15
Applications of Multi-Electrode Array System in Drug Discovery Using Acute and Cultured Hippocampal Slices

Michel Baudry, Makoto Taketani, and Michael Krause

15.1 Introduction

There are currently only limited technologies for discovering, classifying, and testing compounds that could significantly affect cognitive performance in humans. This chapter describes ways in which multi-electrode arrays can successfully be used in this context. The proposed approach emerged from collaborations between research groups at the University of California, Irvine (UCI), the University of Southern California (USC), and Tensor Biosciences. The academic groups have, for a number of years, been developing protocols and analytical software for activating and analyzing electrophysiological responses generated by complex networks in mammalian CNS. This approach represents an effort to record the nearly second-long events that are possibly the substrate of simple cognitive actions; it can also be seen as an attempt to create an experimental platform for practical applications of neural network research. Tensor Biosciences is a startup company that was founded by researchers at UCI and USC and is pursuing a multi-year contract with Matsushita Electric Industrial Co., Ltd. (Panasonic) to develop software for Panasonic's multi-electrode array system (MED64 System) and also build drug discovery platforms by using the MED64 System. Panasonic and its subsidiary, Alpha MED Sciences, have been gradually evolving turnkey hardware and software for stimulating and recording from 64 electrodes placed beneath a brain slice.

Biologically or chemically induced changes in network behavior are ultimately a reflection of effects on synaptic and extrasynaptic activity in brain networks. The latter are not easily predicted by the agent's physiological actions at the molecular and cellular levels. Rather, networks are premier examples of complex systems in which small changes in initial conditions can have large and unexpected consequences. It seems reasonable to assume that actions of psychoactive agents at the network level are substantially larger in magnitude than their actions on individual synapses. This point has been experimentally confirmed for ampakines, a class of compounds that positively modulate AMPA-type glutamate receptors. For instance, ampakine-induced changes on the hippocampal trisynaptic loop were severalfold greater in magnitude than changes in monosynaptic responses within

this circuit (Sirvio et al., 1996). Moreover, compound concentrations necessary to enhance polysynaptic potentials proved to be about four times smaller than those required to increase monosynaptic field EPSPs (Sirvio et al., 1996).

In addition, ascending modulatory systems such as the cholinergic or the monoaminergic systems widely influence cortical network activity. In in vitro preparations, actions of modulatory systems on cortical networks can be mimicked by pharmacological compounds acting pre- or postsynaptically in the target areas of these neuromodulatory systems. We address this issue in this chapter, thereby emphasizing multi-electrode arrays as an important tool to identify and evaluate novel neuropharmacological compounds.

15.2 Brain-on-a-Chip^{TM}

The Brain-on-a-Chip^{TM} technology tests effects of pharmacological compounds on neuronal networks by using Panasonic's MED64 System to monitor network activity in acute or cultured brain slices. The selected preparations display striking similarities to certain network activities as seen in vivo. Thus, studying compounds using multi-electrode array approaches can be used to predict certain in vivo effects of compounds that could not be studied using alternative in vitro techniques. The generally higher throughput of in vitro approaches as compared to in vivo techniques makes multi-electrode array systems a suitable approach for screening larger numbers of compounds. Multi-electrode array technologies provide at least four key advantages over classical technologies.

1. *Use of cultured slices for long-term recordings:* Traditionally, slice electrophysiology experiments are performed in the short period of time during which recording can take place (typically up to 4 to 6 h after slice preparation), which contrasts sharply with the long chronic recordings obtained under in vivo conditions.
2. *Use of co-culture of slices from different brain regions incorporating modulatory systems to various networks:* Another limitation of slice technology is that, in most cases, slice networks have lost a number of inputs, and, in particular, inputs originating from diffuse neuromodulatory systems, such as noradrenergic, serotonergic, and cholinergic neurons, which profoundly modify the in vivo operation of networks. By using co-cultures of slices from various brain regions it is possible to incorporate modulatory influences to many networks.
3. *Use of acute slices exhibiting spontaneous or pharmacologically induced EEG-like rhythms:* In vitro preparations have traditionally been used to create conditions for studying a single synaptic or cellular response, preferably in a single neuron type in isolation. In vivo experiments on the other hand show that many compounds or behavioral situations clearly affect EEG activity. We have developed protocols to recreate EEG-like activity in brain slices on the multi-electrode array similar to those found in vivo. Such protocols therefore

represent a useful extension of in vitro electrophysiology, and may yield more realistic and predictive information regarding the effects of compounds on various network parameters (see also Chapter 18 by Colgin for further illustration of this point).

4. *Use of spatial information in in vitro recordings:* Finally, in vivo recordings have recently evolved toward recording larger and larger numbers of units in an attempt to understand the behavior of large ensembles of neurons. The technology described in this volume has also evolved toward recording a large ensemble of neurons through the use of complex arrays of electrodes (typically 8×8 arrays of geometrically positioned electrodes).

These points are illustrated below where we describe the use of cultured slices for first testing neuroprotective compounds against excitotoxicity, and then for studying long-term plasticity phenomena such as long-term potentiation.

15.3 Slice Culture on a Chip: Neuroprotective Effects of NMDA-R Antagonists

In order to address the question of how neurotoxic agents affect synaptic responses in hippocampal pathways, stable long-term monitoring of synaptic transmission is required. We cultured hippocampal slices directly on the surface of the MED probe, which allowed us to conduct long-term extracellular recordings in CA1 and other hippocampal subfields. Directly culturing hippocampal slices on polyethylenimine-coated probes resulted in a tight adhesion of the slices on the probes, and provided for stable maintenance of the stimulating and recording sites. Typically, one pair of electrodes (one stimulating and one recording electrode) was selected in CA1 to stimulate Schaffer collateral afferents and to extracellularly record excitatory postsynaptic field potentials (fEPSPs) with the best possible quality.

Culturing slices directly on the MED probes bears some significant advantages. For instance, in traditional electrophysiological approaches using glass electrodes it was impossible to track a specific excitatory pathway over many days in cultured slices because of the difficulty of positioning the electrodes in exactly the same manner day by day (although some recent work by Dean Buonomano's laboratory has used some clever approaches to address this issue). A cultured slice on the MED probe elegantly circumvents this problem, as the position of the electrodes relative to the fiber pathways does not change over days. Therefore, the MED probe is exquisitely suited for studying slice cultures.

In agreement with prior studies (Stoppini et al., 1991), slices prepared from postnatal day 10 to 12 rats provided optimal results when tested electrophysiologically after culturing on the MED probe in static conditions with interface levels of culture medium. An example of a slice cultured on the MED probe for one week in vitro as well as positions of the electrodes is depicted in Figure 15.1A. Under our experimental conditions, no obvious morphological changes were noticed during

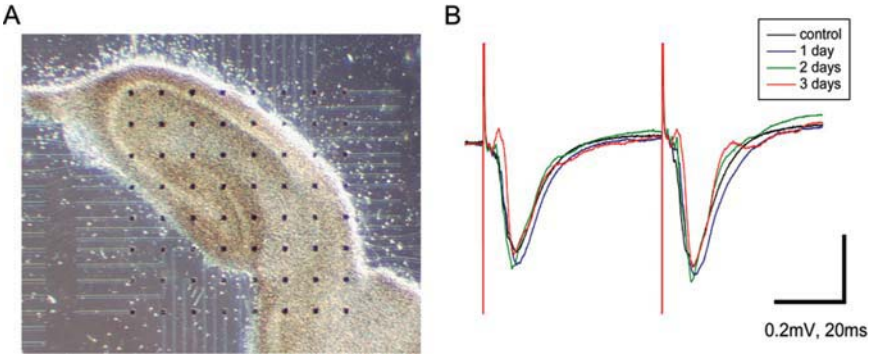

FIGURE 15.1. Experimental design used to perform chronic recording in cultured hippocampal slices: (A) hippocampal slices from 11-day-old rats were cultured and maintained on the MED-64 probes for extended periods of time; (B) paired-pulse stimulations were delivered to various electrodes and extracellular field potentials were recorded. (Reprinted with authorization from Shimono et al., 2001.)

at least two weeks in culture on MED probes. Slight to moderate migration of the cells out of the slice was observed, normally starting after 14 to 20 days. Some flattening of the slices also occurred after two weeks in vitro, which did not appear to interfere with fEPSP recordings. During the first 7 days of in vitro culturing, we observed some increase in the amplitude of fEPSPs; however after 7 to 10 days, the responses stabilized and remained stable over subsequent recording periods (Figure 15.1B). These results were consistent with the findings of Muller et al. As a result, all experiments reported here were done after at least 10 days of culturing slices on the MED probes.

