every 5–15 s at 80–150 μA. Calibration bars = 50 ms and 10 mV. (C) I/O functions for the neuron shown in (A). Initial EPSP slopes were plotted against the stimulation intensity and these points were fit to a sigmoid. Similar I/O functions were observed in response to control and chronically stimulated electrodes. (D) Average asymptotes of I/O functions from 13 slices (25 neurons) showed no significant difference between responses to stimulated and control electrodes (mean = 0.48 ± 0.05 and 0.46 ± 0.05 mV/ms for stimulated and control electrodes, respectively; $p = 0.77$). (E) Mean EPSP slopes in response to stimulated and control electrode at the intensity used for chronic stimulation are not significantly different (mean = 0.23 ± 0.03 and 0.22 ± 0.04 mV/ms for stimulated and control electrodes, respectively; $p = 0.76$).

the electrode tip appeared abnormally darker than the surrounding tissue (Fig. 2A). Since this was never observed in tissue surrounding unstimulated control electrodes, it suggested local tissue alterations produced by the electrical stimulation, potentially caused by high current density in the area surrounding the electrode tip (McCreery et al., 1990; Merrill et al., 2005). To address this problem, we removed the coating from the final 200 μm of the tips of the electrodes (see Section 2), exposing a greater surface area for current discharge. As shown in Fig. 2B, this prevented visible abnormalities in unstained tissue at intensities up to 200 μA. All subsequent experiments were performed using electrodes with exposed tips.

To further determine if stimulation resulted in a loss of neurons or neuronal processes at the site of current injection we performed experiments in which one electrode was stimulated for 2–4 days using the same pattern as above, while the other was used as an unstimulated control. Following stimulation, the slices were fixed and stained with cresyl violet (Roboz Surgical Inst. Co.) or with a neurofilament antibody (Chemicon, cat. #AB5539) in order to evaluate changes in the density of neurons or their processes, respectively. As shown in Fig. 3, we observed no difference between the tissue surrounding the stimulated and unstimulated electrodes. There were no visible inhomogeneities or alterations in the cells or neuronal fibers near the stimulated electrode as compared to the unstimulated electrode (Fig. 3A and B). Additionally, in slices stained with DAPI, we used confocal microscopy to quantify the number of cell bodies in the area (175 by 175 μm; see Fig. 3C and D) surrounding the electrode tips, and found no significant difference in the number of cells observed near the stimulated and unstimulated electrodes (mean = 96 ± 7 and 93 ± 3 for stimulated and control electrodes, respectively; $t_2 = 0.45$, $p = 0.70$; Fig. 3E). Together, these results suggest that the stimulation did not damage the tissue.

3.2. Response to implanted microelectrodes

We next examined the effectiveness of the electrodes in eliciting activity, both with and without chronic stimulation. The above studies indicated that there were no visible morphological changes produced by chronic stimulation, however they did not rule out electrophysiological changes in stimulation effectiveness or axon excitability. As before, we administered stimulation to 13 slices using one electrode and the other was used as a control. Stimulation consisted of 1–4 pulses (at 100 Hz) presented every 5–15 s for 2–4 days using 80–150 μA current intensity. Fig. 4B shows an example of the cellular response elicited by this stimulation pattern. After chronic stimulation in the incubator, whole cell recordings were performed in neurons located approximately mid-way between

