

Cellular electrophysiological changes in the hippocampus of freely behaving rats during local microdialysis with epileptogenic concentration of N-methyl-D-aspartate

Nandor Ludvig* and Hai Michael Tang

Department of Physiology and Pharmacology, State University of New York, Health Science Center at Brooklyn, Brooklyn, NY, USA

[Received 15 July 1999; Revised 2 September 1999; Accepted 2 September 1999]

ABSTRACT: N-methyl-D-aspartate (NMDA) receptor dysfunctions are thought to be involved in the pathophysiology of seizures of hippocampal origin. While the cellular effects of excessive NMDA receptor stimulation have been widely studied *in vitro*, no data are available on the sequence of cellular electrophysiological events that follow the overstimulation of hippocampal NMDA receptors in awake, behaving subjects. Therefore, the present study addressed this problem. Intrahippocampal microdialysis with 500 μ M NMDA was performed in freely behaving rats, and the electrical activity of single neurons in the dialysis area were monitored. In all recorded neurons ($n = 9$), regardless of their type, NMDA induced a long-lasting electrical silence preceded in most cells by a brief but robust firing rate increase. During these firing rate increases, place cells lost the spatial selectivity of their discharges, and a gradual reduction in the amplitude of the action potentials was also observed. Remarkably, electroencephalographic (EEG) seizures developed exclusively after the appearance of cellular electrical silence in the recording/dialysis site. The NMDA-induced electrophysiological changes were reversible. This study demonstrates that the combined single-cell recording–intracerebral microdialysis technique can be readily used for inducing focal epileptiform events in the hippocampus and monitoring the induced cellular electrophysiological events in behaving animals. © 2000 Elsevier Science Inc.

KEY WORDS: Seizure, Action potential, Place cells, EEG.

INTRODUCTION

Temporal lobe complex partial seizures are the single most common type of seizure in the adult population [44]. Approximately 20–50% of patients with such seizures are not satisfactorily controlled with the currently available antiepileptic drugs [11,44]. For these patients, surgical intervention is the only hope for cure. However, these interventions are not without risk and complications [11]. Therefore, searching for new therapeutical strategies for

the treatment of complex partial seizures is one of the most important challenges of epilepsy research.

The prerequisite of developing therapeutically effective strategies for the management of complex partial seizures is to understand the cellular and neurochemical/molecular processes which lead to epileptiform electrical events in temporal lobe structures, such as the hippocampus. Indeed, extensive neuronal loss in this structure is a frequent autopsy finding in patients with complex partial seizures [5,27]. Furthermore, in animals electrical and chemical stimulations of the hippocampus can produce ictal electroencephalographic (EEG) and behavioral symptoms (e.g., motionless stare, subsequent automatisms, etc.) which strongly resemble those occur in human temporal lobe epilepsy [11,21,32]. Therefore, the basic mechanisms of hippocampal epileptogenesis deserves attention.

It is increasingly clear that the neurochemical/molecular mechanisms of hippocampal epileptogenesis are diverse and may involve the complex interactions of ion channels, receptors, second messengers and genes [7,8,12,18]. This report focuses on the N-methyl-D-aspartate (NMDA) receptors, one of the four receptor types through which the effects of glutamatergic/aspartatergic neurotransmission are mediated [29]. In fact, receptor autoradiography and *in situ* hybridization studies both suggest the abundance of NMDA receptors in the dentate gyrus and hippocampus proper [30,39].

The involvement of NMDA receptors in hippocampal epileptiform events has been extensively studied in slice preparations *in vitro* [7,8,9,16], and in whole animals with EEG/microinjection [15,28] and EEG/microdialysis [21,40] techniques, *in vivo*. These investigations have established the basic concepts on the relationship between NMDA receptor activation and seizure genesis in hippocampus. Nevertheless, many problems have remained unresolved. How does NMDA receptor overstimulation alter the firing of hippocampal neurons in natural circumstances, during behavior? How do these cell firing alterations lead to the development of epileptic seizures? These fundamental questions cannot be an-

* Address for correspondence: Nandor Ludvig, M.D., Ph.D., Department of Physiology and Pharmacology, State University of New York, Health Science Center at Brooklyn, 450 Clarkson Avenue, Box 31, Brooklyn, NY 11203, USA. Fax: +1-(718)-270-3103; E-mail: ludvin10@bmc.hscbklyn.edu

swered with the mentioned neuroscience methods. Therefore, we applied the combined single-cell recording–intracerebral microdialysis technique [22,23] to obtain an insight into the cellular electrophysiological changes that take place in the hippocampus during localized perfusions of epileptogenic concentration of NMDA in freely behaving rats. This also provided an opportunity for us to determine whether the combined single-cell recording–intracerebral microdialysis method is a reliable and useful technique to examine the cellular mechanisms underlying hippocampal seizures.

MATERIALS AND METHODS

Animals

Five male Long–Evans rats (250–350 g) were used in this study. They were trained to chase food pellets in a large test chamber according to Muller et al. [34]. The rats were treated according to the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996), and were subjected to experiments approved by the Institutional Animal Care and Use Committee of State University of New York, Health Science Center at Brooklyn. The rats were chronically implanted with a microelectrode/microdialysis probe guide assembly.

Microelectrode/Microdialysis Probe Guide Assembly

The manufacturing of this device has been described previously [22]. In this study, an advanced version of the original design was employed. Each assembly was composed of three custom-made driving screws (Small Parts, Inc., Miami Lakes, FL, USA), a dual row socket (Mill-Max MFG Corp., Oyster Bay, NY, USA), an array of eight nichrome microwires, 25 mm diameter each (California Fine Wire Company, Grover Beach, CA, USA) placed in a 26-G stainless tube, and a microdialysis probe guide (Can-Do Services, Hopewell Junction, NY, USA). The microwires, as well as a larger grounding wire, were connected to the pins of the socket with Silver Print conductive paint (Newark Electronics, Newark, NJ, USA), while the four basic components of the assembly were glued together with Krazy Glue® and dental cement.