Numerous studies have used selective glutamate receptor agonists for producing pathological conditions in the brain in order to study mechanisms of neuronal death and neurotoxicity. In our case, NMDA and AMPA were chosen as excitotoxic agents to compare electrophysiological parameters with traditional parameters of neurodegeneration, and to gain additional information regarding the mechanisms contributing to the loss of synaptic function in excitotoxic hippocampal injury. Long-term incubation of cultured hippocampal slices in the presence of either NMDA or AMPA resulted in concentration-dependent decreases in synaptic responses. Representative I/O relationships and fEPSP recordings before and at various times after incubation in the presence of NMDA (10 μM) or AMPA (1 μM) are shown in Figure 15.2. After 3 h of incubation in the presence of 10 μM NMDA, the maximal amplitude of synaptic responses was reduced to 18 $\pm$ 4% ($n = 3$) of control values and this decrease did not change significantly during subsequent incubation periods (Figure 15.2A). When 1 μM AMPA was applied for 3 h, the decrease in synaptic responses was smaller, and the responses continued to decrease at subsequent time points, and stabilized after one day (Figure 15.2B).

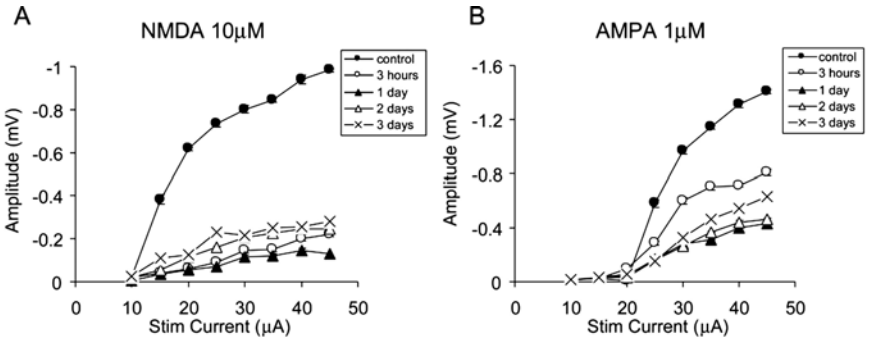

FIGURE 15.2. Chronic effects of NMDA and AMPA on synaptic responses in CA1. After collecting baseline responses, slices were treated with NMDA (10 μM; A) or AMPA (1 μM; B) for the indicated periods of time, and I/O relationships were obtained. The amplitudes of synaptic sponses were plotted as a function of the intensities of stimulation.

The mechanisms underlying the reduction in amplitude of synaptic responses produced by incubation with these two excitotoxins were further studied by applying various concentrations of NMDA and AMPA for two days. As in the control experiments, the plateau values of the respective I/O curves were used for the analysis (Figure 15.3). Fitting the concentration–response curves with the Hill function generated EC50 values of 5.2 ± 0.5 μM for NMDA and 0.81 ± 0.1 μM for AMPA, with Hill coefficients of 1.7 ± 0.2 and 2.6 ± 0.8, respectively. For comparison, we plotted in the same figure concentration–response curves obtained with the PI uptake method (Figure 15.3, open circles and dashed lines). EC50 values of PI uptake method were 21.6 ± 3.4 μM for NMDA and 3.87 ± 0.1 μM for AMPA and Hill coefficients of 3.7 ± 1.0 and 2.0 ± 0.1, respectively. The concentration–response curves obtained with electrophysiology were shifted toward the left and were also less steep (at least for NMDA) than those observed with the PI method, suggesting that these two methods reveal different cellular mechanisms activated by NMDA and AMPA.

To further address this question, we investigated the time-course for recovery of synaptic responses after agonist removal. At the end of incubation in the presence of 10 μM NMDA for 40 min, 3 h, 1 day, and 3 days, the amplitudes of synaptic responses were 48 ± 12, 22 ± 7, 13 ± 3, and $11 \pm 3\%$ of control values, respectively ($n = 3$). However, following 1 h of NMDA washout under the same conditions, synaptic responses were 109 ± 4, 66 ± 5, 52 ± 2, and $18 \pm 3\%$ of the initial amplitude, respectively, indicating that synaptic responses in fact gradually decrease and become irreversibly diminished only after 3 days of continuous treatment with NMDA. Similar results were obtained for AMPA: a 40 min incubation in the presence of 3 μM AMPA led to a large decrease in the amplitude of synaptic responses ($12 \pm 5\%$ of control values) and the responses recovered to up to $93 \pm 2\%$ of control after AMPA was removed for 1 h ($n = 3$); 3 days of incubation in the

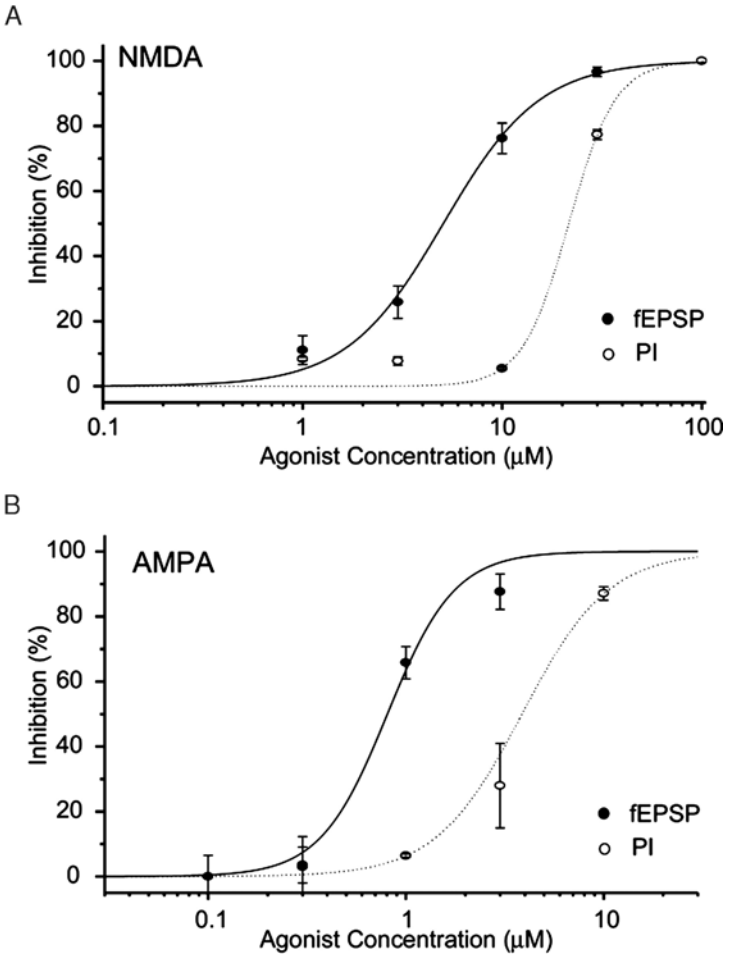

FIGURE 15.3. Chronic effects of various concentrations of NMDA and AMPA on synaptic responses and neuronal damage in CA1. Synaptic responses to maximum stimulation intensities were recorded 48 h after chronic treatment of cultured hippocampal slices with NMDA (A) or AMPA (B). Results were calculated as percentage of inhibition of baseline values and represent means 9 S.E.M. of three experiments (solid circles). Neuronal damage (open circles) was assayed with the PI uptake method. Results were calculated as percentage of maximal damage and represent means ± S.E.M of two experiments. Solid and dashed lines represent the best-fit curves for synaptic responses and PI uptake.

presence of 1 μM AMPA decreased synaptic response amplitudes to 46 ± 4% ($n = 3$) and no recovery was observed even after 24 h of AMPA removal.

