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Application of the microelectrode-array (MEA) technology in pharmaceutical drug research

U. Egert[1)], H. Hämmerle[2) 3)]

[1)] Neurobiologie und Biophysik, Inst. Biol. III, Albert-Ludwigs Universität Freiburg,
[2)] Naturwissenschaftliches und Medizinisches Institut, Reutlingen (NMI)

Introduction

An increasing body of data collected in recent years indicates that the properties and the behavior of individual cells are dynamically modulated by the structure and activity of the embedding network. It is therefore necessary to evaluate manipulations of these, e.g. drug effects on a given neuron, in the context of the activity dynamics of the surrounding neuronal network. Understanding the interplay of cells within small, yet complex networks in vitro could thus improve the predictability of drug effects in the intact organism.

Electrophysiological recording techniques suitable to monitor the activity of neuronal populations in vitro have recently become available. These multi-electrode tools record spike activity and low frequency potentials with substrate integrated electrodes at several sites in the tissue. Besides, they facilitate collection of the sample sizes necessary for statistics. The possibility to electrically stimulate the tissue further expands the range of applications and bioassays, and may thus facilitate the evaluation of pharmacological test substances in industrial research.

The conditions in multielectrode recordings with planar electrode arrays are defined by several biophysical and biological factors. Obviously, the ultimate purpose of such experiments is to answer some biological question. The selection of designs at different scales, materials, data acquisition electronics and production techniques has to follow this goal in mind. From a bottom-up point of perspective, the metal-electrolyte interface and the type of contact to the cells define the interface between cell and electrode. This largely determines the frequency gain function and the signal-to-noise ratio of the recording, which in turn limits the range of biological signals, and thus the questions, that can be the target of the experiment. The Helmholtz-capacitance at the electrode/electrolyte interface defines the electrode impedance and the lower cut-off frequency. To record local field potentials or low-frequency components these should be as low as possible. At any one site in the extracellular space the local potential will be the sum of contributions from all current source within the recording horizon of the electrode. Depending on the tissue under investigation and the relevant time constants different electrode sizes will be suitable. Currently, two different technical

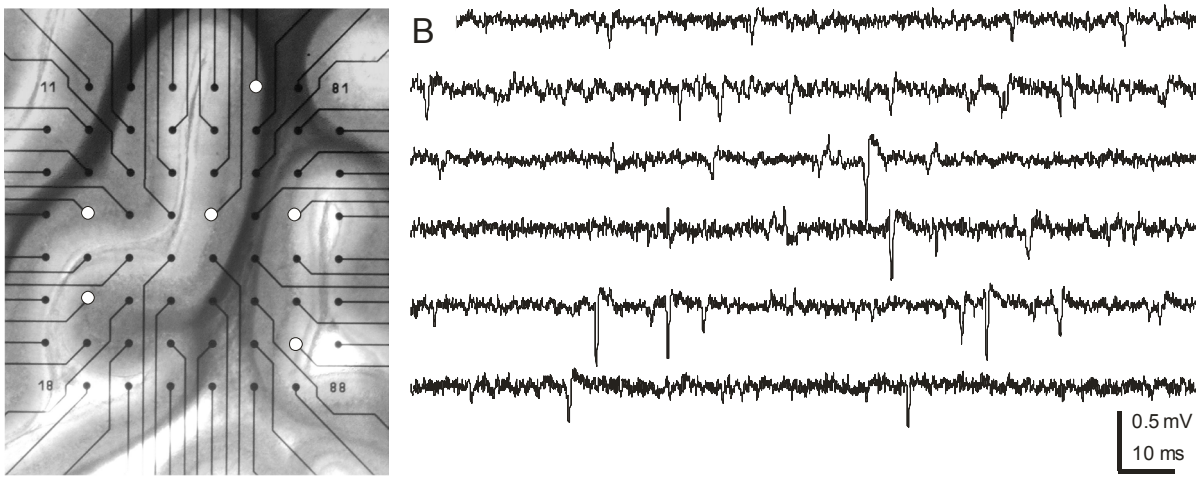

Fig. 1 Spike activity is the basic single neuron activity recorded with extracellular electrodes. In the cerebellum, for example, Purkinje cells are firing spontaneously. (**A**) The micrograph illustrates the structure and dimensions of an acute parasagittal slice of a rat cerebellum on an MEA. (**B**) Raw signal recorded at the positions marked in A. Spikes can be readily detected. The signal to noise ratio is typical for this preparation, and often even higher in organotypic cultures of such slices.

