5
Electrophysiological Monitoring of Hippocampal Slice Cultures Using MEA on Porous Membrane

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Introduction

The development of brain slice preparations and particularly slice culture models that allow maintaining brain networks alive under in vitro conditions for several days, weeks, and even months have brought important new possibilities to study physiological and pharmacological mechanisms in the central nervous system (CNS) (Gahwiler, 1987; Heimrich and Frotscher, 1993; Finley et al., 2004). The simplicity of preparation and maintenance of slice cultures and their versatility in terms of physiological models makes them interesting for large-scale testing of pharmacological compounds. These preparations indeed offer the possibility to control the tissue environment, while conserving in vivo-like characteristics including organization of neuronal networks anatomy, three-dimensional aspect, developmental and functional properties of neurons, or reactivity of the tissue to injury or toxicity (Stoppini et al., 1991). In addition many slices can be obtained from the same animal thereby decreasing the number of animals needed for each experiment. Brain slices and slice cultures have thus been increasingly used over the years as models in studies of synaptic plasticity (Toni et al., 2001; Brager et al., 2002), epilepsy (Chen et al., 2004; Aptowicz et al., 2004; Silva et al., 2003; Kovacs et al., 2002), ischemia (Cater et al., 2003; Cronberg et al., 2004; Perez Velazquez et al., 2003; Barth et al., 1996), regeneration (Lee et al., 2002; Teter et al., 1999; McKinney et al., 1999; Stoppini et al., 1993), demyelination (Roth et al., 1995; Notterpek et al., 1993; Ghoumari et al., 2003; Demerens et al., 1996), toxicity (Shimono et al., 2002; Khaspekov et al., 2004; Xu et al., 2003), apoptosis (Lee et al., 2003; Keynes et al., 2004; Vis et al., 2002)) degenerative diseases (Brendza et al., 2003; Bendiske and Bahr, 2003; Duff et al., 2002; Hay et al., 2004; Murphy and Messer, 2004; Sherer et al., 2003), or even psychiatric and mental disorders (Kawasaki and Tsutsui, 2003).

Another interesting aspect of these preparations is that they are amenable to various methodological approaches. The most widely used readout for functional studies has probably been electrophysiology, through extracellular or intracellular patch clamp techniques (Aptowicz et al., 2004; Leutgeb et al., 2003; Yu et al., 2003; Alix et al., 2003; Thomas et al., 1998b), but protocols for all sorts of analyses

have been successfully applied to slices or slice cultures, including recently live confocal imaging (Kasparov et al., 2002; Dailey and Waite, 1999; Schwartz and Yu, 1995; Miller et al., 1993), transfection methodologies (McAllister 2004; Ibrahim et al., 2000; Thomas et al., 1998a; Bergold et al., 1993; Ehrengruber et al., 2001; Morrison, III et al., 2000a), or genomic analyses (Morrison, 3rd, et al., 2000b; Fan and Tenner, 2004; Morl et al., 2002).

An important advantage in this respect of slice cultures is the possibility to continuously monitor within the same piece of tissue and for several days the changes associated with development, reaction to injury, or long-term pharmacological treatments. One way to do this is through electrophysiological recordings and the use of multiple electrode arrays (van Bergen et al., 2003; Shimono et al., 2002). As these long-term analyses are extremely important for various pharmacological testing procedures such as toxicity analyses, screening for drugs promoting remyelination, regeneration, or synaptic network remodeling, we undertook to develop a long-term recording system, based on the use of multiple electrode arrays applied to interface-type organotypic slice cultures, in which analyses could be carried out in incubatorlike conditions while fully controlling the perfusion medium applied to the tissue. In previous work, we had developed a multi-recording device (Stoppini et al., 1997) for the analysis of spontaneous and evoked electrical activity in organotypic cultures. We have extended this approach and now designed a new type of array onto the surface of a porous membrane or onto a permeable support. This biological/electronic interface, developed within the context of a small company, Biocell Interface, is described here. In particular, we provide evidence for the applicability of this recording interface for analyses of nerve regeneration.

