A technique for repeated recordings in cortical organotypic slices

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Abstract

Electrophysiology studies in vitro have generally focused on forms of plasticity which are rapidly induced and last for minutes to hours. However, it is well known that plasticity at some cellular and synaptic loci are induced and expressed over many hours or days. One limitation in examining these forms of plasticity is the lack of preparations that allow stimulation and recording of the same tissue over a 24 h period or more. Here we describe a simple method for repeated recordings and stimulating the same organotypic slices (different neurons) over a 24 h window. We use the conventional interface organotypic culture method together with a custom chamber, which allows recordings on the intact filter, and DiI to mark the stimulation sites. We show that the health of the neurons, as defined by intrinsic excitability, excitatory and inhibitory input–output curves, and morphology remains unchanged over the 24 h period. This simple technique provides a means to investigate long-term forms of plasticity that may be induced under conditions similar to those observed in vivo. Additionally, it provides the opportunity to perform long-term morphological and pharmacological studies.

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1. Introduction

A fundamental question in neuroscience is what are the neural bases of learning and memory. Much of the research aimed at answering this question has focused on plasticity at the level of the synapses. Indeed, it is generally hypothesized that changes in synaptic strength ultimately underlie learning and memory (Bliss and Collingridge, 1993; Martin et al., 2000). The most studied types of synaptic plasticity have been associative LTP/LTD (Brown et al., 1990; Bear and Abraham, 1996; Buonomano, 1999), and their variants such as spike-timing dependent plasticity (Abbott and Nelson, 2000; Bi and Poo, 2001). While LTP/LTD can be induced in minutes, other forms of plasticity are induced over much longer time scales. For example, homeostatic plasticity is induced over hours and days (Turrigiano et al., 1998; Turrigiano and Nelson, 2004). Additionally metaplasticity (Abraham et al., 2001), and changes in circuit connectivity (e.g., Antonini and Stryker, 1993), are also generally assumed to operate on the time scales of hours and days. However, the study of these and other forms of long-lasting plasticity is in part limited by the lack of preparations that allow recording and stimulation from the same tissue over long periods of time.

Acute slice studies have led to invaluable insights as to the molecular and cellular basis of LTP and LTD. However, acute slices are limited in their ability to study processes that are induced (or expressed) over many hours. The first requirement in studying slower processes is simply long-term survival. Two general types of preparations are currently used that allow neurons to be maintained in vitro for periods of days to weeks: dissociated cell cultures (Banker and Goslin, 1998) and organotypic or tissue cultures (Gahwiler et al., 1997). Each of these techniques has potential advantages and disadvantages (see Section 4). Here we describe an...
additional and simple approach to allow multiple conventional intracellular recordings in the same organotypic slice over 24 h.

2. Methods

2.1. Cortical organotypic slice preparation

Cortical organotypic slices were prepared from seven-day-old Sprague-Dawley rats using the interface method (Muller et al., 1993; Musleh et al., 1997). Briefly, rats were anesthetized with a short exposure to halothane, decapitated and the brain removed. The brain was then placed into ice-cold cutting medium. The cutting medium was composed of (in mM) 3 MgSO4, 10 glucose, 25 HEPES, 10 Trisbase. Hemispheres were then separated with a mid sagittal cut. The sagittal surface of the cortex was placed on a piece of agar and the frontal tip of the cortex was cut. The agar was then glued to a chilled metal base with the sagittal surface of the brain on the base. Coronal slices at thickness of 400 μm were cut from posterior pole. Slices from primary auditory and somatosensory cortex were collected (approximately 1.2–4.8 mm from the occipital tip) and placed on Millicell filters (PICMORG 50; height = 5mm; i.d. = 27 mm) inserts in a six-well cluster dish with 1 ml of culture media. Culture was composed of EMEM plus in mM: 100 K-gluconate, 20 KCl, 26.2 NaHCO3, 1 NaH2PO4, 25 glucose, 2.5 CaCl2 at perfusion rate of 3.5 ml/min, which has been proven important for the long-term health of the slices. Whole-cell recordings were performed under IR/DIC visualization at 23–25 °C, with an Axoclamp 2B amplifier. Recording pipette resistance was 7–9 MΩ. The intracellular solution for the current recordings was composed of (in mM): 100 K-glucanate, 20 KCl, 4 ATP-Mg, 10 phospho-creatine, 0.03 Tris-GTP, 10 HEPES (adjusted pH to 7.3 and osmolarity to 300 mOsm). Series resistance (7–25 MΩ) was compensated manually. Bipolar stimulating electrodes (Frederick haer Co., Bowdoinham, ME) were placed in the layer II/III of sensory cortex, approximately 200–400 μm from the cortical surface. The recording pipette was also placed in layer II/III approximately 500 μm away from stimulating electrodes. Single pulse stimulation was applied with ISO-Flex isolators (AMPI), triggered by a Master-8 stimulator. Each stimulus pulse was 100 μs in duration and from 20 to 130 μA in intensity. Stimuli were delivered every 10 s. EPSPs were recorded twice at each stimulus intensity at resting membrane potential. IPSPs were recorded at a depolarized potential of ~50 mV, in the presence of CNQX (10 μM) and APV (50 μM).