[3)] corresponding author, NMI Reutlingen, Markwiesenstr. 55, 72770 Reutlingen, Germany

approaches are pursued: an implementation of passive metal electrodes, mostly on planar substrates (Gross et al., 1977; Hämmerle et al., 1994; Nisch et al., 1994), and integrated FET arrays (Fromherz, 1999). Of these, the former ones are now well-established, reliable tools and commercially available (Multi Channel Systems GmbH, Reutlingen, Germany; Panasonic, Japan; Plexon, USA).
Single-unit recording in cultures of dissociated neuronal cells allows smaller electrodes of relatively high impedance, the dominant signal frequency being about 1 kHz. Such preparations are now used for drug and toxin screening (Gross et al., 1997), research on pattern and rhythm generation in networks (Jimbo et al., 1999), and lately to control virtual objects based on the activity in neuronal networks (Potter, 2001).

Results

The MEAs used in our laboratories have 60 electrodes of 10-30 µm diameters on a 200 µm grid, with coating of porous titanium nitride to minimize the impedance. The recording horizon of such an electrode has a radius of approx. 60 µm (Egert et al., 2001), the fields of view of the electrodes do not overlap. We analyze, for example, the modulation by dopaminergic drugs of spontaneous spike activity in acute slice preparations from the cerebellum of neonatal rats. Spikes are readily detected in these preparations (**Fig. 1**).
Field potential recordings, however, create different requirements, e.g. in acute and cultured slices from the hippocampus or cortex (Egert et al., 1998; Egert et al., 2001). In such preparations we study the genesis and spread of epileptic activity and its determinants, in-vitro models of neuronal regeneration and spatial aspects