5.1 The Neurosensor Interface System

5.1.1 Description of the BioCell Neurosensor Interface System

The Neurosensor Interface System (NIS) is based on the use of a multi-electrode recording array designed to conduct long-term electrophysiological studies applied specifically to 3-D interface-type organotypic cultures. The system is illustrated in Figure 5.1; it is composed of three main interconnected parts: a console unit which contains the micro-electrode array. This console is connected to a Biocell Interface Signal Conditioner (BCI-100) for stimulation, amplification, and digitalization of recorded signals, and the whole system is hooked up to and controlled by a computer system.

The console unit is a thermoregulated electronic module that houses the removable BioCell interface system. This interface is composed of a disposable cartridge that includes the multi-electrode array maintained in a sandwich between two chambers: the upper chamber, which is the gas perfusion chamber for maintaining the tissue at the interface, and the lower one, which is the solution perfusion chamber (Figure 5.1b). Cultures are grown on a precut and porous disk of

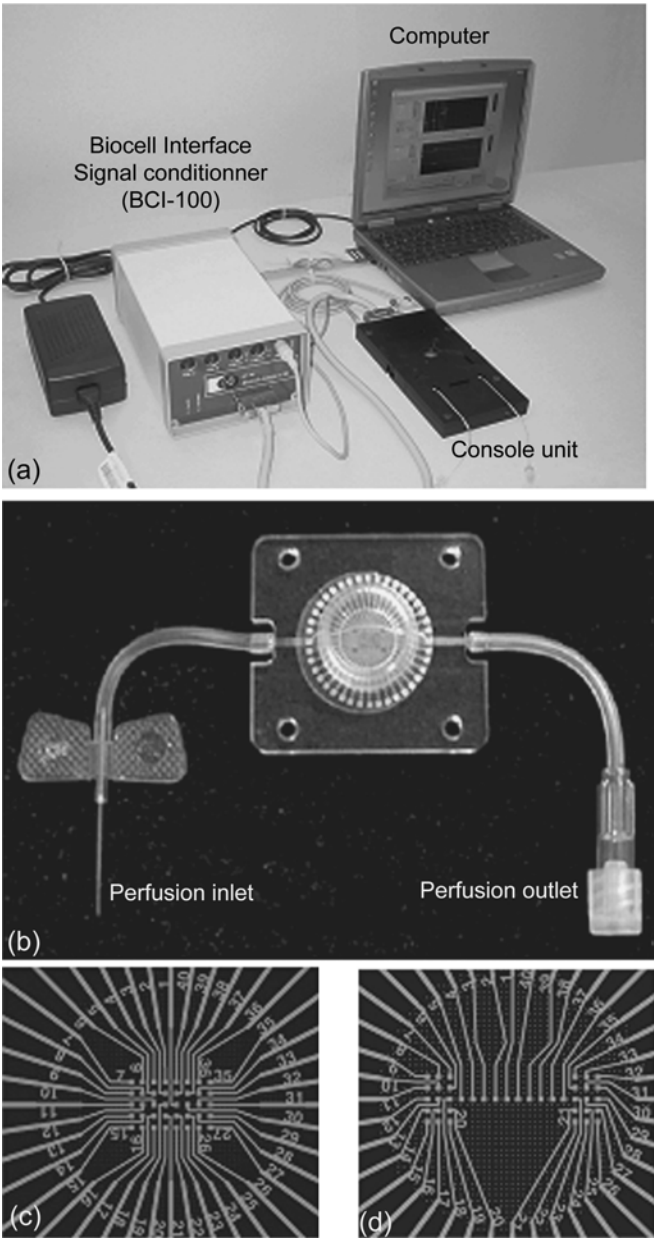

FIGURE 5.1. The Neurosensor Interface System (NIS; arrows) is composed of a console unit connected to the Biocell Interface Signal Conditioner (BCI-100; arrowhead) itself connected to a computer that monitors the experimental parameters (a). The console unit is a thermoregulated electronic module that houses the removable Biocell NIS. The Biocell NIS (b) is a disposable cartridge that contains the multi-electrode array (40 electrodes) built on a porous membrane between two chambers: the gas perfusion chamber and the perfusion chamber. The design of the electrodes can be easily modified depending upon the specific requirements of the experiment or tissue under analysis. Two different designs of multiple electrode arrays are illustrated ((c) and (d)).

membrane (Confetti, 10 mm diameter) that is deposited in the upper chamber on top of the micro-electrode array. The array is built on porous support so that the tissue remains in contact with the solution perfused underneath. The two chambers, the array, and the tissue are then assembled and inserted into the console unit, where the temperature can be precisely controlled.