Surgical Implantation of the Microelectrode/Microdialysis Probe Guide Assembly

The rats were anesthetized with 60 mg/kg i.p. pentobarbital and placed in a stereotaxic apparatus. The skull was exposed, four anchoring screws were placed in the frontal and occipital bones, and a 2-mm diameter craniotomy was made with a dental drill. The coordinates for the center of this hole were: 3.5 mm posterior to the bregma and 3.0 mm left to the midline, according to the atlas of Paxinos and Watson [37]. The dura mater and pia mater were excised. The microelectrode-array, cleaned with 100% ethanol, was introduced stereotaxically through the center of the hole into the brain. The tip of the microelectrode-array was placed 1.5 mm below the surface of the brain, just above the hippocampus. The hole was closed with sterile bone wax, and the assembly was secured to the skull with dental cement applied around the driving screws and the anchoring screws. The grounding wire of the assembly was connected to one of the anchoring screws in the occipital bone with Silver Print conductive paint. Thus, this screw also served as a grounding/reference electrode. The assembly was covered with sterile tape, and the skin was approximated. The animals were introduced into the simultaneous single-cell recording and microdialysis sessions after a 3-day postsurgical recovery.

Simultaneous Single-Cell Recording and Microdialysis in the Hippocampus

In the first day of the recording/dialysis sessions, the experimental animal was transferred to a sound-attenuating Faraday-cage (135 cm width × 135 cm length × 240 cm height). In the center of this cage was a cylindrical test chamber of 70 cm diameter and 50 cm height. The rat was lightly anesthetized with 75 mg/kg i.p. ketamine and the microdialysis probe was inserted in the brain through the probe guide. We used slightly modified versions of the Sved-Curtis concentric probe [43]. The exposed dialysis fiber (18,000 MWCO; 200 μm i.d.) was 800 μm long, extending 400 μm below the tip of the microelectrode array. The horizontal distance between the probe and the electrodes was 400–500 μm. The microdialysis fluid flow was maintained with a syringe pump at a 10 μl/min flow rate and run through a valve (Valco instruments, Houston, TX, USA) and a liquid swivel before entering the inserted probe. The control microdialysis solution was artificial cerebrospinal fluid (ACSF; 150 mM Na, 155 mM Cl, 1.4 mM Ca, 3.0 mM K, 0.8 mM Mg, 1.0 mM P; total osmolarity: 311.2 mOsm; pH = 7.4) filtered through Nylon Acrodisc syringe filters. Immediately after probe insertion, the recording cable was connected to the socket of the assembly. Until the next morning, the brain tissue was allowed to recover from the microtrauma caused by the probe insertion. In this period, the rat rested in a small box inside the cylinder.

In the second day, the electrode/probe unit was advanced into the hippocampus, in 50-μm steps, by rotating the driving screws. This time the extracellular electrical signals, the local EEG waves, and the movement pattern of the rat were monitored, as described [24]. Briefly, the extracellular signals were recorded between microelectrode pairs. The signals were led through a commutator, amplified (10,000×), filtered (between 300 Hz and 10,000 Hz), displayed on oscilloscopes, and digitized at a sampling rate of 40,000 Hz. The EEG waves were recorded between one of the microelectrodes and the grounding/reference screw electrode. These signals were also led through a commutator, amplified 1,000 times, filtered between 0.1 Hz and 50 Hz, and displayed on an oscilloscope. In some experiments, they were also digitized. Movement artifacts were eliminated from the recordings with impedance-lowering operational amplifiers built in the recording cable. The head-position of the animal was followed by a video-tracker (Ebtronics, Elmont, NY, USA) via a camera secured to the ceiling of the Faraday cage. The video-tracker detected a light-emitting diode (LED) built in the recording cable close to the head of the rat, and generated digitized X and Y coordinates for this light source. The digitized extracellular, EEG, and head-position data were collected simultaneously on the hard disk of a computer, in a DOS environment, with the use of the Discovery data acquisition software (DataWave Technologies, Inc., Longmont, CO, USA). In addition, the behavior of the rat in the test chamber was watched on a monitor.

The electrode/probe unit was advanced until action potentials with at least 2.5 times higher amplitude than the 60–70 μV background noise were recorded over an at least 30 min period. Then the type of each recorded neuron was identified. According to well-established criteria [4,14,24,38], fast-firing interneurons, non-spatial complex-spike (pyramidal) cells, and place cells were identified. Then the NMDA perfusion started.

NMDA Perfusions

The rat was placed into the cylindrical test chamber, and the valve was switched to direct the NMDA solution to the brain. Based on our previous EEG/microdialysis studies [21], the concentration of the epileptogenic NMDA solution was selected to be