To validate the ability of our system to discriminate drugs with different neuroprotective properties, the potency of two distinct noncompetitive NMDA receptor antagonists was determined. MK-801 is a known high-affinity antagonist, whereas

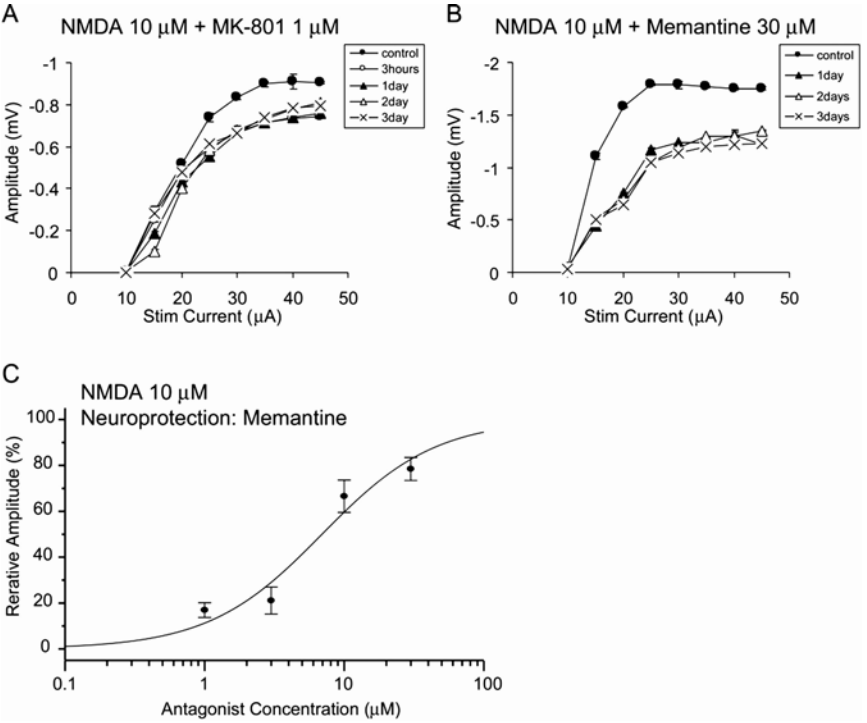

FIGURE 15.4. Effects of MK801 and memantine on NMDA-induced decrease in synaptic responses in CA1. After collecting baseline responses, slices were treated with 10 μM NMDA and 1 μM MK801 (A) or 10 μM NMDA and 30 μM memantine (B) for the indicated periods of time. Input/output relationships were obtained and the amplitudes of the synaptic responses were plotted as a function of the intensities of stimulation (typical cases are shown). Synaptic responses elicited by stimulation at maximum intensity were recorded 72 h after chronic treatment of cultured hippocampal slices with 10 μM NMDA in the presence of various concentrations of memantine (C). Results were calculated as percentage of responses recorded under control conditions (i.e., in the absence of NMDA) and represent means ± S.E.M. of three experiments.

memantine is an uncompetitive low-affinity antagonist and is currently used in clinical treatments for Alzheimer's disease (Parsons et al., 1999). Based on the concentration–response analysis performed above (Figure 15.3A), 10 μM NMDA was chosen as the concentration of agonist for the antagonist tests. Inasmuch as we were interested in evaluating the neuroprotective effects of these antagonists in chronic application, we did not study their effects before the first 3 h time point.

After performing control measurements, slices were incubated in culture media containing 10 μM NMDA and 1 μM MK-801 for various periods of time. Under these conditions, synaptic responses were only slightly depressed from 3 h up to 3 days of incubation (Figure 15.4A), indicating that 1 μM MK-801 almost

completely protected synaptic transmission against NMDA-mediated neurotoxicity, a result in good agreement with previous studies using different markers of synaptic damage (Peterson et al., 1989; Pringle et al., 2000; Kristensen et al., 2001). Relative amplitude of synaptic responses measured at stimulation intensities corresponding to the plateau of I/O curves after 3 days of incubation was $91 \pm 6\%$ of control ($n = 3$).

When memantine was used as an antagonist, the pattern of protection was relatively similar, as synaptic responses were significantly protected for up to 3 days of incubation in the presence of 10 μM NMDA and 30 μM memantine. However, the degree of protection was much smaller than that provided by MK-801, as only $78 \pm 5\%$ ($n = 3$) of the initial amplitude was preserved under these conditions (Figure 15.4B). The concentration dependency of memantine protection was studied using 10 μM NMDA and various concentrations of memantine. The concentration–response curve was fitted with the Hill equation and provided values for IC50 of 6.9 ± 1.6 μM, with a Hill coefficient of 1.1 ± 3 (Figure 15.4C).

Despite its relatively high affinity, MK-801 is not used clinically to protect CNS from neurodegenerative and other kinds of disorders. One of the reasons for its inadequateness for medical treatment is related to the almost irreversible nature of its binding to the NMDA receptors (Parsons et al., 1999), and some of its neurotoxic properties. In our experiments, incubation of the slices in the presence of 1 μM MK-801 alone resulted in decreased synaptic responses after 1 day. Interestingly, synaptic responses were not affected after 3 h of incubation. However, after 1, 2, and 3 days of incubation, the amplitudes of responses were decreased to 83 ± 6, 79 ± 11, and $76 \pm 10\%$ ($n = 3$) of control, respectively. These decreases in amplitude were not significantly different from the decrease in amplitudes produced by 1 μM MK-801 in the presence of 10 μM NMDA. In contrast to MK-801, long-term incubation in the presence of memantine even at 30 μM did not result in a significant decrease in synaptic transmission.

Results obtained with chronic application of NMDA receptor agonist or antagonists are summarized in Figure 15.5. Control responses were measured in 23 slices and were compared with the results obtained after incubation during 3 days in the presence of NMDA, NMDA plus antagonists, or antagonists alone. Our results indicate that the large (70 to 80%) decrease in synaptic responses produced by incubation in the presence of 10 μM NMDA could be almost completely prevented ($91 \pm 6\%$, $n = 3$) by co-incubation with 1 μM MK-801 (not significantly different from control values). Protection was substantially weaker for memantine even at higher concentrations, as only $78 \pm 5\%$ ($n = 3$) of synaptic responses remained after treatment with NMDA and 30 μM memantine ($p < .05$ as compared to control values). However, it is important to note that incubation with memantine (30 μM) but not MK-801 (1 μM) in the absence of NMDA resulted in responses that were statistically identical to those obtained under control conditions (control vs. memantine: $p = .267$, control vs. MK-801: $p = .010$).

These data clearly demonstrate that chronic multi-site recordings in an in vitro preparation represent a new methodology to assess the properties and mechanisms of neuronal damage. This technology should be of interest to a wide range of

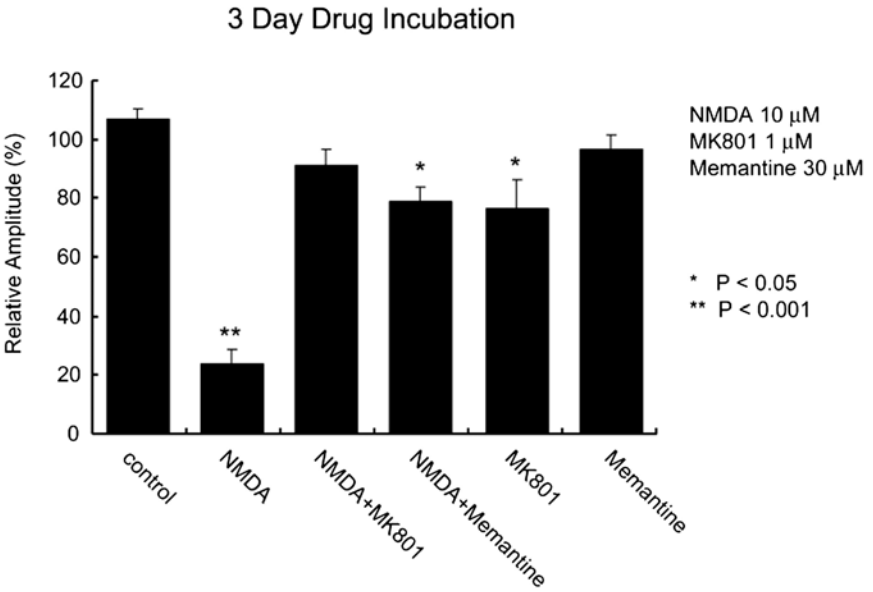

FIGURE 15.5. Comparison of the effects of various chronic treatments on synaptic responses in CA1. Synaptic responses to stimulation at maximal intensity were recorded in cultured hippocampal slices three days after treatment with various drugs or combinations of drugs. Results represent amplitudes of synaptic responses; they are expressed as percentage of the values recorded under control conditions and are means ± S.E.M. (control: $n = 23$; other cases: $n = 3$, $^{*}p < 0.05$, $^{**}p < 0.001$).

neuroscientists and should provide a new and powerful method to study the chronic effects of drugs or other experimental manipulations in an in vitro preparation.