2.3. Techniques for repeated recordings

The recording chamber was made from SylGuard (Dow Corning) and designed to house the filters on the IR-DIC microscope stage. This allowed perfusion and electrophysiological recordings to take place from cells on the intact filter. After the experiment on the first day the orientation of the filter in the chamber was marked, and the filter was removed and placed in a new dish containing 0.5 ml of new culture media and 0.5 ml of the culture media from which it was removed. The dish was placed in a separated incubator (also set 35 °C and humidified with 5% CO2 and 95% O2) for 24 h. The second incubator only housed slices that had been placed on the rig and exposed to nonsterile conditions in order to avoid the potential infections of the slices in the ‘home’ incubator. The stimulation electrode was painted with a tiny amount of the finely ground DiI crystals (Molecular Probes). Specifically, a fine paintbrush was used to pick up DiI crystals and then was used to stroke the tip of the stimulating electrode. A digital picture was taken after we placed the stimulating electrode on the slice (Fig. 1A). After recording, we removed the electrode and took a picture again. As shown in Fig. 1B, the DiI crystals were left on the tissue at the site of the electrode placement. Twenty-four hours later, the same filter was removed from the holding incubator and placed
inside the recording chamber in the same position as marked one day before. As shown in Fig. 1C, DiI successfully marked on the site of electrode placement over the 24 h period. The next day the filter was replaced in the chamber in the original orientation. Then the stimulating electrode was repositioned in the slice based on the DiI markings and picture (Fig. 1D).

2.4. Biocytin histochemistry

In order to verify the general morphology of neurons both at time 0 and 24 h, biocytin (0.5%) was included in the pipette solution during some of the whole-cell recordings. Following the experiment, the slices, still on the filter were fixed overnight in 0.1 M PBS (pH 7.4) with 4% paraformaldehyde at 4 °C and then transferred to 30% sucrose and kept at 4 °C. During the staining, the slices were rinsed in 0.1 M PBS three times and incubated with 0.4% Triton-X 100 in PBS for 20 min. Slices were then incubated in 0.1 M PBS with 1:200 avidin-Oregon Green 488 conjugate at 4 °C in the dark overnight. The next day, the slices were rinsed with 0.1 M PBS three times, mounted on a glass slide with mounting medium (Vector laboratory). The labeled neurons were observed using a fluorescence microscope (Nikon Microphot-FXA) with FITC filter and photographed at 200× magnification. Individual neurons were traced by using PhotoShop 6.0 software.

2.5. Data analysis

Electrophysiology data was acquired using DataWave software at a sampling rate of 10 kHz. The acquired data was analyzed using custom written Matlab code. EPSP slopes were defined as the maximal slope calculated using a 3 ms sliding window. The I–O data was fitted with a sigmoid function (Marder and Buonomano, 2003). The sigmoid was characterized by three parameters: asymptote, gain (related to the slope of the linear portion of the sigmoid), and threshold (intensity at which the response corresponds to 50% of the asymptote). Neurons with input–output data that were not accurately fit with a sigmoid were discarded in the final analysis. Slopes of the IPSPs were calculated in the same manner as the EPSPs except the minimum slope was used. All the statistical data are expressed as mean ± S.E.M. The statistical difference between groups was estimated using the Student’s t-test.

3. Results

The general protocol was to transfer the slices from the incubator to the chamber where the slices are perfused with ACSF for the first recording session, which lasted approximately 1 h. The slices were then transferred back to the culture dish, and returned to the incubator. Eighteen to 14 h later, the slices were brought back to the recording chamber for the second recording session. Our first goal was to establish that the tissue was not infected as the result of the first recording session. Although infections were occasionally observed at 24 h, a careful cleaning of the recording chamber between sessions and a clean holding incubator generally eliminated the occurrence of infections. Our next concern was determining whether there were any significant changes in the general physiology of the slices. Below we describe the results of the intrinsic excitability, synaptic and morphological studies of neurons at time 0 and 18–24 h later.

Shown in Fig. 2A and B is an example of a neuron that was recorded in layer II/III at time 0, and of a different neuron at the 24 h time point. A total of 18 neurons were labeled, 9/10 neurons acquired from time zero displayed typical pyramidal neuron morphology. Shown in Fig. 2C, neurons exhibited pyramidal-like soma with basal dendrite tree and a long apical dendrite. All eight of the neurons labeled at the 24 h time point were pyramidal neurons (Fig. 2D). Thus based on morphological and electrophysiological properties we are confident that the data presented below are from pyramidal neurons. Additionally, at the qualitative level the morphological data suggested that neurons from both groups exhibited similar pyramidal neuron morphology.