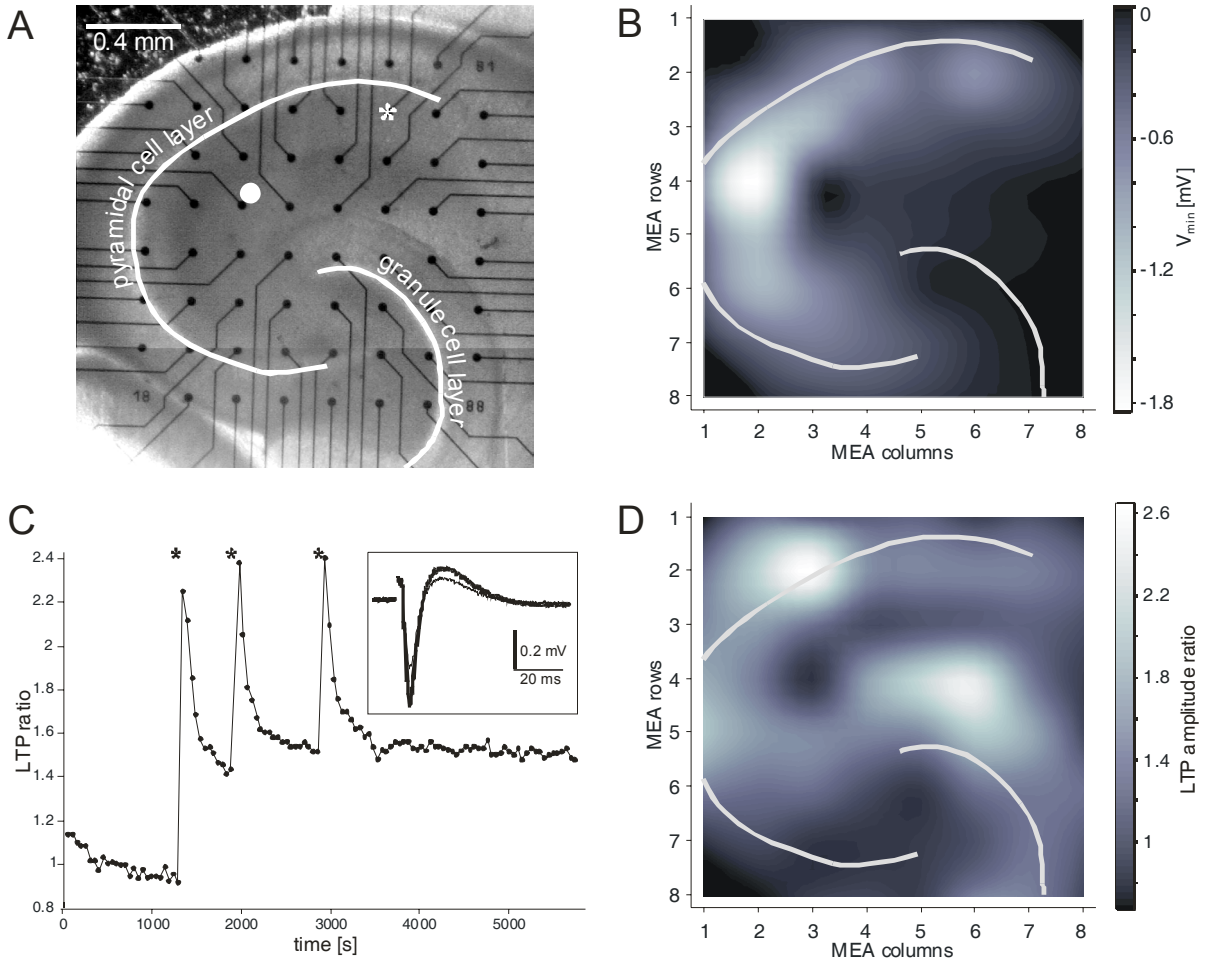

Fig. 2 Analysis of long-term potentiation (LTP) in an acute slice of the hippocampus. The distribution of field potential properties and changes in hippocampal slices (**A**) can be assessed with MEAs. (**B**) Population spike amplitudes induced by electrical stimulation (at the white dot in A) can reach more than 2 $mV_{peak\text{-}peak}$, with pronounced minima in the pyramidal cell layer and the proximal apical dendrites. (**C**) Brief bursts of stimuli (*, three repetitions) induced LTP, drastically increasing the response amplitude. The insert shows a typical response profile (thin line: before LTP induction, thick line: recorded at 3100 s). The responses recorded at the asterisk in A increased temporarily by up to 140% (post titanic potentiation) and persistently by more than 50%. (**D**) The distribution of the increase of the response amplitude is visualized as an interpolated pseudocolor image. Significant increases of the response are mainly found in areas activated by orthodromic or antidromic activation of synapses in the CA region and the dentate gyrus.

of synaptic plasticity, like long-term potentiation and paired pulse facilitation (**Fig. 2**). The frequency composition of field potentials are quite variable, ranging from low frequency response components, with time constants up to hundreds of milliseconds, to spikes with up to 3.5 kHz. The corresponding MEA design should allow coverage of an appropriate area of the tissue and should have electrodes with low impedance to record low frequency components. Such electrodes also allow the application of electrical current stimuli with low voltages, avoiding electrolysis.

Besides nerve cells, muscle cells are well known electrogenic cells, using electrical activity to transmit excitation. In cardiology, the spread of activity, its embryonic development and its pathological conditions, such as arrhythmias, are highly important issues (**Fig. 3**). Here, the continuously contracting tissue poses quite a challenge for recordings with conventional microelectrodes. It is very difficult, and often impossible, to determine spatial patterns of excitations spread, details of the waveform and long term changes with classical means, let alone simultaneous measurements. The advent of MEAs thus marks significant progress, supported by the fortuitous situation that the contraction is essentially isometric at the tissue surface adhering to the substrate. Substrate integrated electrodes thus not only facilitate the management of multiple electrodes, but also create a unique mechanical situation forming a highly stable recording situation. This