15.4 Chronic LTP Assay for Memory Enhancers

15.4.1 Long-Term Recording of Long-Term Potentiation in Cultured Hippocampal Slices

As discussed above, direct culturing of hippocampal slices on MED probes resulted in a tight adhesion of the slices on the probes, thus providing for stable maintenance of the stimulating and recording sites and thereby long-term extracellular recording in hippocampal field CA1 (Shimono et al., 2000). LTP was induced by delivering trains of high frequency to one of two inputs converging on one set of pyramidal cells in CA1, with the other input being used as control (Figure 15.6). Field EPSPs evoked by stimulation on either test or control pathway were recorded before and 1 h, 1 day, and 2 days after delivering the tetanus stimulations to the test pathway. Although the fEPSPs evoked in the control pathway remained stable

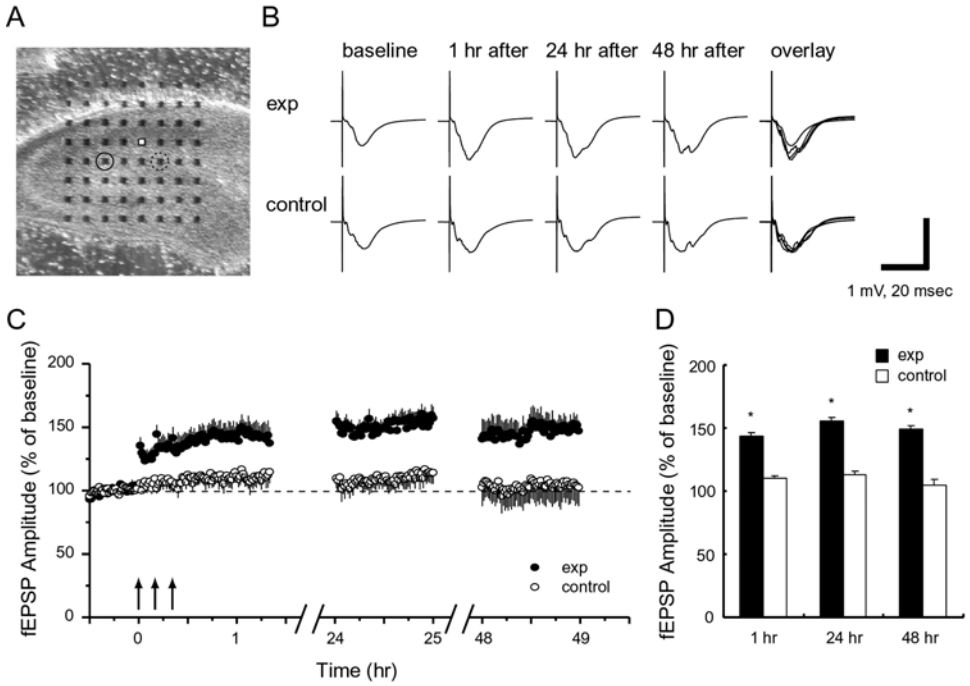

FIGURE 15.6. Long-lasting recording of long-term potentiation in cultured hippocampal slices. (A) Micrograph of a hippocampal slice cultured on an MED probe. Interelectrode distance is 150 μm. The recording electrode is indicated by a white square, and stimulation electrodes are indicated by a solid circle (tetanized pathway (exp)) and a dotted circle (control pathway). (B) fEPSPs evoked by Schaffer fiber were recorded in field CA1 before and after tetanus stimulation. fEPSPs evoked by stimulation of the tetanized pathway (exp) are shown on the top row and fEPSPs evoked by stimulation of the control pathway (control) are shown on the bottom row (each response is the average of 10 sweeps; typical examples are shown). (C) Summarized graph of long-lasting LTP recording. The maximum amplitude of fEPSP was determined and calculated as percentage of averaged baseline values (means ± S.E.M., $n = 8$). Arrows indicate time of high-frequency stimulation. (D) LTP amplitude was averaged over a 10 min period at 1 h, 24 h, and 48 h after high-frequency stimulation, and was expressed as percentage of baseline values.

during recording, potentiation of the fEPSPs evoked in the test pathway started immediately after tetanus and, in a subset of slices, lasted for more than 2 days without any significant decay over this time period (Figure 15.6B). Potentiation lasting more than 1 h occurred in 27 out of 38 slices tested, and the number of slices in which LTP lasted more than 1 day was 15 out of 27 slices. Data obtained from long-term recordings of LTP that lasted more than 2 days ($n = 8$) are summarized in Figure 15.6C. Field EPSP amplitudes in test and control pathways represented 143 ± 3% and 110 ± 2% of their respective pretetanus levels after 1 h, 156 ± 3% and 113 ± 2% after 1 day, and 149 ± 3% and 105 ± 4% after 2 days, respectively.

Differences between responses recorded in the test and control pathways at 1 h, 1 day, and 2 days after tetanus were highly significant ($p < .0001$, $n = 8$).

15.4.2 Effects of APV on LTP Induction in Cultured Hippocampal Slices

To determine whether long-lasting LTP induced in slices cultured on MED probes was similar to LTP typically induced in acute or cultured hippocampal slices, tetanus stimulation was applied in the presence of the NMDA receptor antagonist, D-APV. When APV (50 μM) was applied 30 min before and during high-frequency stimulation, no LTP was observed (relative amplitudes of test and control pathways were 114 ± 1% and 112 ± 1% 1 h after tetanus and 103 ± 2% and 102 ± 1% 3 h after APV washout; $n = 11$). On the other hand, when high-frequency stimulation was delivered 3 h after APV washout, LTP was again elicited and to the same degree as in naive slices (147 ± 3% and 116 ± 3% in test and control pathways, respectively; $n = 5$; data not shown). Such potentiation also lasted more than one day.

To further assess the effects of APV on synaptic plasticity, synaptic responses were determined one day following high-frequency stimulation in the presence of APV, and APV washout. Synaptic responses remained unmodified as compared to their baseline values whether or not the slices had been treated with APV, and whether or not they had been tetanized in the presence of APV. This result clearly indicates that, under our conditions, high-frequency stimulation did not produce an NMDA receptor-independent form of long-term potentiation. To eliminate the possibility that the lack of modification of synaptic responses was due to some deleterious effects of tetanization in the presence of APV, we tested the effects of high-frequency stimulation one day after APV washout. LTP was elicited in six out of eight slices and the magnitude of LTP was similar to that produced in naive slices (average relative amplitudes expressed as percentage of initial baselines were 153 ± 3% and 116 ± 2% after 1 h in the test and control pathways, respectively; means ± S.E.M. of six experiments).

Trains of high-frequency stimulation in cultured hippocampal slices elicit a long-lasting form of LTP that, in a significant number of slices, can be recorded for several days. This form of LTP was completely blocked by APV, an antagonist of NMDA receptors. This blockade was completely reversible as LTP could be induced 3 hr or 24 hr after APV washout. Thus, this form of LTP exhibits similar features as typical LTP elicited in CA1 of acute or cultured hippocampal slices. In this regard, our data regarding the probability and the amplitude of LTP in hippocampal slices cultured for 10 days are in good agreement with previous results.

Our results also indicate that LTP in field CA1 of a subset of cultured hippocampal slices can occur without showing any significant decay over more than two days, a result reminiscent of what has been reported for in vivo LTP in field CA1 (Staubli and Lynch, 1987). This finding indicates that this experimental model

might be ideally suited to study mechanisms underlying LTP maintenance and consolidation. Furthermore, as cultured slices have been successfully used with various mutant mice, our results open the way for a wide range of studies related to long-term recordings of synaptic modifications resulting from a broad spectrum of manipulations.

15.5 Co-Culture on a Chip: Effects of Neuromodulators

Many psychoactive agents act by modulating one or more of the diffuse ascending systems: that is, serotonin, acetylcholine, dopamine, and norepinephrine. These compounds facilitate or retard transmission but generally do not act as agonists or antagonists and often have only subtle effects on transmitter binding. Because of this, detection of modulatory agents is often best accomplished with an assay that incorporates synaptic transmission. This presents a severe problem in the case of the diffuse systems because the pertinent axons are both very sparse and disconnected from their cell bodies. It is thus difficult to establish that a stimulating electrode is in contact with the targeted fibers or that an evoked response reflects monosynaptic transmission involving the intended projections. One solution to these problems is to co-culture the cell bodies that give rise to the diffuse system of interest with an appropriate anatomical target of the system. However, co-cultures have not been widely used with the cultured slice technique recently introduced by Stoppini et al. (1991). The method we developed uses slices prepared from rat brains in the second postnatal week, a time point at which the major anatomical systems have been laid down. Moreover, the slices gradually take on a much more adultlike state than is the case with traditional organotypic cultures.

