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REVIEW

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Biological application of microelectrode arrays in drug discovery and basic research

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Abstract Electrical activity of electrogenic cells in neuronal and cardiac tissue can be recorded by means of microelectrode arrays (MEAs) that offer the unique possibility for non-invasive extracellular recording from as many as 60 sites simultaneously. Since its introduction 30 years ago, the technology and the related culture methods for electrophysiological cell and tissue assays have been continually improved and have found their way into many academic and industrial laboratories. Currently, this technology is attracting increased interest owing to the industrial need to screen selected compounds against ion channel targets in their native environment at organic, cellular, and sub-cellular level.

As the MEA technology can be applied to any electrogenic tissue (i.e., central and peripheral neurons, heart cells, and muscle cells), the MEA biosensor is an ideal in vitro system to monitor both acute and chronic effects of drugs and toxins and to perform functional studies under physiological or induced pathophysiological conditions that mimic in vivo damages. By recording the electrical response of various locations on a tissue, a spatial map of drug effects at different sites can be generated, providing important clues about a drug's specificity.

In this survey, examples of MEA biosensor applications are described that have been developed for drug screening and discovery and safety pharmacology in the field of cardiac and neural research. Additionally, biophysical basics of recording and concepts for analysis of extracellular electrical signals are presented.

Keywords Drug discovery · Safety pharmacology · Organotypic tissue culture · Cell culture · Ion channel · Microelectrode array · Field potential · Electrophysiology

Abbreviations *AP* action potential · *DG* dentate gyrus · *EC* entorhinal cortex · *ECG* electrocardiogram · *ERG* electroretinogram · *LFP* local field potentials · *MEA* microelectrode array · *PSTH* peri-stimulus–time histogram · *SNR* signal-to-noise ratio

Introduction

Bioelectricity has attracted the interest of many scientists since Galvani published his impressive experiments with frog's legs in 1791. Nowadays, bioelectricity is investigated in relation to pathways and regulatory circuits of many physiological functions and information coding in neuronal systems. Knowledge on the functional regulation of the underlying electrical excitability and its role in diseases and therapeutic manipulations are of great importance in both medicine and pharmacology. Currently, increased interest is brought to the molecular level of bioelectricity by the pharmaceutical industry, as an increasing number of diseases have been shown to be related to dysfunction of ion channels or to affected regulatory pathways [1]. Therefore ion channels attract special attention as a target class for drug discovery, and adequate electrophysiological methods and instrumentations are required to investigate the modulation of specific ion currents as a measure for the pharmacological activity of a compound. This has resulted in the ongoing development of electrophysiological methods and instrumentation that allow automated monitoring of ion channel function in higher throughput screening-compatible formats. The introduction of these new techniques, especially automated patch clamping [2, 3], opens new perspectives for ion channel drug discovery [4]. However, these methods prefer cell-

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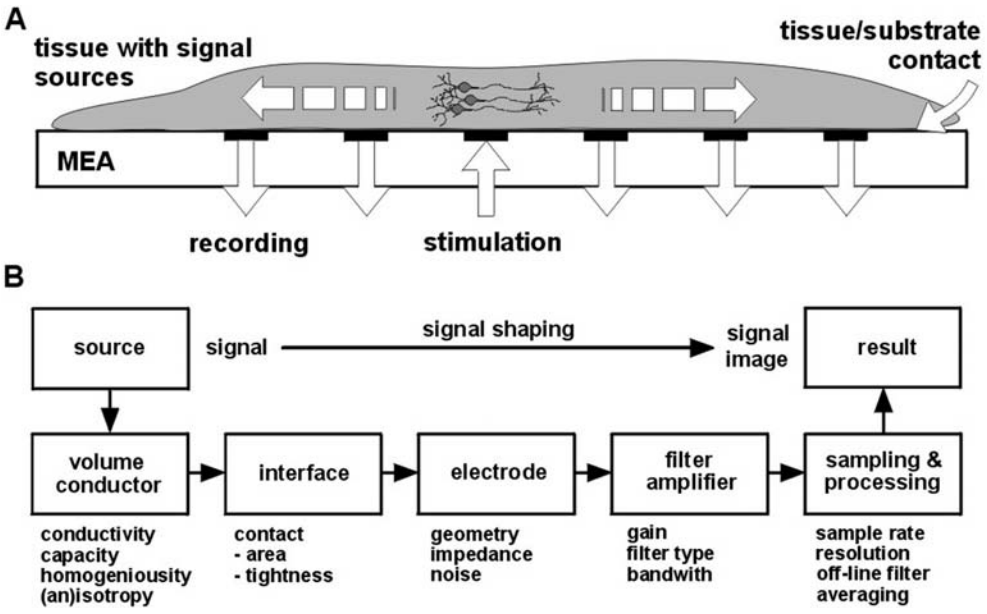