3.1. Intrinsic neuronal properties at 0 and 24 h

The input resistance, resting membrane potential and excitability of a sample of neurons were characterized at zero and 24 h. The mean resting potential at time zero was −72.7 ± 1.7 mV (n = 14). The mean resting membrane potential of neurons that were recorded on the second session at 24 h was −71.8 ± 1.3 mV (n = 12). There was no significant difference of the resting membrane potential between the two
sessions ($t_{25} = 0.81, p = 0.42$). Fig. 3A–B shows the excitability of two different cells from the 0 and 24 h recording sessions. Excitability was examined with 500 ms current pulses of $-0.1, 0.1, 0.2$ and $0.3 \text{ nA}$. Both neurons exhibited regular spiking patterns with accommodation. The mean input resistance is plotted in Fig. 3C. The input resistance of the neurons was similar between the two recording sessions (zero: $215.7 \pm 18.9 \text{ M} \Omega$, $n = 14$; 24 h: $219.4 \pm 22.8 \text{ M} \Omega$, $n = 12$; $t_{25} = 0.18; p = 0.85$). Fig. 3D shows the input–output curves from neurons recorded at both sessions. There was no significant difference between the numbers of spikes elicited at each intensity for both groups of cells ($F_{1,94} = 0.001, p = 0.97$). These results indicate that basic synaptic excitation was unaltered over the 24 h period.

3.2. Synaptic input–output functions at 0 and 24 h

To address whether the synaptic properties were altered by the repeated recording sessions, we characterized the synaptic input–output curves of neurons during the first ($t = 0$) and second recording sessions ($t = 18–24$ h). Input–output curves were obtained with extracellular stimulation, using 6–10 different stimulus intensities. The slope of the monosynaptic EPSP was used as the output measure (see Section 2). Fig. 4A and B shows the raw data from two neurons recorded at the 0 and 24 h session. Fig. 4C shows the data in panels A and B in the form of input–output curves. The mean asymptote and ‘gain’ of the sigmoid used to fit the input–output function are shown in Fig. 4D. There was no significant change in these measurements between the two groups (asymptote $t_{25} = -0.17, p = 0.86$; gain $t_{25} = 0.16, p = 0.88$). These results indicate that basic synaptic excitation was unaltered over the 24 h period.

3.3. Evoked inhibition at 0 and 24 h

To investigate whether synaptic inhibition was altered by the repeated recordings over the 24 h period, we recorded monosynaptic IPSPs in the presence of CNQX (10 μM) and APV (50 μM) at 0 and 24 h. Since it has been reported that application of CNQX and APV can itself induce homeostatic synaptic plasticity (Turrigiano et al., 1998; Kilman et al., 2002), we conducted IPSP measures at 0 and 24 h in different slices (note that we still recorded from the 24 h slices at 0 h, but not in the presence of CNQX/APV). All the IPSPs were recorded at a membrane potential of $-50 \text{ mV}$. As shown in Fig. 5A, the monosynaptic IPSPs were similar in the 0 and 24 h groups at each stimulus intensity. There was no significant difference between IPSP amplitude at any intensity between the groups (Fig. 5B, $F(1, 63) = 0.16, p = 0.68$, ANOVA). The IPSPs could be completely abolished by bath
Fig. 4. Synaptic input–output curves 24 h apart. Parts (A) and (B) show the raw data for the input–output curve recorded at zero and 24 h, respectively. The upper traces are five samples at different stimulating intensities. Blue, green, cyan, black and red color represents the intensities at 30, 40, 50, 60, and 70 µA, respectively. The straight vertical line in the beginning of the trace indicates the time of stimulation (time zero). The voltage raster provides the complete data. Each sweep represents the response to a stimulus. Two sweeps were obtained at each intensity. (C) The input–output curves derived for panel A, B and fitted with a sigmoid. (D1 and D2) The mean of asymptote and the ‘gain’ (slope of the sigmoid fitting curve) calculated at zero and 24 h. (For interpretation of the references to colour in this figure legend, please refer to the web version of the article)

Fig. 5. Synaptic inhibition 24 h apart. Part (A) shows the raw data of monosynaptic IPSPs recorded at membrane potential of −50 mV together with bath application of CNQX (10 µM) and APV (50 µM) at zero and 24 h. Traces represent four samples at 30, 50, 70, and 90 µA stimulus intensities. The straight vertical lines on the traces indicate the time of stimulation. Part (B) shows the average value of the slopes that were calculated at each intensity from three preparations (7–10 neurons).

application of 10 µM bicuculline (10 µM, data not shown). The results indicated that the monosynaptic inhibition was unaltered during the repeated recordings 24 h apart.