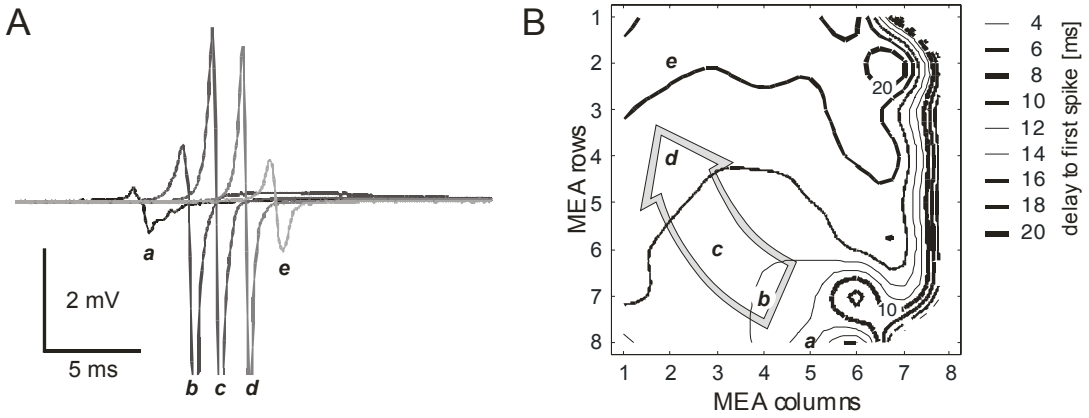

Fig. 3 Spread of excitation in a cell culture of neonatal cardiac myocytes (DIV 6). Based on recordings from 60 electrodes in this confluent cell layer, we reconstructed the spread of excitation through the tissue. (**A**) The delays and changes of the spike shape were readily visible in these sample traces recorded at 5 positions along the path of propagation (a-e in B). (**B**) In this case, the origin of excitation lay in the bottom right corner of the MEA (approx. at the asterisk). Excitation spread towards the top-left corner, as indicated by the arrow. The isochrones (interpolated based on 60 recording positions) give the delay of the spike minimum at each position with respect to the earliest peak. Only a thin layer of cells that did not visibly contract covered the right side of the array (columns 7 & 8). The minima in A were clipped graphically.

allows us the observation and analysis of physiological processes in active cardiac tissue over periods of several days, enabling a new class of experiments on cardiac development (Igelmund et al., 1999).

Even though planar, passive MEA now prove their value in neurophysiological and cardiological research, some limitations persist, that restrain the field of application. One such limit is that it is not easily possible to match the geometry of the tissue with the layout of the electrode array, which is often complex and variable. Although desirable, it is frequently not essential to record from vast numbers of equidistant electrodes, but rather important to record from particular positions with high sampling density, and only lower density at other locations, in each case matching the electrode distribution to the tissue geometry. A new technical approach currently developed at the NMI offers a large number of potential recording sites, from which an arbitrary subset can be selected by activating these electrodes (**Fig. 4**) (Bucher et al., 2001). The principle is illustrated in Fig. 4 A. A thin layer of amorphous silicone normally electrically insulates two layers of conducting thin-film leads. These in turn are insulated against the electrolyte except at their intersections. Each intersection is a potential electrode that becomes active only when the layer of silicone is locally irradiated with a laser. Light then creates charge carriers, locally rendering the silicone a conductor, thus connecting the leads and activating the electrode above the intersection. With this device it will be possible to select electrodes at appropriate sites by a laser scanning device, depending on the needs of the experiment.

We expect that the availability of these techniques, of the corresponding data acquisition and data analysis tools, and an increasing number of experimental protocols will contribute to our understanding of signal propagation and information processing, as well as pathological conditions thereof. It facilitates the search for putative neuro- and cardio-active drugs in the pharmaceutical industry.
Many of the problems, approaches, techniques and applications were defined and addressed in a joint project of the NMI, Multi Channel Systems, the University of Freiburg and the University of Cologne[1)].

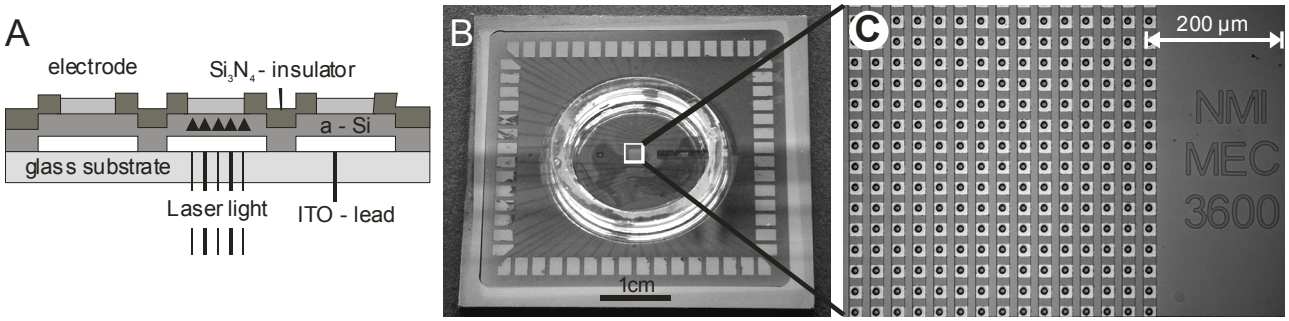

Fig. 4 New developments will extend the range of applications of currently available passive MEAs. (**A**) In a novel multilayer structure focal laser illumination induces local charges in a thin layer of amorphous silicone, which insulates two layers of leads. Each of their intersections can form an electrode, which is activated by the laser. (**B**) Light-addressable high-density arrays will allow the user to select a subset of electrodes from, in this prototype, 3600 potential electrodes, at positions optimally suited for the preparation and question of the experiment. (**C**) The black dots in this magnification of the white square in B are the putative electrodes that are deinsulated towards the buffer reservoir.

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[1)] see http://www.brainworks.uni-freiburg.de/projects/mea/index.html for details