The multi-electrode array is a network of 40 micro-electrodes 30 ìm thick and made of pure gold by plasma evaporation (Figures 5.1c and d); each electrode is insulated by biocompatible photoresist. This electrode network is embedded on a permeable and transparent membrane so that perfusion solution from the lower chamber can reach the tissue placed on the array. The position of the electrodes can be visualized and adjusted using a dissecting microscope or directly through the camera integrated within the connector. An inlet and outlet tubing system allows gas (to the upper chamber) and medium (to the lower chamber) to be perfused through the appropriate chambers at defined rates. Each electrode can be assigned as stimulating, recording, or earth/ground electrode. They also have variable gains. A golden metallic film layer arranged around the lower chamber makes the reference electrode. The stimulation and triggering can be performed either by the computer or by an external stimulator.

The Biocell Interface Signal Conditioner receives signals from the network of electrodes in contact with the tissue. A software utility allows the user to easily configure and set up the conditioner. Parameters that can be controlled include: the selection of acquisition channels, adjustment of the impedance of electrodes, conversion to AC or DC recording, amplification gain, adjustable offset, and analog/digital conversion. All commands for setting experimental and electrophysiological recording parameters as well as for data analysis are computerized.

The computer and software system includes a board that drives acquisition of digitized signals and stimulation. A data acquisition software package (Multiplexor) allows stimulation of up to 8 pairs of electrodes and recording from up to 8 electrodes among the 40-electrode matrix. Processing software allows online analysis of acquired data. A graphical software tool, compatible with Windows, allows users to configure and set up the conditioner. Every configuration can be saved on disk for further use. This software can be used to control the electrode parameters such as: amplitude of stimulation pulses (0 to 4092 mV), pulse duration (100 μs to 1 sec), pulse rate (1 pulse/day to 100 pulses/sec), temperature of the experiment, and perfusion parameters.

The online analysis software is composed of several panels for the visualization and analysis of the activity collected by the eight selected channels. One panel is for spontaneous activity (Figure 5.2a), a second one for evoked activity (Figure 5.2b), and the third panel is used for online quantification of response parameters such as amplitudes, latency, or slope (Figure 5.2c).

5.1.2 Properties of the BioCell-Interface System

This multi-electrode array system was designed for large-scale applications and long-term analyses such as required for drug testing applied to various models