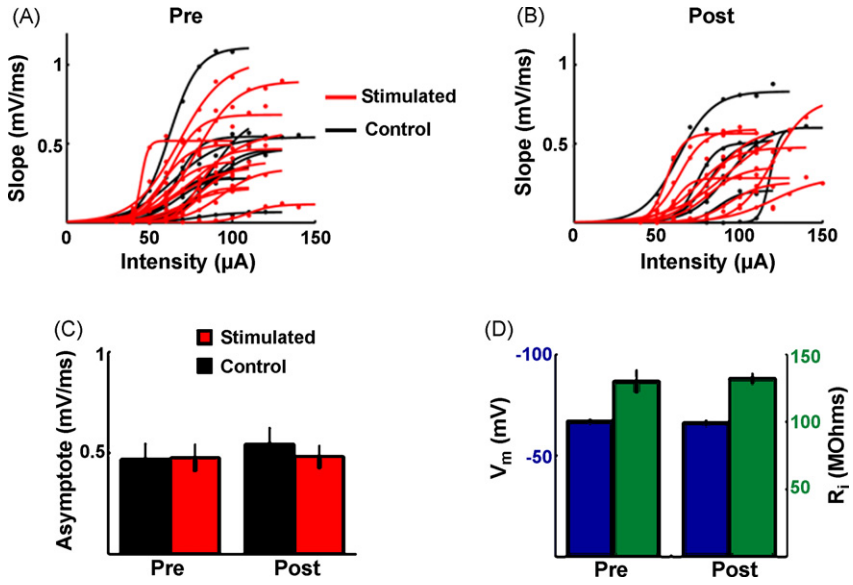

Fig. 5. Repeated recordings before and after chronic stimulation show similar responses. (A) I/O functions from 1 to 3 neurons in 4 slices in response to the control electrode (black) and the electrode to be used for chronic stimulation (red). (B) I/O functions from 1 to 2 neurons in the same 4 slices in response to the control and stimulated electrode after 2–4 days of chronic stimulation consisting of a burst of 4 pulses (at 100 Hz) at 80 μA given every 10 s. (C) No significant difference in the mean maximal EPSP responses to the control and stimulated electrodes, as measured by the asymptote of the I/O function, was observed either before (mean = 0.47 ± 0.08 and 0.48 ± 0.07 for unstimulated and stimulated electrodes, respectively; $p = 0.94$) or after (mean = 0.55 ± 0.08 and 0.48 ± 0.06 for unstimulated and stimulated electrodes, respectively; $p = 0.53$) chronic stimulation. Additionally, there was no significant difference between the pre- and post-stimulation responses for the electrode used for the chronic stimulation ($p = 0.96$). (D) Resting membrane potentials (in blue) and input resistances (in green) of neurons recorded pre- and post-stimulation were not significantly different (mean resting membrane potential = −67 ± 1 mV and −66 ± 1.4 mV for pre- and post-stimulation recordings, respectively; $p = 0.63$; mean input resistance = 130 ± 9 MΩ and 133 ± 4 MΩ for pre- and post-stimulation recordings, respectively; $p = 0.82$; Fig. 5D) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

both electrodes (1–3 neurons per slice). Single pulses from both the unstimulated and stimulated electrodes were effective in eliciting postsynaptic responses at a range of stimulation intensities (Fig. 4A). As it has been shown previously with traditional stimulating electrodes, polysynaptic activity and spiking were evoked at some current intensities (Buonomano, 2003), indicating that there was sufficient excitation to evoke a monosynaptic response as well as network activation. It should also be noted that previous work has shown these complex responses to be the result of combined excitatory and inhibitory conductances (Johnson and Buonomano, 2007a). To quantify the effectiveness of the stimulated and control electrodes, we plotted an input–output (I/O) function from each electrode and fit the points with a sigmoid (Marder and Buonomano, 2003). An example of the similarity of the I/O functions from stimulated and unstimulated electrodes for one neuron is shown in Fig. 4C. Using the asymptote of the I/O function as a measure to compare maximal synaptic strength between the two electrodes, we found no differences between the stimulated and control electrodes (mean = 0.48 ± 0.05 and 0.46 ± 0.05 mV/ms for stimulated and control electrodes, respectively; $t_{48} = 0.30$, $p = 0.77$; Fig. 4D); thus demonstrating that the effectiveness of the electrode was not altered by chronic stimulation.

In addition to quantifying the asymptote of the I/O function we compared the initial slopes of the EPSP response at the intensity used for chronic stimulation. Interestingly, we observed no difference between the response to the stimulated and unstimulated electrodes (mean = 0.23 ± 0.03 and 0.22 ± 0.04 mV/ms for stimulated and control electrodes, respectively; $t_{47} = 0.30$, $p = 0.76$; Fig. 4E) (see Section 4).

3.3. Repeated recordings before and after chronic stimulation

To further determine if chronic stimulation produced any long-term changes or damage to the tissue we took advantage of the capability of repeated recordings from the same slice (Dong and Buonomano, 2005) to further demonstrate that the electrode's effectiveness does not change after chronic stimulation. Before stimulation, I/O functions were plotted from 2 to 3 neurons per slice (3 slices). As expected, since we are recording from different cells, there was significant individual variation in the I/O functions between neurons and electrodes (Fig. 5A); however, the average responses are not significantly different as measured by the mean asymptote values (mean = 0.47 ± 0.08 and 0.48 ± 0.07 for unstimulated and stimulated electrodes, respectively; $t_{23} = 0.08$, $p = 0.94$; Fig. 5C). After 2–4 days of stimulation (same pattern as above), input–output functions were repeated (using different neurons), and there was no significant difference between the unstimulated and stimulated electrodes (mean = 0.55 ± 0.08 and 0.48 ± 0.06 for unstimulated and stimulated electrodes, respectively; $t_{14} = 0.64$, $p = 0.53$; Fig. 5B). Additionally, the mean asymptote values from the stimulated electrodes were not significantly different from the mean values before stimulation ($t_{22} = 0.05$, $p = 0.96$; Fig. 5C). Comparison of mean resting membrane potential and input resistance of neurons recorded pre- and post-stimulation revealed no significant difference (mean resting membrane potential = −67 ± 1 mV and −66 ± 1.4 mV for pre- and post-stimulation recordings respectively; $t_{26} = 0.49$, $p = 0.63$; mean input resistance = 130 ± 9 MΩ and 133 ± 4 MΩ for pre- and post-stimulation recordings respectively; $t_{26} = 0.23$, $p = 0.82$; Fig. 5D), and also demonstrates that these values are similar to what has previously been observed (Johnson and Buonomano, 2007a). Together with the above results, these experiments demonstrate that our stimulation method provides a safe and effective technique for chronic stimulation.