Figure 15.7 consists of micrographs of sections through two cultured hippocampal slices, one (left side) co-cultured with the median raphe nucleus of the brainstem and the other co-cultured with the medial septal nucleus. The raphe–hippocampal sections have been processed for immunocytochemistry using antibodies against serotonin and the septal–hippocampal sections were processed for acetylcholinesterase histochemistry. Serotonergic and cholinergic cell bodies can be seen in the boxes marked "B" in the survey micrographs in the top panels of the figure. Labeled fibers arising from these neurons are evident in the higher-power images in the middle panels. The bottom panels show the serotonergic and cholinergic fibers forming dense plexuses above and below the cell body layers within hippocampus, the pattern of innervation found in situ. The dense puncta in these figures correspond in size, appearance, and distribution to axon terminals. In all, cultured slices prepared relatively late in development provide excellent targets for the diffuse projections.

Success in the co-culture experiment opened the way to tests of how the diffuse ascending projections affect network-level operations in the hippocampus and retrohippocampal cortex. Figure 15.8A shows a septal/hippocampal culture sitting atop a 64-electrode array and Figure 15.8B provides typical recordings of spontaneous physiological activity. Note the triplets of large biphasic waves that

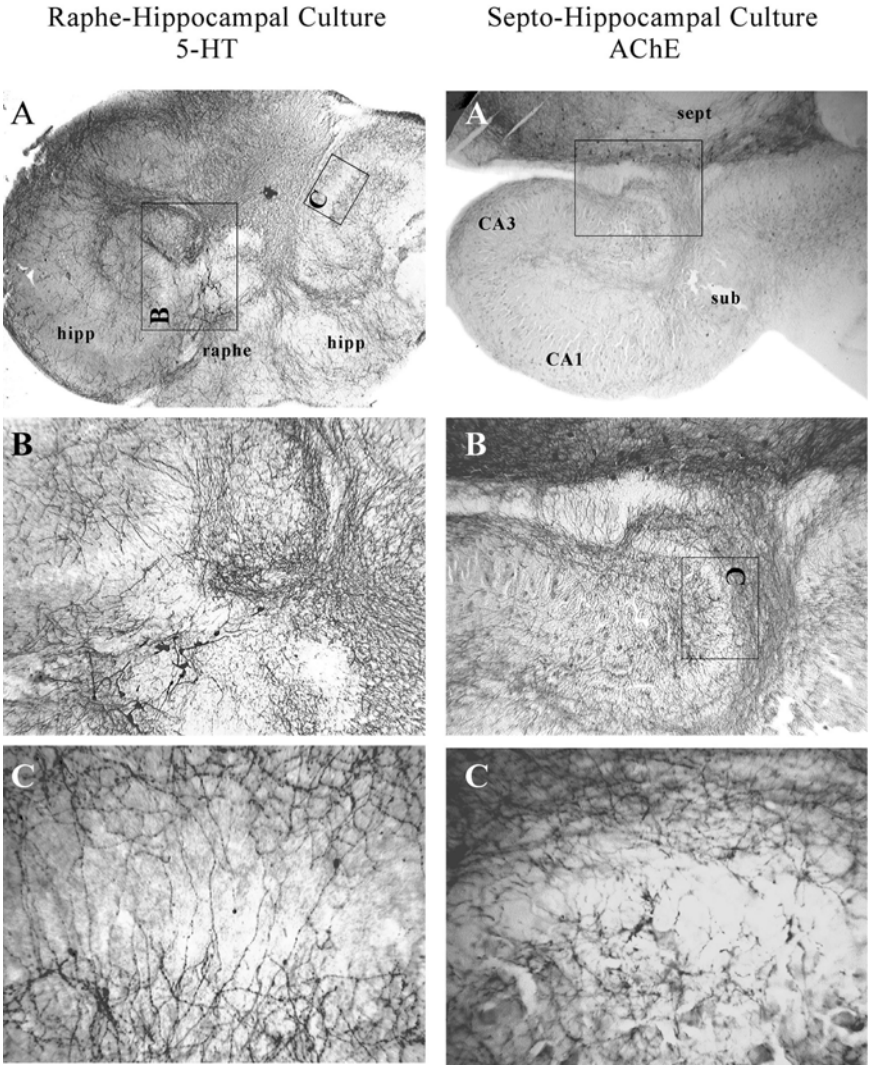

FIGURE 15.7. Co-cultures of slices. Micrographs of sections through two cultured hippocampal slices, one (left side) co-cultured with a slice from the median raphe nucleus of the brainstem and the other co-cultured with a slice from the medial septal nucleus. The raphe–hippocampal sections have been processed for immunocytochemistry using antibodies against serotonin and the septal–hippocampal sections were processed for acetylcholinesterase histochemistry. Serotonergic and cholinergic cell bodies can be seen in the boxes marked "B" in the survey micrographs in the top panels of the figure. Labeled fibers arising from these neurons are evident in the higher-power images in the middle panels. The bottom panels show the serotonergic and cholinergic fibers forming dense plexuses above and below the cell body layers within the hippocampus, the pattern of innervation found in situ. The dense puncta in these figures correspond in size, appearance, and distribution to axon terminals.

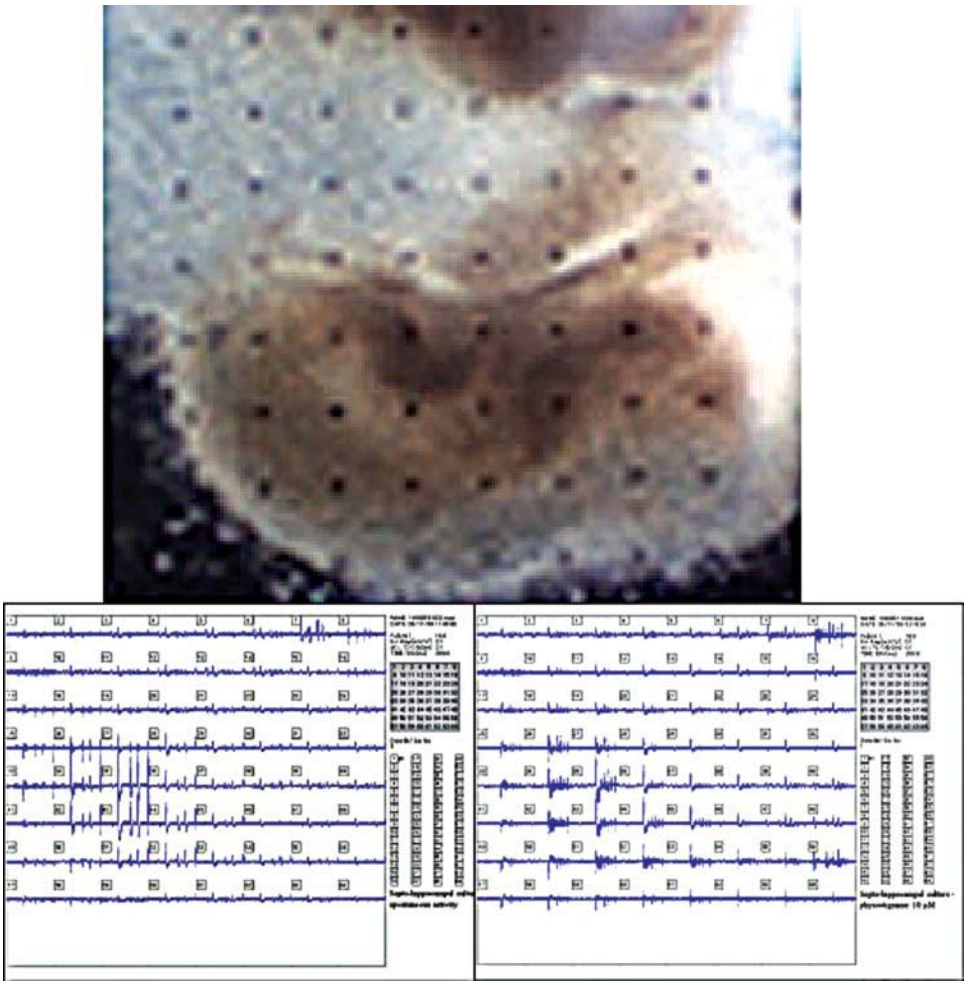

FIGURE 15.8. Recordings in co-cultures: (A) micrograph of a septal/hippocampal culture sitting atop a 64-electrode array. (B) Typical recordings of spontaneous physiological activity. Note the triplets of large biphasic waves that are particularly prominent at electrodes #36 and #44. Based on their shape and distribution, these potentials appear to be synchronous postsynaptic responses, that is, the result of a sizeable number of CA3 pyramidal neurons firing at about the same time. (C) Results obtained with physostigmine, a psychoactive drug that modulates cholinergic transmission by blocking the catalytic enzyme acetylcholinesterase. As is evident, the drug triggered rhythmic EEG activity at sites throughout the hippocampus, an effect similar to that it produces in vivo.