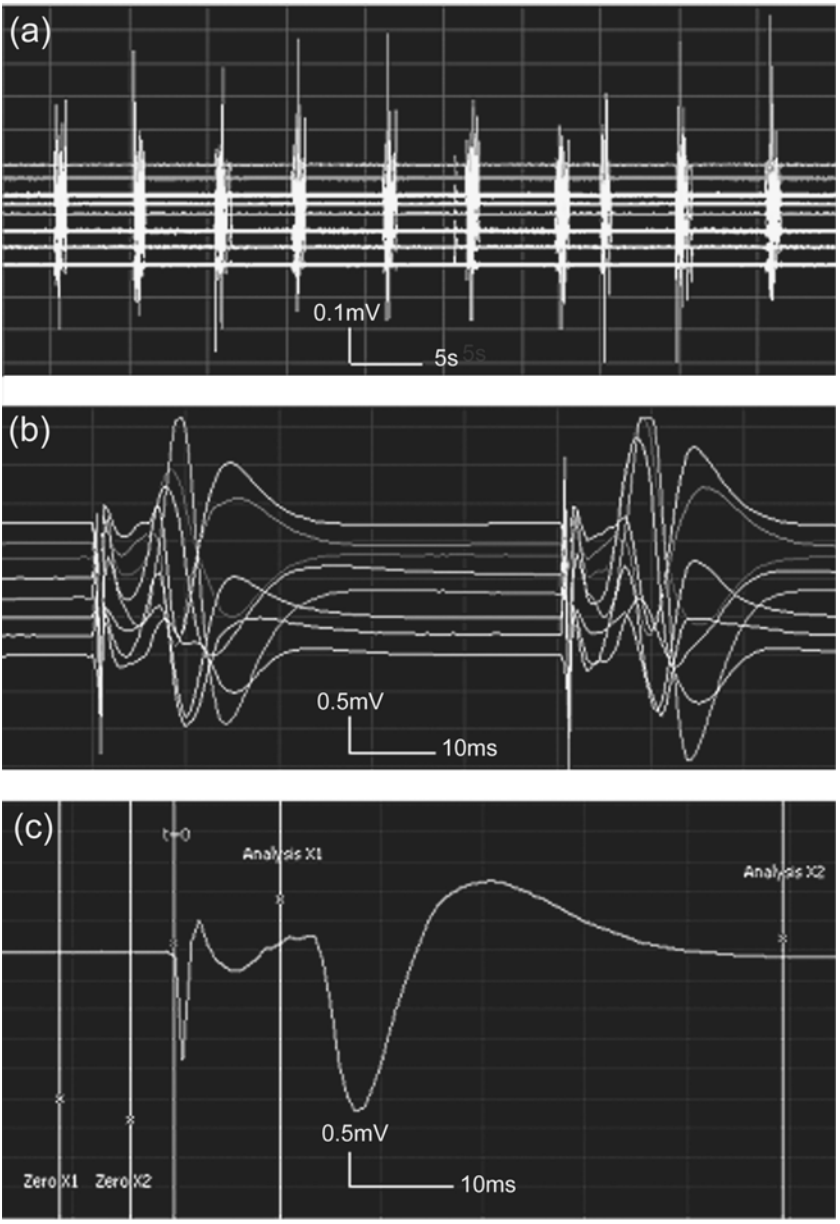

FIGURE 5.2. Software panels used for the analysis of electrical activity. The BioCell software combines different windows that allow the manipulator to visualize and analyze individually all selected channels. In the upper panel (a) are shown the eight recording channels which in this case display spontaneous epileptic activity induced by a strong electrical stimulation. The intermediate panel (b) illustrates the evoked synaptic activity recorded by eight selected channels in response to paired-pulse stimulation of CA3. The lower panel (c) is the trace analysis window for online analysis of the parameters of evoked responses.

developed in organotypic slice cultures. An objective was to have a simple system, easy to use, that could be handled. The main advantages include:

- Use of low cost, disposable cards that exclude possible artifacts coming from residual products from previous experiments and that can be prepared at the same time from the same animal to be run in parallel using multiple simultaneous systems.
- Possibilities to apply different patterns of stimulation in different locations for several days and to record activity simultaneously from several electrodes over prolonged periods of time.
- Possibility to continuously perfuse the tissue with culture medium or artificial CSF solutions, while keeping the cultures in a sterile environment, with control of the temperature and no need for a CO2 incubator.

5.2 Pharmacological Applications

5.2.1 Stability of Evoked Activity

One important objective for the design of this multi-electrode recording system was to be able to perform analyses of synaptic activity over prolonged periods of time in a simple and reproducible manner. For this the design of the recording chamber was adapted so as to maintain incubatorlike conditions at a much smaller scale. Figure 5.3 illustrates recordings obtained in the CA1 area of hippocampal organotypic slice cultures by stimulation of CA3 neurons. Over a period of six hours in this experiment, synaptic activity could be elicited without major changes in the size and characteristics of responses. In another protocol that we use regularly, synaptic activity is recorded for periods of 20 minutes a day over several consecutive days. Figure 5.3b shows the mean amplitude of the responses recorded in this way on the eight available channels for a period of seven days in one experiment. The stimulation intensity was maintained constant throughout the experiment. Considering the numerous factors susceptible to affect excitability or survival of cultures over this period of time, the stability obtained in this experiment is of particular interest and clearly indicates that the quality of the survival of the slice cultures maintained in the recording chamber has been optimized. The possibility to reach such stability constitutes therefore an important advantage for carrying precise pharmacokinetic or toxicological studies of a given molecule on prolonged periods of time.