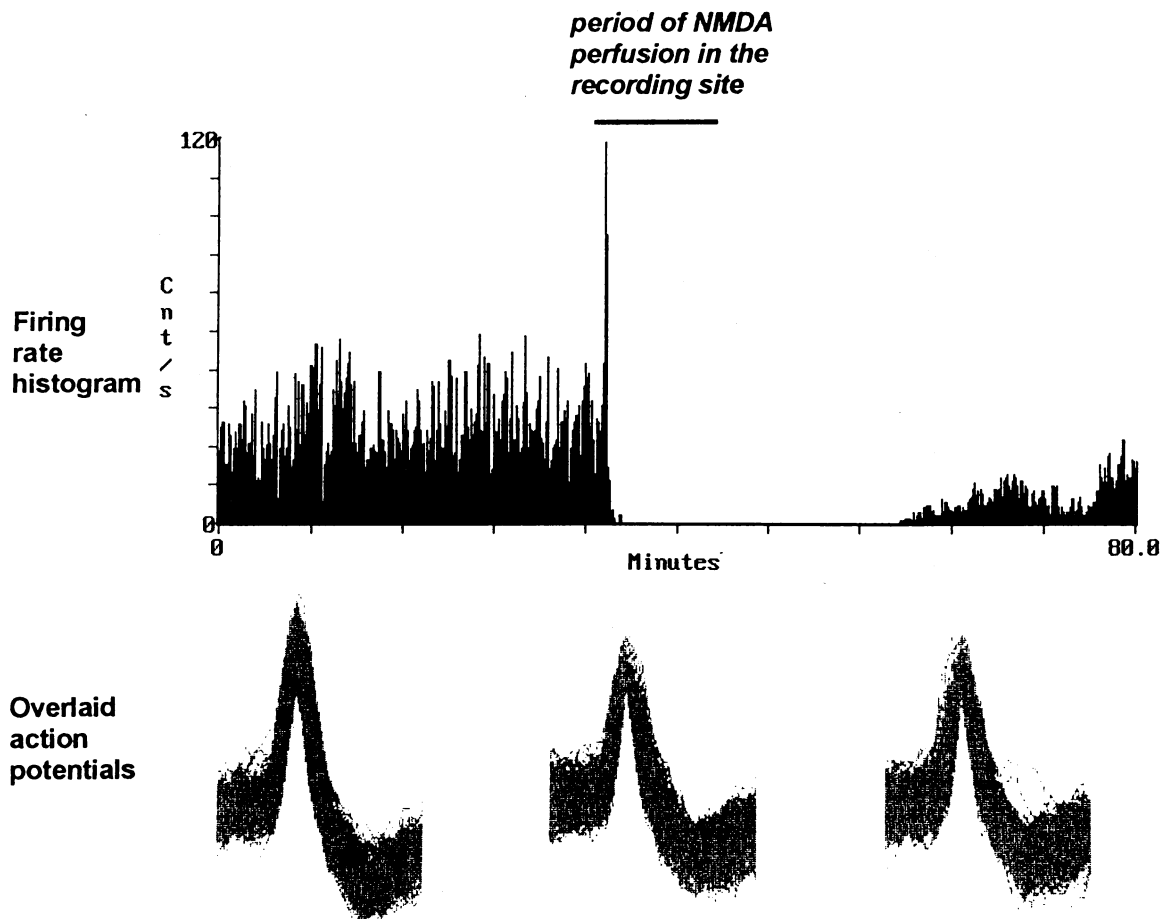


FIG. 1. The biphasic effect of intrahippocampal microdialysis with $500 \mu\text{M}$ N-methyl-D-aspartate (NMDA) on the firing of an interneuron in the recording site. The Y axis of the histogram represents the number of action potentials per second (cnt/s); the X axis represents the data collection time (80 min). During data collection, the rat was moving around in the test chamber. Note that the fairly constant, 20–40 action potential/s basal firing rate dramatically changes during the NMDA exposure: a brief firing rate increase to as high as 120 action potential/s is followed by an approximately 20 min electrical silence. As the drug is washed out from the recording/dialysis site, the cell regains its electrical activity. The overlaid action potentials of the cells (800- μs segment for each; maximum amplitude: $250 \mu\text{V}$; negativity up) are also shown. Each waveform comprises 600–800 individual action potentials, as they were displayed before during and after the NMDA perfusion. The similarity of the waveforms prove that the recordings were made from the same single neuron.

$500 \mu\text{M}$. The pH of the drug solution was brought to 7.4. Due to the distance between the valve and the inserted probe, the drug solution reached the recording/dialysis site after a 20–25 min delay. Each NMDA perfusion lasted for 10–15 min, after which the drug was washed out from the recording/dialysis site with ACSF. Data files were generated for the periods before, during and after the hippocampal NMDA exposure.

Histology

After the end of the experiments, the animals were euthanized with 100 mg/kg i.p. pentobarbital. Their brain was removed, frozen in liquid N_2 , and sectioned with a cryostat. Forty- μm thick sections were collected, Nissl-stained, and examined with a light microscope to verify the localization of the track of the microelectrode/microdialysis probe unit. These units were identified to be in the CA1 ($n = 2$) and CA3 ($n = 3$) regions, with the dialysis fiber partially penetrating into the dentate gyrus. No excitotoxic lesions were observed in the histological preparations.

Data Analysis

The extracellular, EEG, and behavioral data were analyzed both on-line and off-line. The on-line analysis included: (1) observing the rat's behavior on the video monitor, (2) printing the EEG traces displayed on one of the oscilloscopes and examining the waves, and (3) documenting visible changes in the firing pattern of the detected cells. The off-line analysis was accomplished with the CP Analysis program package of DataWave Technologies and the Mapmaker program of ESCO (Mt. Kisco, NY, USA). First, the action potentials were discriminated, as described [22]. Using the discriminated action potentials, firing rate histograms (Fig. 1) and firing rate maps (Fig. 2) were generated. A detailed description of these maps is available elsewhere [23]. The action potentials belonging to a single neuron were overlaid and examined (Fig. 1). Important segments of the single-cell recordings, alone (Fig. 3) or together with simultaneously obtained EEG recordings (Fig. 4), were played back and examined. In addition, for each cell its maximum firing rates (spike per

second) before and after the NMDA perfusion were calculated with the CP Analysis program. These values were compared with paired *t*-test. Since the basal firing frequency of the cells varied within a wide range, the logarithm of the maximum firing rates were used, as recommended by Motulsky [33].

RESULTS

The action potentials of nine cells were recorded in the 5 animals. Out of the nine neurons, one was identified as interneuron, six as non-spatial pyramidal cell, and two as place cell. In all neurons, the NMDA perfusions induced firing pattern changes which do not occur spontaneously (Figs. 1–4). These drug-induced firing pattern changes developed in four rats 25.0 ± 2.7 min (mean \pm SEM) after switching the valve to direct the drug solution to the brain, while in the fifth animal the effect developed after a 41-min delay. These delays were due to the slow flowing of the drug solution from the valve to the probe and to the diffusion of the NMDA molecules from the probe to the recorded cells.