are particularly prominent at electrodes #36 and #44. Based on their shape and distribution, these potentials appear to be synchronous postsynaptic responses, that is, the result of a sizeable number of CA3 pyramidal neurons firing at about the same time. Figure 15.8C shows the first results obtained with physostigmine, a psychoactive drug that modulates cholinergic transmission by blocking the catalytic enzyme acetylcholinesterase. As is evident, the drug triggered rhythmic EEG activity at sites throughout the hippocampus, an effect similar to that it produces in vivo. Analyses now in progress indicate that multiple frequencies are promoted by physostigmine and that the response to the drug is regionally differentiated. But as they stand, the above results demonstrate the feasibility of using cultured slices to detect compounds that modulate ascending diffuse projections. Rhythmic activity of a type not seen in conventional slices has also been recorded in raphe–hippocampal preparations (data not shown) and experiments with modulatory drugs will begin shortly.

15.5.1 Studying the Modulation of Cholinergic Rhythmic Activity in Acute Ventral Hippocampal Slices

In this section we present results obtained with acute hippocampal slices recorded with the MED64 System. It has recently been shown that rhythmic activity in the gamma frequency band (20 to 40 Hz) could be induced and studied in the CA3 area of ventral hippocampal slices (Fisahn et al., 1998; Fellous and Sejnowski, 2000; for review see McBain and Fisahn, 2001). This was an interesting discovery as rhythmic activity in the slice showed some resemblance to that recorded in vivo, where it is observed during memory formation (Fell et al., 2001), sleep–wake states (Llinas and Ribary, 1993; Maloney et al., 1997), and visual activity (for review see Singer, 1999). To induce rhythmic activity in ventral hippocampal slices, we used the cholinergic agonist carbachol, allowing us to study rhythmic activity in isolation in the brain slice preparation.

Depending on the interelectrode space on the MED64 probe (most of our recordings were done using a probe with 150 μm interelectrode space), rhythmic activity can be monitored in many subregions of the hippocampal slice. It is our (Shimono et al., 2000) as well as other investigators' (Fisahn et al., 1998; Traub et al., 2000) experience that rhythmic activity shows the largest spectral power in the CA3 region. We therefore concentrated our investigation on this region. Because our recordings also contain data from other regions (e.g. the CA1 region), a comparison of compound effects on different regions would be possible. To record from acute slices, we adapted the MED64 System to a perfusion system, allowing the addition of compounds together with artificial cerebrospinal fluid. Slices in that system were kept in a warm humidified atmosphere of 95% O_2: 5% CO_2. In the presence of carbachol, rhythmic activity was persistent for hours, and 5 μM of the agonist in the bath was found to be a moderate concentration, where spectral power or frequency had not reached their maximal values. We therefore used this concentration in most of our recordings to study modulation of rhythmic activity

by a variety of pharmacological compounds. Spectral power and frequency ranged approximately from 5 to 600 μV^2 and 18 to 40 Hz, respectively.

We were mostly interested in the aminergic modulation of cholinergic rhythmic activity in acute hippocampal slices, and therefore focus in this section on the serotonergic modulation of these rhythms. The hippocampus receives a moderate to high serotonergic innervation, originating from the dorsal and medial raphe nuclei (Moore and Halaris, 1975; Azmitia and Segal, 1978; Oleskevich and Descarries, 1990; McQuade and Sharp, 1997). Activating the serotonergic system in the raphe nucleus in vivo desynchronizes the hippocampal encephalogram (Assaf and Miller, 1978; Nitz and McNaughton, 1999). We therefore asked the question whether and how 5-HT modulates cholinergic-induced rhythmic activity in ventral hippocampal slices (Krause and Jia, 2005).

15.5.2 Involvement of 5-HT$_{1A}$ and 5-HT$_2$ Receptors in the Modulation of Cholinergic Hippocampal Rhythmic Activity

Experiments in our laboratory showed (Krause and Jia, 2005) that 5-HT decreased the spectral power, but not the frequency, of carbachol-induced rhythmic activity in a reversible manner (Figure 15.9). This modulation was concentration-dependent

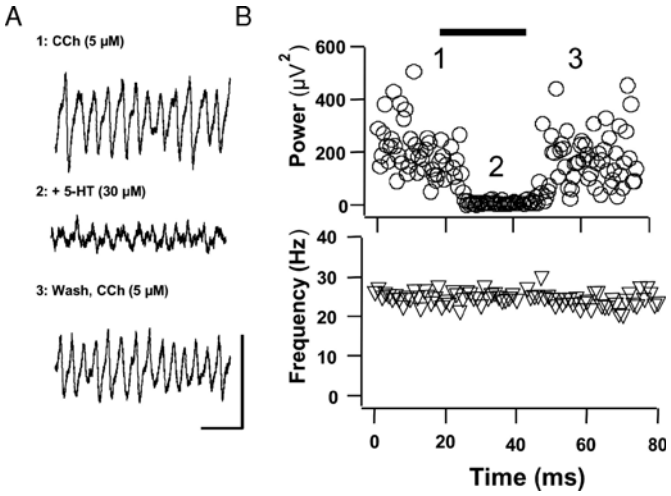

FIGURE 15.9. Carbachol-induced rhythmic activity in ventral hippocampal slices and its suppression by 5-HT: (A) carbachol (CCh, 5 μM) in the bath leads to sustained rhythmic activity (1). Adding 5-HT (30 μM) suppresses spectral power of this rhythmic activity (2). This effect is reversible (3). (B) Diagrams showing power and frequency of CCh-induced rhythmic activity before, during, and after 5-HT application (indicated by the black horizontal bar). The numbers indicate time points were sweeps were taken in A. Calibration: 50 μV, 100 msec. (Edited from Krause and Jia, 2005, with permission from Elsevier.)