4. Discussion

In present study we described a method that enables multiple recording sessions from organotypic slices. We used a number of electrophysiological measures to examine any potential changes in slice health produced by multiple sessions. There was no significant difference between the mean input resistance (Fig. 3C) and excitability (Fig. 3D) from the first to second session. Additionally the input–output curve of the monosynaptic EPSPs and IPSPs did not change over the 24 h period. As is characteristic of organotypic slices synaptic stimulation also produced polysynaptic responses (Buonomano, 2003). The polysynaptic responses also appeared similar over the 24 h period, but a quantitative comparison is difficult due to the inherent variability of the polysynaptic EPSPs. Although there are some normal developmental changes occurring in the circuitry over the first 14–21 DIV (De Simoni et al., 2003), we did not observe any significant changes of the circuitry over a 24 h period.

Many neurophysiological studies would benefit from multiple recordings. These include plasticity studies that are induced over many hours, and pharmacology studies, such as protein-synthesis inhibitor experiments. Two general types
By incorporating a number of improvements, one approach has been to perform ‘chronic’ recordings in organotypic slices, as well as site-specific stimulation. These techniques, which are still being perfected, offer a powerful means to study network dynamics. While organotypic and dissociated cultures address the problem of maintaining neural tissue alive for days and weeks, they do not address the issue of multiple recording sessions. One approach to this problem has been to perform ‘chronic’ recordings in dissociated cell cultures, activation of a single excitatory neuron can often trigger an action potential in a postsynaptic neuron via a single suprathreshold EPSP (e.g., Bi and Poo, 2001). A phenomenon assumed not to occur normally in cortical circuits, since the average EPSP amplitude in pyramidal neurons is under 1 mV (Mason et al., 2003; Markram et al., 1997; Reyes and Sakmann, 1999). Additionally, organotypic slices exhibit a number of other potential advantages. Firstly, the general cytoarchitecture, laminar relationships, synaptic strengths, and excitatory-inhibitory balance are similar to that of acute slices (Gahwiler et al., 1997; Dantzker and Callaway, 1998; Debanne et al., 1999; Klostermann and Wahl, 1999; Yamada et al., 2000). Secondly, polysynaptic responses are well preserved in organotypic slice, providing the opportunity for network dynamics studies (Buonomano, 2003). Thirdly, the development of organotypic slices seems to parallel the stages of development in vivo (De Simoni et al., 2003). Finally, a number of studies have reported equivalent forms of plasticity in organotypic cultures (Debanne et al., 1994; Pavlidis et al., 2000; Hayashi et al., 2000; Daoudal et al., 2002; Montgomery et al., 2001; Leutgeb et al., 2003). These studies suggest that normal forms of synaptic plasticity are operating in organotypic slices.

While organotypic and dissociated cultures address the problem of maintaining neural tissue alive for days and weeks, they do not address the issue of multiple recording sessions. One approach to this problem has been to perform ‘chronic’ extracellular recordings in dissociated cell cultures (Jimbo et al., 1999; Potter and DeMarce, 2001) and organotypic slices (Shimono et al., 2002). These techniques use substrate-integrated multi-electrode arrays, in which tissue is cultured on a substrate with embedded electrodes. These fixed electrodes provide a means to perform multi- or single-unit extracellular recordings in dissociated cultures, or field recordings in organotypic slices, as well as site-specific stimulation. These techniques, which are still being perfected, offer a powerful means to study network dynamics. However, there have been few studies, which have recorded repetitively or continuously from neurons over a 24 h period. Substrate-integrated recordings also do not offer the ability to perform intracellular recordings, which will be necessary to analyze changes in subthreshold plasticity and inhibition. A second approach recently described (Leutgeb et al., 2003), is to use an ‘adult-culture’ technique. This technique relies on acute hippocampus slices prepared from animals age of p25 to p30. By incorporating a number of improvements to the culturing and recording techniques the author were able to induce field LTP and record for approximately 6–8 h. However, this technique does not involve returning slices to the incubator and is likely to be limited to ≪12 h of recording.

5. Conclusions

The technique described here offers a novel approach to study neural processes over the course of 24 h. Since slices are replaced in the incubator, it is likely this technique will allow more than two recording sessions, and inter-session intervals of a few days. Currently we are examining in vitro experience-dependent plasticity protocols, in which differential pathways are stimulated in phase or out of phase, to determine if we can observe the emergence of pathway selectivity in vitro. This type of plasticity, analogous to cortical plasticity and reorganization (Buonomano and Merzenich, 1998) is likely to occur over a period of days, and will require preparations similar to that described here.

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References