The predominant cellular electrophysiological action of NMDA was a biphasic effect. Namely, the drug first induced a brief, 5–120 s firing rate increase, which was followed by a 8–14-min period when the cell did not discharge action potentials. This cellular electrical silence was always reversible, and after washing out the drug from the recording site with ACSF, the neurons regained their electrical activity (Fig. 1). This biphasic effect was apparent in six of the nine cells. The NMDA-induced initial firing rate increase from 15.45 ± 9.0 action potential/second to 46.57 ± 20.82 action potential/sec (mean \pm SEM) was statistically significant ($p < 0.05$; $t = 4.034$; $df = 5$). In the remaining three cells the drug induced solely electrical silence. Thus, electrical silence developed in all recorded cells.

In the place cells, the NMDA perfusion increased not only the rate of the location-specific discharges, but also the rate of the background firing. Thus, as the rat moved around during the initial NMDA exposure the place cells fired at every location. As a result, the spatial selectivity of the firing of these neurons was lost. This is demonstrated in Fig. 2.

The cellular electrophysiological effects of NMDA included not only firing frequency changes, but also alterations in the waveform of the action potentials. However, this was apparent only in those neurons ($n = 3$) which responded to the NMDA exposure with a very large initial firing rate increase. In these cells, the drug caused a gradual, 17–29% decrease in the amplitude of the action potentials (Fig. 3).

In all rats, the NMDA perfusion caused epileptiform EEG spikes. The duration of these EEG seizures varied between 5 and 40 s, and were not accompanied with behavioral symptoms. The most striking feature of these EEG seizures was that they never developed during the periods when the cells increased their firing. Instead, these abnormal EEG events developed only after the appearance of the cellular electrical silence. This phenomenon is clearly demonstrated in Fig. 4.

DISCUSSION

In this study, intrahippocampal microdialysis was performed in freely moving rats and the firing of single neurons in the dialysis area was monitored. For 10–15-min periods, the control microdialysis fluid was replaced with 500 μ M NMDA. It was previously shown that approximately 15% of the perfused NMDA molecules diffuses out from the microdialysis probe to the surrounding tissue [21]. This manipulation resulted in a brief, initial firing rate increase and a subsequent, longer-lasting electrical silence in the majority of the cells, while the rest of the recorded neurons

responded solely with electrical silence. The pH of the NMDA solution was 7.4 and its osmolarity was 311.7 mOsm. During the NMDA perfusions, as fine cellular functions could be followed in the dialysis area as the firing of place cells. The observed firing pattern changes, which do not occur spontaneously in hippocampal neurons, developed in the intervals when the NMDA molecules passed through the microdialysis fiber. After washing out the drug from the recording/dialysis site, the electrical activity of the cells recovered. In similar experimental conditions, potassium ions or lidocaine were found to cause different cellular electrophysiological effects [22,23]. Therefore, it can be concluded that the firing pattern changes described in this study were not due to technical error, cell death, or non-specific drug action, rather they were due to specific, NMDA-induced pharmacological effects.

It should be emphasized that while we recorded the action potentials of single neurons at the tip of the microelectrode array, the NMDA molecules diffusing out of the nearby microdialysis probe affected the microenvironment of many cells and axon terminals/fiber varicosities within the recording/dialysis site. As a consequence, the described cellular NMDA effects could be due to several mechanisms. First, these effects could be due to direct stimulation of the NMDA receptors located on the dendrites and/or soma of the recorded cells. However, they could also be due to stimulation of NMDA receptors located on the dendrites and/or soma of neighboring neurons. Indeed, postsynaptic NMDA currents operate in both pyramidal cells and interneurons in the CA1 region [31]. Furthermore, the observed NMDA effects could also be due to stimulation of presynaptic NMDA receptors located on neighboring axon terminals and fiber varicosities. In fact, in the hippocampus NMDA receptor stimulation has been found to increase the release of γ -aminobutyric acid and noradrenaline and to decrease that of acetylcholine and serotonin [45]. It is likely that all of these mechanisms contributed to the detected NMDA effects, perhaps to a different extent for each cell. We add, that although the length of the microdialysis fibers was as short as 800 μ m, a spillover of a portion of the diffused NMDA molecules to the dentate gyrus might also took place. These may explain the variability of the magnitude and duration of the firing rate increases and why biphasic and solely silencing cellular effects were both observed. Unfortunately, our recording/dialysis method was not exploited in its full potential. Simultaneous ensemble recordings from a large number of neurons, neurotransmitter analysis of the dialysates, and staining of the dialysis area were not conducted. Therefore, the presumably complex synaptic mechanisms of the NMDA actions revealed in this study await for future experiments.

The EEG seizures induced by hippocampal NMDA perfusions developed exclusively after a period of cellular electrical silence within the perfusion site. This electrical silence might be due to interneuron-mediated inhibition, depolarization block, or, as suggested by Fig. 4, spreading depression [19]. The phenomenon showed that in a hippocampal epileptogenic focus the very neurons which are responsible for generating the electrographic seizures can actually cease to fire during the emergence of the paroxysmal spikes. It is possible that the initial firing rate increase of the neurons in the focus induce similar firing rate increases, and eventual hypersynchronization, in neighboring hippocampal and synaptically connected extrahippocampal circuitries. When the number of neurons recruited into this pathophysiological network activity reaches a critical mass, electrographic seizure develops, even though the neurons in the focus already terminated their firing. This is consistent with the idea that “. . . complex partial, and perhaps even simple partial, seizures would seem to require the propagation of the ictal discharge to adjacent and distant structures. . . .” [12]. Clearly, to solve this problem simultaneous, multichannel EEG and single-cell recordings in distant hippocam-