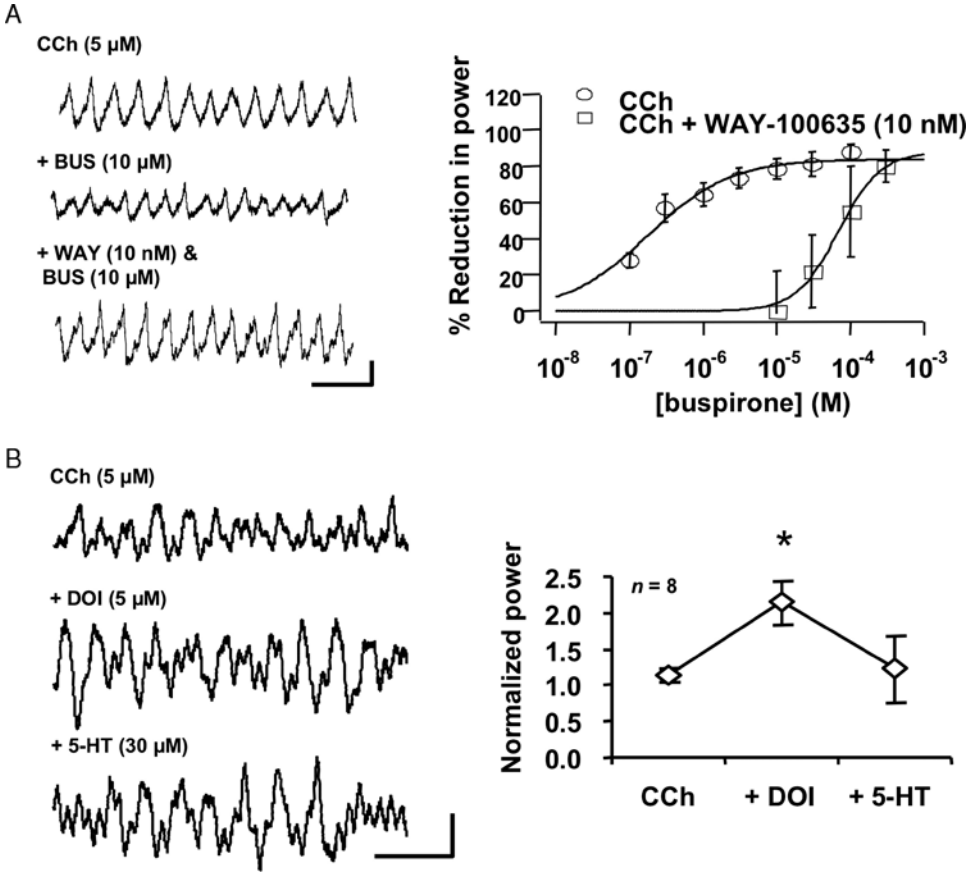

FIGURE 15.10. CCh-induced activity is differentially modulated by 5-HT_{1A} and 5-HT_2 receptors. (A) The 5-HT_{1A} agonist buspirone (BUS) suppresses CCh-induced rhythmic activity in a concentration-dependent manner. The suppression is reversed by the 5-HT_{1A}-selective antagonist WAY-100635 (WAY, 10 nM). (B) The 5-HT_2 agonist DOI (DOI, 5 μM) increases CCh-induced activity. 5-HT (30 μM) fails to decrease rhythmic activity in the presence of DOI. Calibration: 50 μV, 100 msec. (Edited from Krause and Jia, 2005, with permission from Elsevier.)

with an estimated IC_{50} for 5-HT of 4 μM (data not shown). We next used receptor-specific agonists and antagonists to determine the receptor subtype(s) underlying the suppression of carbachol-induced rhythmic activity. Interestingly, we found a differential modulation of carbachol-induced activity by 5-HT_{1A} and 5-HT_2 receptors (Figure 15.10). Whereas the 5-HT_{1A} agonists buspirone (estimated IC_{50}: 0.25 μM) and 8-OH-DPAT (not shown) mimicked the effect of 5-HT on rhythmic activity, the 5-HT_2 receptor agonist DOI increased spectral power of the rhythms. In addition, 5-HT no longer decreased spectral power in the slice in the presence

of DOI. The 5-HT_{1A} antagonist WAY-100635 or the 5-HT_2 antagonist ritanserin alone had no effect on carbachol-induced rhythmic activity.

On the basis of the long-lasting effect of 5-HT showing no desensitization we excluded the involvement of 5-HT_3 receptors. However, we cannot exclude the involvement of other 5-HT receptors. From these results we concluded that 5-HT shows differential effects on carbachol-induced rhythmic activity in ventral hippocampal slices with 5-HT_{1A} activation exerting a predominant effect to elicit a decreased spectral power of the rhythm.

15.5.3 Testing the Ability of MM223, a Novel 5-HT_{1A} Agonist to Modulate Carbachol-Induced Rhythmic Activity

We next used our system to test and compare a novel selective 5-HT_{1A} agonist, MM223 (Mokrosz et al., 1999; Bojarski et al., 2002), in its ability to modulate carbachol-induced rhythmic activity. Many 5-HT_{1A} ligands such as buspirone, gepirone, or WAY-100635 are 1-arylpiperazines. In contrast, MM223 is a 1,2,3,4 tetrahydroisoquinoline amide derivative, representing a novel class of 5-HT_{1A} ligands (Figure 15.11A). Therefore, we wanted to test MM223's ability to affect carbachol-induced rhythmic activity. This compound exhibited a Ki of 0.95 nM at the 5-HT_{1A} receptor, and of 452 nM at the 5-HT_2 receptor, as assessed in radioligand binding studies (Bojarski et al., 2002). In contrast, buspirone shows a Ki of $\sim$20 nM at the 5-HT_{1A} receptor (Sharif et al., 2004). Using the lip retraction test (in rat), MM223 was found to be a partial agonist at postsynaptic 5-HT_{1A} receptors (Mokrosz et al., 1999). MM223 mimicked the effect of buspirone and 5-HT in suppressing carbachol-induced rhythmic activity, and its action was antagonized by WAY-100635, thereby confirming its agonist behavior at a presumed 5-HT_{1A}-mediated physiological action (Figure 15.11B). Using a Scatchard analysis on the data shown in Figure 15.11C, we found Ki values of 3.44 μM for buspirone, and 0.73 μM for MM223, indicating that MM223 was more potent in decreasing carbachol-induced rhythmic activity than buspirone (Figure 15.11D). Our results using the MED64 System therefore confirmed the results from receptor binding studies mentioned above.

Taken together, these data show that carbachol-induced rhythmic activity in ventral hippocampal slices can be used to evaluate pharmacological features of novel compounds.

15.6 Conclusions

The Brain-on-a-Chip™ technology bridges the gap between biochemical and single-cell testing and behavior by determining the effects of compounds on living slices of brain containing intact networks of neurons—a largely unexplored area essential to understanding drug effects on complex human behavior. The Brain-on-a-Chip™ technology is an information-rich and physiologically relevant

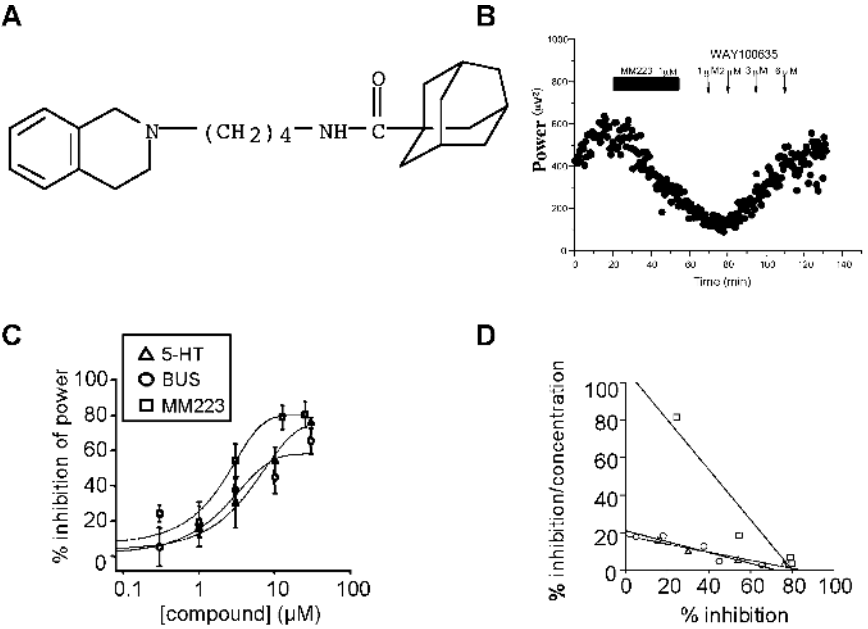

FIGURE 15.11. The novel 5-HT_{1A}-selective agonist MM223 suppresses CCh-induced rhythmic activity more potently than buspirone or 5-HT: (A) structure of MM223. (B) Suppression of CCh-induced activity by MM223 (1 μM) is reversed by WAY-100635. (C) Concentration-dependence of CCh-induced inhibition of spectral power by 5-HT, buspirone (BUS), and MM223. MM223 suppresses CCh-induced rhythmic activity more potently than BUS or 5-HT. (D) Scatchard plot of the data shown in (C) indicates linear relationships between the percentage of inhibition per concentration of agonist and the percentage of inhibition itself as a measure for the Ki value. See legend in (C) for symbols representing all three compounds.

in vitro brain assay system that addresses all four major drawbacks of conventional electrophysiology:

1. *Acute and chronic measurements:* We have shown that the MED64 can generate more temporal information about the activity of neuronal circuits than any conventional methods. We have developed methods to culture and maintain living brain tissue slices directly on the multi-electrode chips for up to several weeks. This enables us to study the chronic or long-term effects of drugs on brain activity. Whereas most conventional neurotoxicity tests can only measure the extent of cell death in response to potentially toxic drugs, we now have a functional assay capable of detecting a much wider range of drug side effects.
2. *Access to local and modulatory activity:* We can directly study the effects of drug candidates on all major neurotransmitter pathways present in the brain. We extended our chronic culture technology to co-cultures of various types of brain tissues to study the important modulatory projections from the serotonergic,

dopaminergic, cholinergic, and adrenergic neurons in the brainstem. For example, when the tissue from raphe nuclei containing serotonergic neurons was cultured next to hippocampal tissue for several days, the serotonergic neurons extended projections into the hippocampus, where they established functional serotonergic synapses. This allowed us to study the effects of drugs, such as selective serotonin reuptake inhibitors (SSRIs), on endogenously released modulatory transmitters, that is, 5-HT, in the hippocampus. This truly revolutionary approach enables us to study the effects of virtually all drugs for most major psychiatric disorders in a physiologically relevant in vitro system.