5.2.2 Drug Testing

The main type of applications for which this system was designed was to perform pharmacological analyses of the effects of compounds on excitability and synaptic transmission when applied to models that require long-term monitoring of activity. An important condition therefore is to be able to apply drugs to the tissue through an efficient perfusion system that does not alter stability. At the same time, the amount

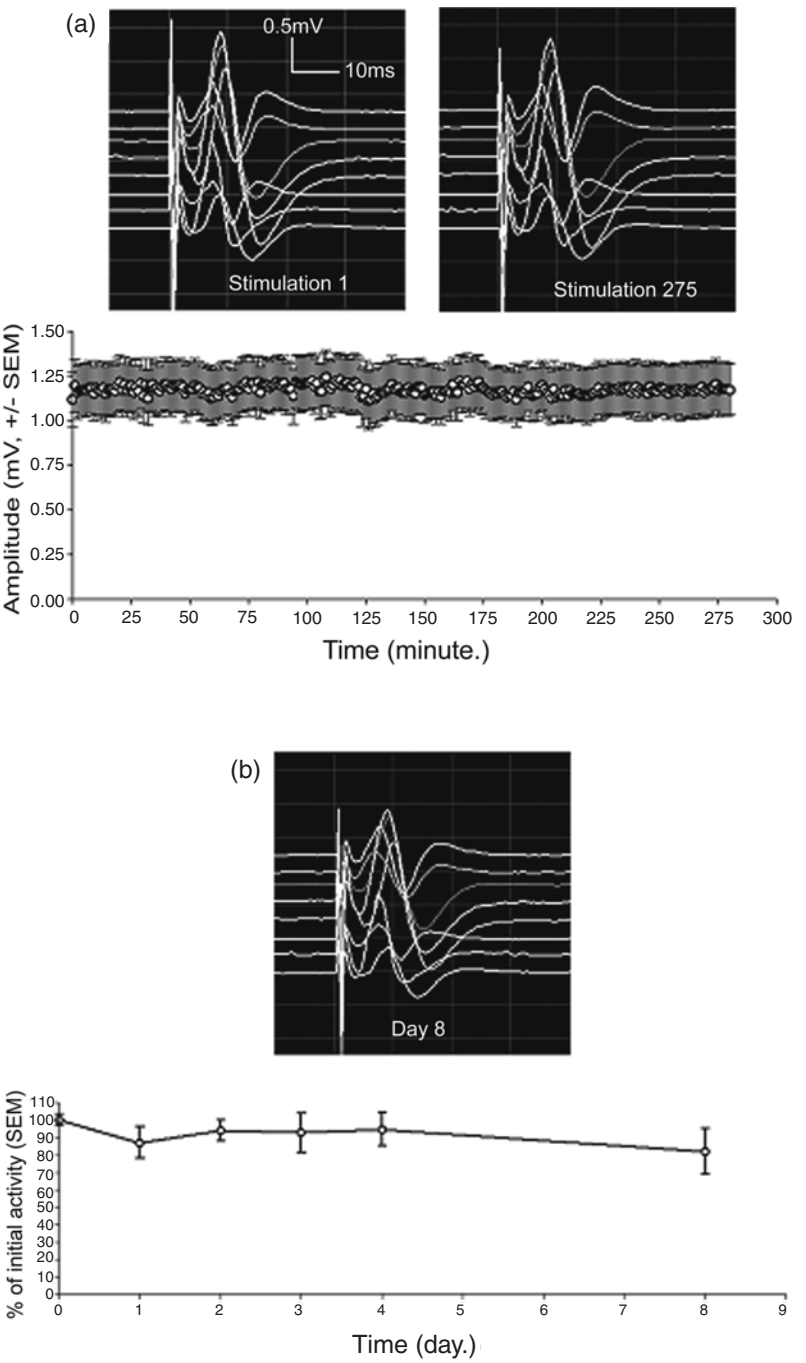

FIGURE 5.3. Stability of evoked activity. Illustration of the synaptic responses amplitude recorded in CA1 hippocampal organotypic slice cultures on eight channels over a period of several hours. The lower panel illustrates another protocol in which synaptic responses were recorded for 20 minutes each day over a period of eight days and represented as the average amplitude (±SEM) obtained during each recording daily session (b).

of medium to be perfused has to remain as small as possible as the quantities of substances to be tested are usually limited. A compromise has therefore to be found for each condition so as to be able to exchange the medium in the chamber, but without excessive perfusion volumes and while keeping the recording stability. Figure 5.4 illustrates the results of classical pharmacological experiments performed using drugs that affect the excitability or mechanism of synaptic transmission. In Figure 5.4a, two antagonists of glutamate receptors, NBQX (10 micromolar), a blocker of AMPA-type receptors, and D-AP5 (50 micromolar), an NMDA receptor blocker, were applied to the tissue at a perfusion rate of 10 ml/min. As expected these antagonists block excitatory synaptic transmission. The effect was obtained within a few minutes of drug application, which is about as fast as that obtained when testing drugs in regular submerged or interface chambers using tissue slices. Also washout of the drugs resulted in a complete recovery with a very comparable time course, indicating an efficient perfusion system that does affect stability of recording despite the fact the array is built onto a porous membrane.