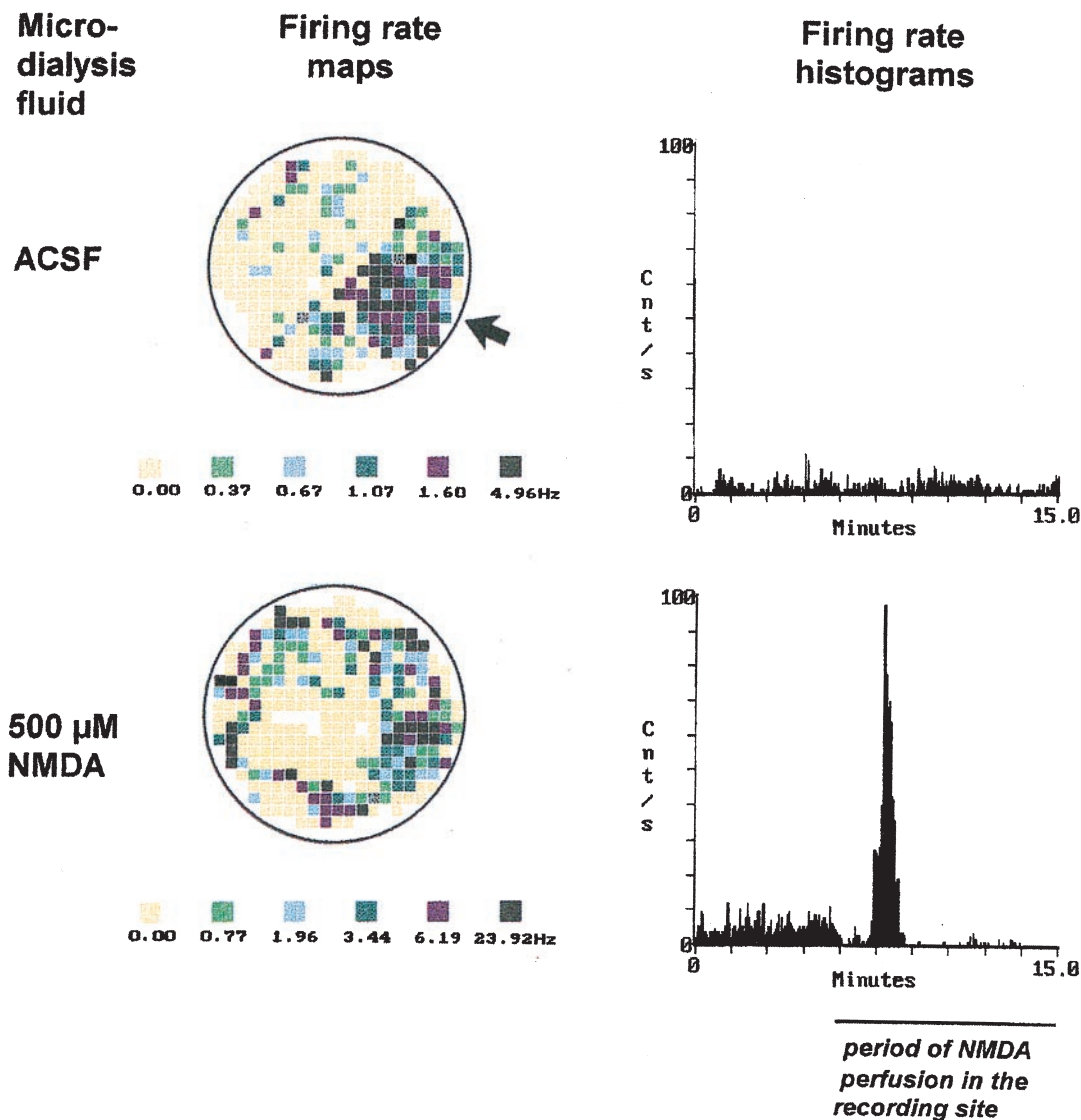


FIG. 2. The spatial selectivity impairing effect of intrahippocampal microdialysis with 500 μ M N-methyl-D-aspartate (NMDA) on the firing of a local place cell. Firing rate maps and the corresponding firing rate histograms are shown, as indicated. The data were obtained during microdialysis with artificial cerebrospinal fluid (ACSF) and a subsequent (15 min) microdialysis with the NMDA solution, while the rat was moving around in the cylindrical test chamber. The firing rate maps demonstrate the average firing rates of the same place cell in the areas of the pixels. The color code for the firing rate are given below the maps. Yellow represents the areas where the cell did not fire; black represents the areas where the cell fired with the highest firing rates; white represents areas which the rat did not visit. Note that in the control condition, when ACSF is perfused, the cell displays high frequency firing only in a restricted location of the test chamber (arrow). During NMDA perfusion this characteristic firing pattern is lost: the cell exhibits spatially diffuse electrical activity. Also note on the histograms the dramatic but brief firing rate increase during the NMDA exposure and the subsequent electrical silence.

pal areas, as well as in other limbic regions, are needed. It should be added, however, that cellular firing rate changes analogous to those described in this study have not been observed in the few extracellular recordings from temporal lobe epilepsy patients [1,2].

An interesting finding of this study was the loss of spatial selectivity of the firing of place cells during the initial NMDA exposure. We observed a similar effect in these neurons when we perfused high concentration of K^+ in their extracellular milieu [23]. Place cells are pyramidal cells, which manifest a unique firing pattern during movement in a large space. Namely, they

markedly increase their firing rate at specific spatial sites, while produce only low frequency background discharges at other locations [24,34,35,36]. It has been proposed that the network of hippocampal place cells forms a cognitive map [35,36]. Based on the recent finding that these cells can flexibly terminate and develop their spatial firing during prolonged environmental exposure, an alternative theory was presented [24]. Accordingly, the high frequency firing of hippocampal place cells may serve to create permanent memory engrams for specific spatial sites in extrahippocampal areas, possibly in the association cortex. Regardless of