4. Discussion

Cortical computations are an emergent property of local cortical networks composed of tens of thousands of recurrently

connected neurons. While great strides have been made in understanding the synaptic and cellular properties of the component elements of neural networks, there is a significant explanatory gap in how computations emerge from these elements. For example, while it is known that exposure to patterned stimuli plays an instructive role in normal cortical plasticity and organization, how patterned stimulation ultimately modulates synaptic, cellular and network plasticity to produce functional computations is not understood.

An experimental preparation system to bridge traditional in vitro and in vivo studies would be instrumental in understanding how activity patterns shape cortical responses. The ideal preparation would need to have long-term viability, and demonstrate similar network dynamics and plasticity to that observed in vivo. Also important is that the method for delivering patterned stimuli must assure that the same pathways which 'experienced' the patterned stimulation can later be tested for plasticity or altered behavior.

Although both dissociated and organotypic cultures fit the criteria of having long-term viability, organotypic cultures show a number of potential advantages over dissociated cultures. First, organotypic slices maintain much of the laminar organization and synaptic connectivity characteristic of the intact cortex (Caeser et al., 1989; Annis et al., 1993; Debanne et al., 1995), which may be important in the flow of information through the cortical circuitry and ultimately affect the expression of plasticity. In contrast, in dissociated cultures in addition to the lack of a laminar organization, a number of neural properties appear to be altered compared to the in vivo state. For example, in dissociated cultures autapses can be observed. Additionally, a single presynaptic input can be capable of eliciting an action potential in a postsynaptic neuron (Bi and Poo, 1998), whereas the EPSP/C amplitudes observed between pyramidal neurons is under 2 mV/100pA, both in acute (Markram et al., 1997) and organotypic slices (Debanne et al., 1995; Pavlidis and Madison, 1999). Second, many of the same types of plasticity observed in vivo and in acute slices have been demonstrated in organotypic slices. For example, a number of studies have reported the existence of LTP and LTD (including STDP) (Debanne et al., 1994, 1998; Hayashi et al., 2000; Pavlidis et al., 2000; Montgomery et al., 2001; Daoudal et al., 2002; Leutgeb et al., 2003) and homeostatic plasticity (Aptowicz et al., 2004; Karmarkar and Buonomano, 2006) in organotypic slices; thus suggesting that normal forms of associative synaptic plasticity are functional in organotypic slices.

A previous study using the technique described here has shown that chronic stimulation produces a decrease in the average level of spontaneous dynamics (Johnson and Buonomano, 2007a)—a finding consistent with homeostatic plasticity studies suggesting that networks can up or down regulate their activity in response to manipulations that alter global levels of activity (Turrigiano and Nelson, 2004; Karmarkar and Buonomano, 2006). However, in the current study it is of interest that the comparison of the I/O functions and EPSP slopes between the stimulated and unstimulated electrodes did not reveal any significant difference (Fig. 4). Some models of cortical plasticity would have predicted that the stimulated pathway would become dominant over the unstimulated pathway (Bienenstock et al., 1982; Buonomano and Merzenich, 1998). It is possible that the absence of plasticity reflects the use of a fairly simple nonassociative stimulation protocol, or alternatively, that we are evoking more complex changes that are not immediately obvious by the measures used here, for example changes in the timing or robustness of polysynaptic activity. Indeed, ongoing experiments reveal that the use of associative patterns produces differential plasticity of the network activity in response to the stimulated pathways (Johnson and Buonomano, 2007b).

4.1. Other chronic stimulation methods

The method described here provides a simple and effective manner to chronically stimulate organotypic slices. It should be noted that although MEAs have been primarily used with dissociated cultures, they can also be used with organotypic slices (Shimono et al., 2002; Pfeffer et al., 2004), and thus provide an alternative method for chronic stimulation. However, this approach is limited to the use of the roller-tube technique, as opposed to the interface culturing technique used here, and the former produces slices that are only two to three cell layers thick and results in more dramatic changes in neural network architecture.

A number of additional techniques may also prove effective in providing long-term chronic stimulation, including the use of optical approaches such as photosensitive ion channels (Boyden et al., 2005). However, one potential advantage of the method described here, in addition to its relative simplicity and the fact that genetic manipulations are not required, is the relative ease in which the same afferents are stimulated in the incubator and on the electrophysiology rig. Specifically, using chronically implanted electrodes that remain attached to the slice culture facilitates the detection of the effects of the network's 'experience'.

Here we have described a method using implanted electrodes to provide experience to cortical organotypic cultures. We have shown that this method can be used to easily and reliably provide patterned activity for hours or days while the culture remains in the incubator. We believe this method will prove valuable in studying experience-dependent plasticity and other long-term forms of learning.

Acknowledgements

We thank Marianne Ciluffo and the Microscopic Techniques Laboratory of the Brain Research Institute at UCLA for technical knowledge and performing the staining procedures, and Balaji Jayaprakesh and Alcino Silva for technical assistance and use of the confocal microscope. This research was supported by the NIH (MH60163).

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