3. *Real signal:* Our data indicate that we can use a more realistic activity profile of living brain circuits than other, less complex methods. Our in vitro tissue slices exhibit endogenous dynamic oscillations similar to those found in living brains. In fact, we were able to chemically induce and manipulate these oscillations to produce the three major types of EEG waves: beta, gamma, and theta. Thus, the readout of our in vitro assay is physiologically relevant to the situation in a living brain. In addition, the dynamic oscillations are 10 to 100 times more sensitive to drugs than monosynaptic responses typically recorded in conventional slice electrophysiology. These features, for the first time, make it possible to study the effects of behavioral-relevant dosages of drugs in vitro.
4. *Network:* We generate much more spatial information about the activity of neuronal circuits than conventional methods. We use a 2-D array of 64 microelectrodes embedded in a special chip (MED64 probe) to simultaneously study the entire oscillating neuronal network containing a number of different cell types, synapses, and localized receptor types, all in a single experiment. In fact, we routinely generate movies of neuronal activity within neuronal circuits on a millisecond time scale resolution to directly observe the effects of drugs on the various neurotransmitter pathways within the central nervous system.

We believe that all these features will be continuously improving and that this type of approach will become more and more widely used to bridge the gap between in vitro and in vivo approaches.

Acknowledgments. The authors wish to thank Yusheng Jia, Michael Lee, Frank Tsuji, Lam Ho, and Ken Shimono for their help with some of the experiments, and A. Bojarski for the generous gift of MM223.

References

Assaf, S.Y. and Miller, J.J. (1978) The role of a raphe serotonin system in the control of septal unit activity and hippocampal desynchronization. *Neuroscience* 3: 539–550.

Azmitia, E.C. and Segal, M. (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.* 179: 641–667.

Bojarski, A.J., Mokrosz, M.J., Minol, S.C., Koziol, A., Wesolowska, A., Tatarczynska, E., Klodzinska, A., and Chojnacka-Wojcik, E. (2002). The influence of substitution at aromatic part of 1,2,3,4-tetrahydroisoquinoline on in vitro and in vivo 5-HT(1A)/5-HT(2A) receptor activities of its 1-adamantoyloaminoalkyl derivatives. *Bioorg. Med. Chem.* 10: 87–95.

Fell, J., Klaver, P., Lehnertz, K., Grunwald, T., Schaller, C., Elger, C.E., and Fernandez, G. (2001). Human memory formation is accompanied by rhinal-hippocampal coupling and decoupling. *Nat. Neurosci*. 4: 1259–1264.

Fellous, J.M. and Sejnowski, T.J. (2000). Cholinergic induction of oscillations in the hippocampal slice in the slow (0.5–2 Hz), theta (5–12 Hz), and gamma (35–70 Hz) bands. *Hippocampus* 10: 187–197.

Fisahn, A., Pike, F.G., Buhl, E.H., and Paulsen, O. (1998). Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro. *Nature* 394: 186–189.

Fix, A.S., Horn, J.W., Wightman, K.A., Johnson, C.A., Long, G.G., Storts, R.W., Farber, N., Wozniak, D.F., and Olney, J.W. (1993). Neuronal vacuolization and necrosis induced by the noncompetitive N-methyl-D-aspartate (NMDA) antagonist MK(+)801 (dizocilpine maleate): A light and electron microscopic evaluation of the rat retrosplenial cortex. *Exp. Neurol.* 123: 204–215.

Krause, M. and Jia, Y. (2005). Serotonergic modulation of carbachol-induced rhythmic activity in hippocampal slices. *Neuropharmacology*. 2005 Mar; 48(3): 381–390. Epub 2005 Jan 25.

Kristensen, B.W., Noraberg, J., and Zimmer, J. (2001). Comparison of exitotoxic profiles of ATPA, AMPA, KA and NMDA in organotypic hippocampal slice cultures. *Brain Res.* 917: 21–44.

Llinas, R. and Ribary, U. (1993). Coherent 40-Hz oscillation characterizes dream state in humans. *Proc. Natl. Acad. Sci. U. S. A.* 90: 2078–2081.

Maloney, K.J., Cape, E.G., Gotman, J., Jones, B.E. (1997). High-frequency gamma electroencephalogram activity in association with sleep-wake states and spontaneous behaviors in the rat. *Neuroscience* 76: 541–555.

McBain, C.J. and Fisahn, A. (2001). Interneurons unbound. *Nat. Rev. Neurosci.* 2: 11–23.

McQuade, R. and Sharp, T. (1997). Functional mapping of dorsal and median raphe 5-hydroxytryptamine pathways in forebrain of the rat using microdialysis. *J. Neurochem.* 69: 791–796.

Mokrosz, M.J., Bojarski, A.J., Duszynska, B., Tatarczynska, E., Klodzinska, A., Deren-Wesolek, A., Charakchieva-Minol, S., and Chojnacka-Wojcik, E. (1999). 1,2,3,4-tetrahydroisoquinoline derivatives: A new class of 5-HT1A receptor ligands. *Bioorg. Med. Chem.* 7: 287–295.

Moore, R.Y. and Halaris, A.E. (1975). Hippocampal innervation by serotonin neurons of the midbrain raphe in the rat. *J. Comp. Neurol.* 164: 171–183.

Nitz, D.A. and McNaughton, B.L. (1999). Hippocampal EEG and unit activity responses to modulation of serotonergic median raphe neurons in the freely behaving rat. *Learn. Mem.* 6: 153–167.

Oleskevich, S. and Descarries, L. (1990). Quantified distribution of the serotonin innervation in adult rat hippocampus. *Neuroscience* 34: 19–33.

Parsons, C.G., Danysz, W., and Quack, G. (1999). Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist—A review of preclinical data. *Neuropharmacology* 38: 735–767.

Peterson, C., Neal, J.H., and Cotman, C.W. (1989). Development of N-methyl-D-aspartate excitotoxity in cultured hippocampal neurons. *Dev. Brain Res.* 48: 187–195.

Pringle, A.K., Self, J., Eshak, M., and Iannotti, F. (2000). Reducing conditions significantly attenuate the neuroprotective efficacy of competitive, but not other NMDA receptor antagonists in vitro. *Eur. J. Neurosci.*12: 3833–3842.

Sharif, N.A., Drace, C.D., Williams, G.W., and Crider, J.Y. (2004). Cloned human 5-HT1A receptor pharmacology determined using agonist binding and measurement of cAMP accumulation. *J. Pharm. Pharmacol.* 56: 1267–1274.

Shimono, K., Brucher, F., Granger, R., Lynch, G., and Taketani, M. (2000). Origins and distribution of cholinergically induced beta rhythms in hippocampal slices. *J. Neurosci*. 20: 8462–8473.

Singer, W. (1999). Neuronal synchrony: A versatile code for the definition of relations? *Neuron* 24: 49–65, 111–125.

Sirvio, J., Larson, J., Quach, C.N., Rogers, G.A,, and Lynch, G. (1996). Effects of pharmacologically facilitating glutamatergic transmission in the trisynaptic intrahippocampal circuit. *Neuroscience*. 74: 1025–1035.

Staubli, U. and Lynch, G. (1987). Stable hippocampal long-term potentiation elicited by 'theta' pattern stimulation. *Brain Res.* 435: 227–234.

Stoppini, L., Buchs, P.A., and Muller, D. (1991). A simple method for organotypic cultures of nervous tissue. *J. Neurosci. Meth.* 37: 173–182.

Traub, R.D., Bibbig, A., Fisahn, A., LeBeau, F.E., Whittington, M.A., and Buhl, E.H. (2000). A model of gamma-frequency network oscillations induced in the rat CA3 region by carbachol in vitro. *Eur. J. Neurosci*. 12: 4093–4106.