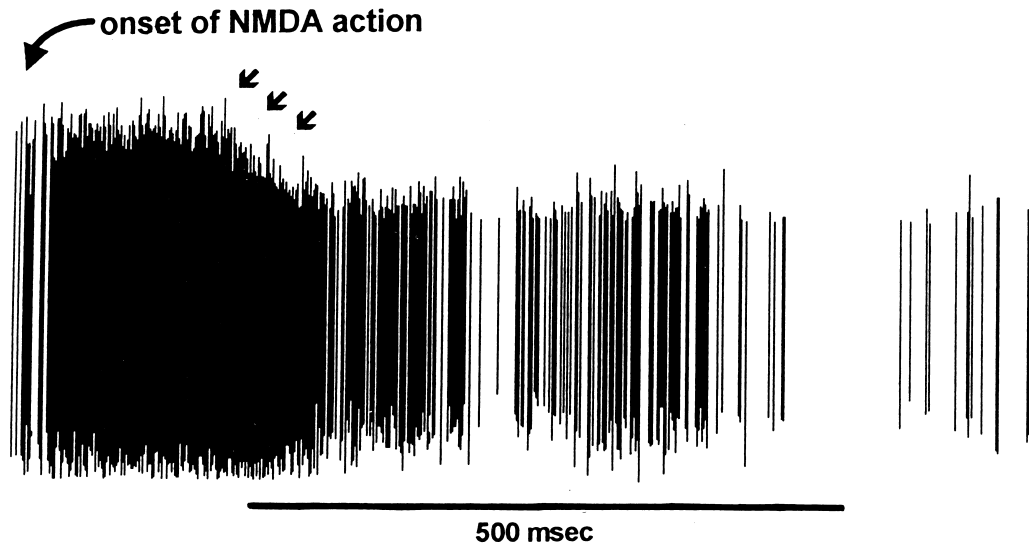


FIG. 3. The gradually decreasing amplitude of the action potentials of a hippocampal interneuron during microdialysis with $500 \mu\text{M}$ N-methyl-D-aspartate (NMDA) in the recording site. Each vertical line is an action potential generated by the cell; time calibration as indicated. Note the robust increase of the frequency of the action potentials shortly after the NMDA exposure, and that the amplitude of the action potentials (maximum = $250 \mu\text{V}$) is decreasing (arrowheads). The gradual nature of this decrease indicates waveform changes in the same cell.

which theory is accurate, the neurophysiological characteristics of place cells suggest that they are parts of the cognitive system and are involved in learning and memory formation. Considering the well-established role of NMDA receptors in plastic hippocampal processes [26], it is important to understand how these receptors

contribute to the determination of place cell firing. The effects of NMDA receptor blockade on place cell firing was examined by Kentros et al. [17]. The present study showed that excessive NMDA receptor stimulation in the hippocampus deteriorates the delicate electrical activity of place cells by increasing their non-

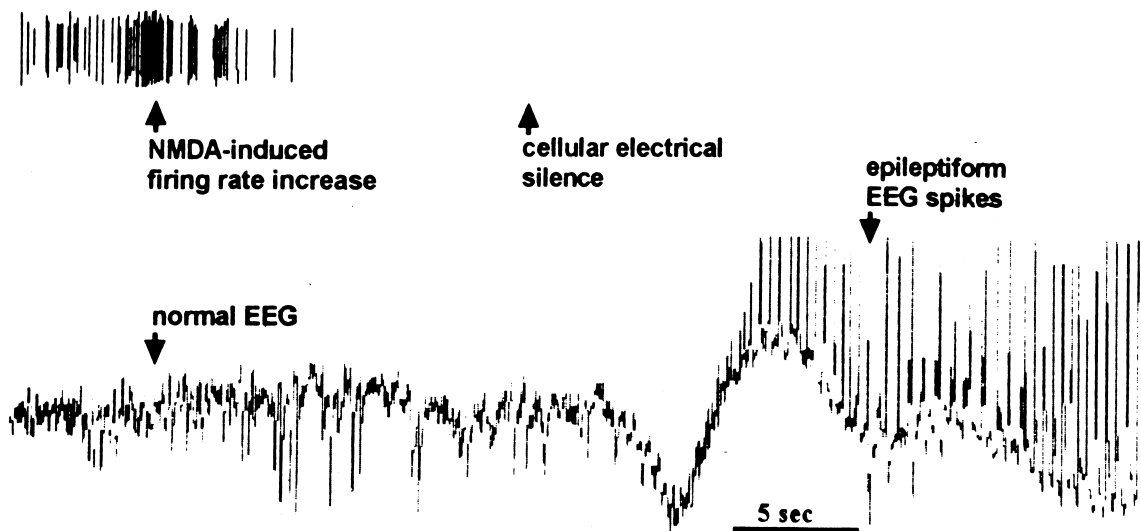


FIG. 4. The development of epileptiform electroencephalogram (EEG) spikes during cellular electrical silence induced by intrahippocampal microdialysis with $500 \mu\text{M}$ N-methyl-D-aspartate (NMDA) in a freely moving rat. Traces of single-cell activity and EEG waves, recorded simultaneously from the dialysis site, are shown. Upper trace: action potentials from a non-spatial pyramidal cell; lower trace: local EEG waves. Time calibration as indicated. Maximum amplitude: $270 \mu\text{V}$ for single-cell recordings; $1,500 \mu\text{V}$ for EEG recordings. Note that the NMDA-induced firing rate increase is followed by cellular electrical silence, and it is clearly this period when the epileptiform spikes develop. Also note the DC potential shift, a possible sign of spreading depression, prior to the epileptiform EEG activity.

spatial background firing. Such cellular dysfunction may well contribute to the amnesia with regard to complex partial seizures. Whether lower, subepileptogenic concentrations of NMDA also exert a deteriorating effect on place cell firing is a problem that deserves further studies, especially since they may shed new lights on the relationship between seizures and cognition.