Figure 5.4b is another illustration of the same type of experiments, but carried out with TTX, an antagonist or sodium channel that prevents generation of the action potential. Although the acute effect of TTX could easily be monitored in these experiments, we curiously noticed that applications for longer periods of time clearly affected the survival of the tissue. Washout of the compound after one to two days of application reproducibly resulted in partial recovery of synaptic activity. This example illustrates therefore the possible interest of this system for toxicological analyses of the effect of various compounds on brain tissue survival or functionality.

5.3 Use of the Neurosensor Interface for Studies of Nerve Regeneration

Stoppini and collaborators, using rat hippocampal slice cultures, showed that sectioning of the Schaffer collaterals (i.e., the axons of CA3 pyramidal neurons connecting the dendrites of CA1 cells) resulted in a sprouting and regrowth of lesioned axons that re-established synaptic connections between the two areas and that this capacity to regenerate was age dependent (Stoppini et al., 1997). By recording the amplitude of the activity elicited across the lesion in the days following the section, they were able to show a progressive recovery that occurred within a few days in one- to two-week-old tissue, but required weeks or was only partial in older cultures. The approach used, however, made it difficult to follow recovery within the same slice for the required period of time.

Furthermore, as this property may be of interest for the development of proregenerative drugs, we investigated the possibility of using the BioCell multi-electrode array system to monitor regeneration in this paradigm. Figure 5. illustrates the progressive changes in amplitude of the responses evoked across the lesion as a function of time. This experiment was performed by selecting four electrodes