Another intriguing observation was that the amplitude of the action potentials of the most intensely firing neurons decreased. The variability of the amplitudes of hippocampal pyramidal cells, including place cells, has been known [4,14,24,38], but this study is the first to demonstrate that such amplitude changes can be induced by pharmacological means in the behaving animal. This supports the idea that the production of action potentials in hippocampal neurons is not a uniform process. In these cells, differential back-propagation of axonally originated spikes to the distal dendrites [25] and differential forward-propagation of dendritically originated spikes to the soma [46] can both take place. These processes may be influenced by excessive NMDA receptor stimulation, resulting in descending action potential amplitudes when very high firing rates are sustained.

Our study demonstrated the ability of the combined single-cell recording–intracerebral microdialysis method to create an epileptogenic focus in the hippocampus of awake, behaving animals and to monitor the developing cellular electrophysiological events. Indeed, since we introduced the integration of EEG and microdialysis techniques into epilepsy research in 1990 [20] many other groups adopted this experimental approach [10,13,40,41]. Thus, with further improvements, the methodology described in this paper can contribute to the understanding of the cellular and neurochemical/molecular bases of hippocampal epileptogenesis. Furthermore, although integrative cellular neuropharmacological techniques are not yet used in antiepileptic drug research [3], in the future, such methods can help to recognize and characterize novel drugs with antiepileptic potential. Finally, the relative ease to electrophysiologically monitor and pharmacologically manipulate a discrete brain site suggests that this arrangement may have therapeutic implications, as well. Chronically implanted depth electrodes [42] and intracerebral catheters [6] have long been used in human patients. These two devices can be combined and equipped with a miniature, portable electrophysiological analysis/drug delivery system. This would make it possible to monitor the electrical activity of an epileptic focus and, based on the characteristics of the incoming signals, pharmacologically control the function of the abnormal tissue. Whether the use of such hybrid neuroprosthetic devices is a viable strategy for the management of intractable epileptic seizures remains to be investigated.

ACKNOWLEDGEMENTS

This study was supported by National Institutes of Health grants AA10814 and MH56800 and by a Research Investment Initiative Grant from State University of New York, Health Science Center at Brooklyn to Nandor Ludvig.

REFERENCES

- Babb, T. L.; Carr, E.; Crandall, P. H. Analysis of extracellular firing patterns of deep temporal lobe structures in man. *Electroencephalogr. Clin. Neurophysiol.* 34:247–257; 1973.
- Babb, T. L.; Wilson, C. L.; Isokawa-Akesson, M. Firing patterns of human limbic neurons during stereoencephalography (SEEG) and clinical temporal lobe seizures. *Electroencephalogr. Clin. Neurophysiol.* 66:467–482; 1987.
- Bialer, M.; Johannessen, S. I.; Kupferberg, H. J.; Levy, R. H.; Loiseau, P.; Perucca, E. Progress report on new antiepileptic drugs: A summary of the fourth Eilat conference (EILAT IV). *Epilepsy Res.* 34:1–41; 1999.
- Buzsaki, G.; Penttonen, M.; Nadasdy, Z.; Bragin, A. Pattern and inhibition-dependent invasion of pyramidal cell dendrites by fast spikes in the hippocampus *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* 93:9921–9925; 1996.
- Dam, A. M. Epilepsy and neuron loss in the hippocampus. *Epilepsia* 21:617–629; 1980.
- Del Bigio, M. R. Biological reactions to cerebrospinal fluid shunt devices: A review of the cellular pathology. *Neurosurgery* 42:319–325; 1998.
- Dichter, M.; Ayala, G. Cellular mechanisms of epilepsy: A status report. *Science* 237:157–164; 1987.
- Dingledine, R.; McBain, C. J.; McNamara, J. O. Excitatory amino acid receptors in epilepsy. *Trends Pharmacol. Sci.* 11:334–338; 1990.
- Dorandeu, F.; Wetherell, J.; Pernot-Marino, I.; Tattersall, J. E. H.; Fosbraey, P.; Lallemand, G. Effects of excitatory amino acid antagonists on dendrotoxin-induced increases in neurotransmitter release and epileptiform bursting in rat hippocampus *in vitro*. *J. Neurosci. Res.* 48:499–506; 1997.
- During, M. J.; Spencer, D. D. Adenosine: A potential mediator of seizure arrest and postictal refractoriness. *Ann. Neurol.* 32:618–624; 1992.
- Engel, J. Jr. *Seizures and epilepsy*. Philadelphia: F. A. Davis Company; 1989.
- Engel, J. Jr.; Dichter, M. A.; Schwartzkroin, P. A. Basic mechanisms of human epilepsy. In: Engel, J. Jr.; Pedley, T. A., eds. *Epilepsy: A comprehensive textbook*. Philadelphia: Lippincott-Raven Publishers; 1997:499–512.
- Engel, J. Jr. Research on the human brain in an epilepsy surgery setting. *Epilepsy Res.* 32:1–11; 1998.
- Fox, S. E.; Ranck, J. B. Jr. Electrophysiological characteristics of hippocampal complex-spike cells and theta cells. *Exp. Brain Res.* 41:399–410; 1981.
- Hallak, M.; Irtenskauf, S. M.; Janusz, C. A.; Cotton, D. B. Stimulation and inhibition of N-methyl-D-aspartate receptors in rats: Developing a seizure model. *Am. J. Obstet. Gynecol.* 169:695–700; 1993.
- Isokawa, M.; Levesque, M. F. Increased NMDA responses and dendritic degeneration in human epileptic hippocampal neurons in slices. *Neurosci. Lett.* 132:212–216; 1991.
- Kentros, C.; Hargreaves, E.; Hawkins, R. D.; Kandel, E. R.; Shapiro, M.; Muller, R. U. Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. *Science* 280:2121–2126; 1998.
- Lason, W.; Turchan, J.; Przewlocki, R.; Machelska, H.; Labuz, D.; Przewlocka, B. Effects of pilocarpine and kainate-induced seizures on N-methyl-D-aspartate receptor gene expression in the rat hippocampus. *Neuroscience* 78:997–1004; 1997.
- Leao, A. A. P. Spreading depression. In: Purpura, D. P.; Penry, J. K.; Woodbury, D. M.; Tower, D. B.; Walter, R. D., eds. *Experimental models of epilepsy*. New York: Raven Press; 1972:174–196.
- Ludvig, N.; Mishra, P. K.; Burger, R. L.; Lasley, S. M.; Dailey, J. W.; Jobe, P. C. Combination of intracerebral microperfusion/microdialysis and EEG techniques: A new tool for studying the pathology of experimental seizures. *Epilepsia* 31:659; 1990.
- Ludvig, N.; Mishra, P. K.; Yan, Q. S.; Lasley, S. M.; Burger, R. L.; Jobe, P. C. The paradoxical effect of NMDA receptor stimulation on the electrical activity of the sensorimotor cortex in freely behaving rats: Analysis by combined EEG-intracerebral microdialysis. *Synapse* 12:87–98; 1992.
- Ludvig, N.; Potter, P. E.; Fox, S. E. Simultaneous single-cell recording and microdialysis within the same brain site in freely behaving rats: A novel neurobiological method. *J. Neurosci. Meth.* 55:31–40; 1994.
- Ludvig, N.; Chao, K.; Altura, B. T.; Altura, B. M.; Fox, S. E. Manipulation of pyramidal cell firing in the hippocampus of freely behaving rats by local application of K⁺ via microdialysis. *Hippocampus* 6:97–108; 1996.
- Ludvig, N. Place cells can flexibly terminate and develop their spatial firing. A new theory for their function. *Physiol. Behav.* 67:57–67; 1999.
- Magee, J. C.; Johnston, D. A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* 275:209–213; 1997.
- Malenka, R. C.; Nicoll, R. A. NMDA-receptor-dependent synaptic

- plasticity: Multiple forms and mechanisms. *Trends Neurosci.* 16:521–527; 1993.
27. Mathern, G. W.; Babb, T. L.; Armstrong, D. L. Hippocampal sclerosis. In: Engel, J. Jr.; Pedley, T. A., eds. *Epilepsy: A comprehensive textbook*. Philadelphia: Lippincott-Raven Publishers; 1997:133–155.
 28. McMaster, O. G.; Du, F.; French, E. D.; Schwarcz, R. Focal injection of aminooxyacetic acid produces seizures and lesions in rat hippocampus: Evidence for mediation by NMDA receptors. *Exp. Neurol.* 113:378–385; 1991.
 29. Meldrum, B. S. The role of glutamate in epilepsy and other CNS disorders. *Neurology* 44(suppl. 8):S14–S23.
 30. Monyer, H.; Burnashev, N.; Laurie, D. J.; Sakmann, B.; Seeburg, P. H. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12:529–540; 1994.
 31. Morin, F.; Beaulieu, C.; Lacaille, J.-C. Membrane properties and synaptic currents evoked in CA1 interneuron subtypes in rat hippocampal slices. *J. Neurophysiol.* 76:1–16; 1996.
 32. Moshe, S. L.; Ludvig, N. Kindling. In: Pedley, T. A.; Meldrum, B. S., eds. *Recent advances in epilepsy*. New York: Churchill Livingstone; 1997:21–44.
 33. Motulsky, H. *Intuitive biostatistics*. Oxford: Oxford University Press; 1995.
 34. Muller, R. U.; Kubie, J. L.; Ranck, J. B. Jr. Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. *J. Neurosci.* 7:1935–1950; 1987.
 35. Muller, R. A quarter of a century of place cells. *Neuron* 17:813–822; 1996.
 36. O'Keefe, J.; Nadel, L. *The hippocampus as a cognitive map*. Oxford: Clarendon Press; 1978.
 37. Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates* (2nd ed.). New York: Academic Press; 1986.
 38. Ranck, J. B. Jr. Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. *Exp. Neurol.* 41:461–555; 1973.
 39. Schroder, H. Cellular and subcellular distribution of receptors in the entorhinal-hippocampal system: Morphologic and biochemical aspects. *Hippocampus* 3:139–148; 1993.
 40. Sierra-Paredes, G.; Sierra-Marcuno, G. Microperfusion of picrotoxin in the hippocampus of freely moving rats through microdialysis probes: A new method of induce partial and secondary generalized seizures. *J. Neurosci. Meth.* 67:113–120; 1996.
 41. Smolders, I.; Khan, G. M.; Manil, J.; Ebinger, G.; Michotte, Y. NMDA receptor-mediated pilocarpine-induced seizures: Characterization in freely moving rats by microdialysis. *Br. J. Pharmacol.* 121:1171–1179; 1997.
 42. Spencer, S. S.; Sperling, M. R.; Shewmon, D. A. Intracranial electrodes. In: Engel, J. Jr; Pedley, T. A., eds. *Epilepsy: A comprehensive textbook*. Philadelphia: Lippincott-Raven Publishers; 1997: 1719–1747.
 43. Sved, A. F.; Curtis, J. T. Amino acid neurotransmitters in nucleus tractus solitarius: An in vivo microdialysis study. *J. Neurochem.* 61:2089–2098; 1993.
 44. Williamson, P. D.; Engel, J. Jr. Complex partial seizures. In: Engel, J. Jr.; Pedley, T. A., eds. *Epilepsy: A comprehensive textbook*. Philadelphia: Lippincott-Raven Publishers; 1997:557–566.
 45. Vizi, E. S.; Kiss, J. P. Neurochemistry and pharmacology of the major hippocampal transmitter systems: Synaptic and nonsynaptic interactions. *Hippocampus* 8:566–607; 1998.
 46. Wong, R. K. S.; Stewart, M. Different firing patterns generated in dendrites and somata of CA1 pyramidal neurons in guinea pig hippocampus. *J. Physiol.* 457:675–687; 1992.