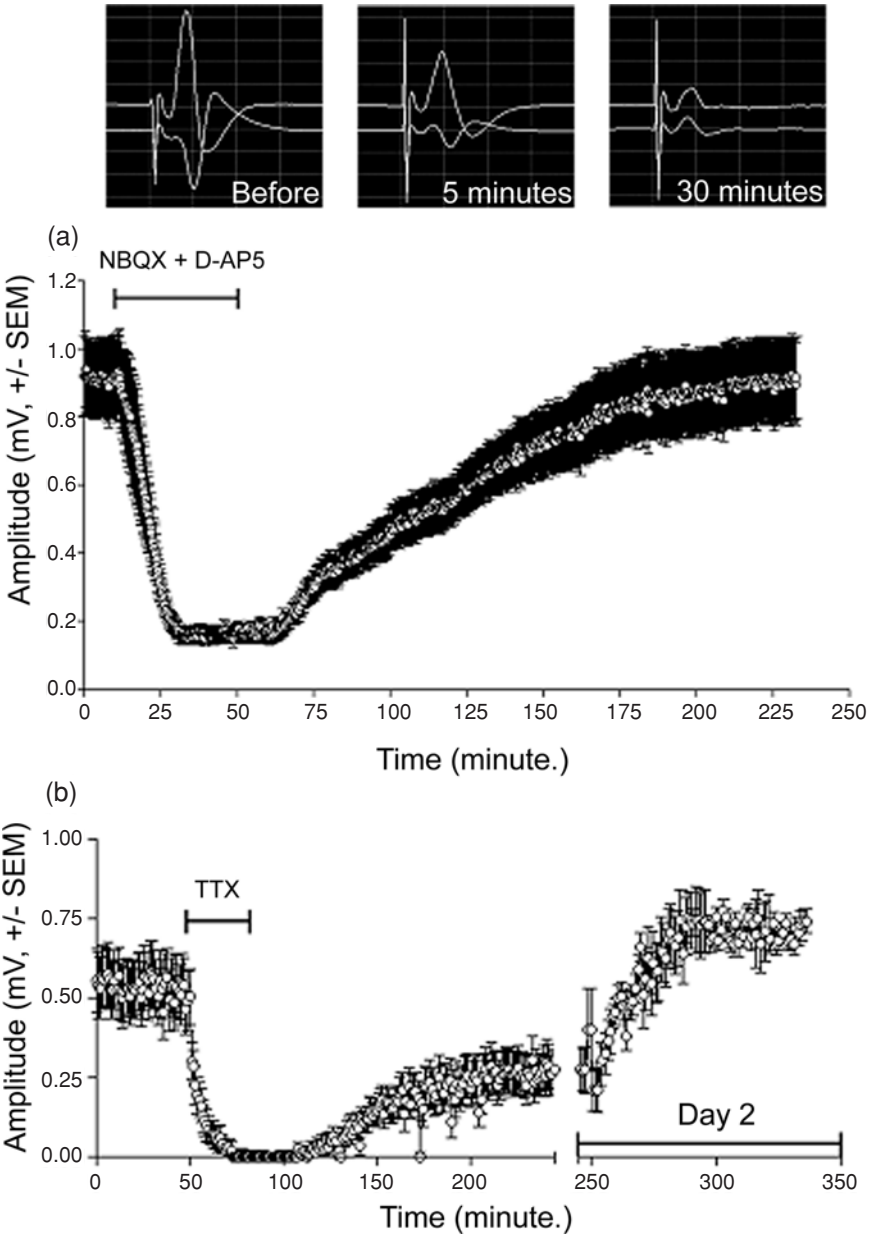

FIGURE 5.4. Pharmacological applications. (a) Illustration of an experiment in which 50 microM of D-AP5 and 10 microM of NBQX were applied to the perfusion medium at a rate of 10 ml/min. Note the time course of activity blockade and recovery illustrating the possibility to rapidly exchange the medium of the chamber, with low volumes and slow perfusion rates without affecting stability. (b) Other example illustrating the acute blockade of excitation by TTX (1 μM).

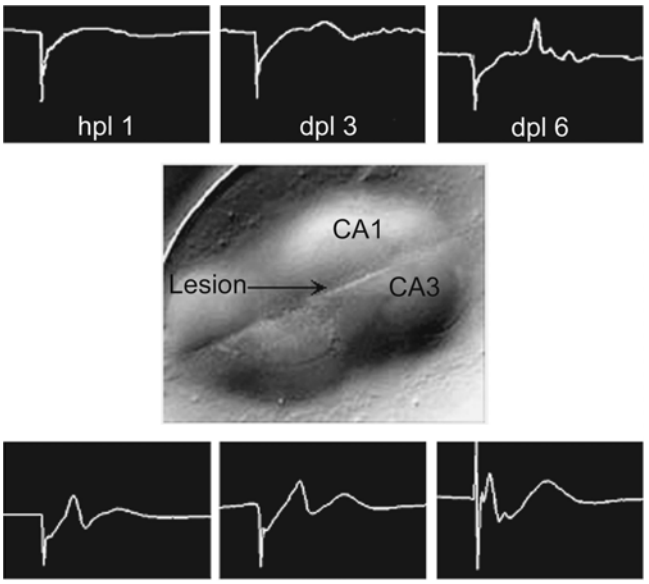

FIGURE 5.5. Paradigm used for studies of nerve regeneration using the Biocell Neurosensor Interface. Slice cultures were cut using a razor blade between CA3 and CA1 areas. Continuous monitoring of activity shows a complete elimination of evoked synaptic responses across the lesion immediately after the lesion and aa progressive recovery of the amplitude of the evoked responses within the days postlesion (center and right upper panels). In contrast, activity recorded within the CA3 region is not modified over time indicating the good viability of the tissue following the section.

located in the CA1 area for the recording of the synaptic responses evoked by the regenerating axons and four others close to the site of stimulation, but within the CA3 area, to verify the viability of the tissue.

These experiments were also carried out using mice rather than rats in order to be able to take advantage of the existence of possible transgenic animals. The slice culture was then cut with a sterile razor blade and synaptic activity across the cut monitored over several days. The results so obtained confirmed the initial observation made in rats, demonstrating the capacity of CA3 cells to sprout through the lesion and to form new synaptic contacts in the CA1 field. They also provided evidence for a similar age dependency of regeneration (Figure 5.6). Although evoked responses across the lesion recovered about 60% of their initial amplitude in young cultures after 10 days (10 to 15 days old), the recovery was only 20% in the older ones. In contrast, the electrodes localized close to the site of stimulation but within the CA3 area remained constant over the entire recovery period independently of the age of the cultures, showing that the tissue remained viable even in the older slices and that the defect in regeneration was not due to a potential damage of older cells. These experiments indicate therefore that the BioCell interface system is of potential

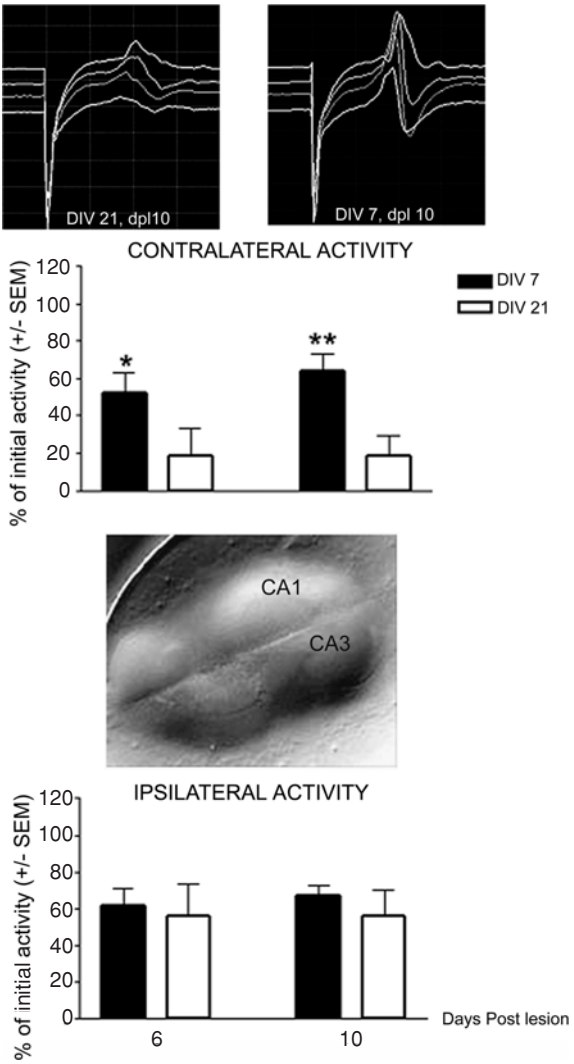

FIGURE 5.6. Age-dependent capacity to regenerate across a lesion in mouse hippocampal cultures. The graph illustrates the percentage of recovery of activity across the lesion obtained 6 days after the lesion in mouse slice cultures in which the lesion was carried out either 7 days or 21 days after explantation. Again the synaptic activity elicited in the CA3 areas does not change over time, indicating that the defects of regeneration observed in older tissue are not due to a greater susceptibility or damage of CA3 cells.

interest for the development of large-scale testing of drugs that could have application in promoting regeneration. It allows continuous and reproducible monitoring of activity even in the case where specific manipulations of the tissue are required.

5.4 Conclusions

We described in this chapter the main characteristics of a new multi-electrode array system that make it of interest for the development of drug-testing approaches. As we showed here, this system is relatively simple and easy to handle; it is characterized by an excellent stability of recordings, capacity to easily run pharmacological and toxicological experiments by exchanging the perfusion medium and monitoring effects on synaptic transmission or tissue viability for several days or weeks, and the possibility to apply this approach to even more complex physiological models such as lesion-induced regeneration or tissue re-myelination.

In comparison to other or previous multi-electrode arrays, the main advantage of the Neurosensor Interface System is probably the design based on a low-cost, disposable cartridge and membrane array that renders its use simple, reliable, and compatible for simultaneous large-scale applications. In contrast, one disadvantage might be the relatively limited number of electrodes available for simultaneous recordings of activity and the difficulty to exploit the system in such a way as to perform single-cell spike recordings or unit recordings. The objective however, when designing this array was to privilege a development compatible with large-scale screening of compounds rather than analysis of signal integration or single-cell properties within a complex network.

The system has therefore been developed to ensure versatility and applicability to numerous different biological models susceptible to be of relevance for drug development.

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