Fig. 1A,B Stimulation and recording of field potentials by microelectrode arrays (MEA). **A** A brain slice is attached to the planar surface of the MEA with its embedded electrodes, which can be used both for stimulation and recording. After stimulation a two-dimensional spread of evoked activity in the tissue occurs and can be recorded with high spatial and temporal resolution. Prerequisite for reliable signal transmission is a close contact of the tissue to the substrate. **B** The recorded, stored, and analyzed signal is an image of the original signal of the cellular sources shaped by several characteristic parameters of the components of the signal chain

based assays with expression cell lines, and none of them enable the electrophysiological screening of selected compounds against ion channel targets in an intact cellular environment. This can be achieved by the use of micro-electrode arrays (MEA, Fig. 1) – a well-accepted method enabling stimulation and recording of bioelectricity with high spatial and temporal resolution in cell and tissue cultures.

Since its introduction 30 years ago by Thomas and coworkers [5], the MEA technology and the related culture methods for electrophysiological cell and tissue assays have been continually improved (for a review on MEA history and applications see ref. [6]). Since computer capacity has become large enough for online-processing of data streams generated by tens of electrodes, and measurement systems and microelectrode arrays have become commercially available, the technique now has found its way into many academic and industrial laboratories (for an overview on MEA and equipment suppliers see ref. [7]).

The focus of our MEA-related work within the framework of several joint projects over the last 10 years has been the development of high-quality arrays with novel electrodes (Fig. 2) suitable for long-term monitoring with sufficient signal-to-noise ratio [8, 9, 10, 11, 12, 13], the establishment of novel applications [14, 15, 16, 17, 18, 19, 20], and the analysis of the data [21, 22, 23]. With respect to the need of the pharmaceutical industry for test systems suitable for functional secondary screening of compounds and regulatory requirements concerning safety issues in the course of approval of new drugs, we recently established new biosensor applications using brain, retina, and cardiac tissue. Here we give an overview on these MEA applications. Additionally, biophysical considerations on generation, spread, and recording of field potentials and concepts for the analysis of extracellular electrical signals are presented.

Recording of field potentials in cell and tissue cultures

Electrical activity of cells in neuronal and cardiac tissue is always accompanied by the flow of current through the extracellular fluid surrounding the cellular signal sources. Related to the current is an extracellular voltage gradient that varies in time and space according to the time course of the temporal activity and spatial distribution and orientation of the cells. MEAs offer the unique possibility for non-invasive recording of this activity of cells and networks from, in our lab, as many as 60 recording sites simultaneously.

Recording experiments using MEAs can mostly be considered as following one or a combination of the following motivations:

I. to gain information about interactions between electrogenic cells at different locations in the same tissue, which may be used to analyze the spatio-temporal dynamics of activity or the representation of information in neuronal networks;
II. to reduce the time required for an experiment by simultaneously recording at several sites in parallel, and thus sample the distribution of electrophysiological behavior efficiently, which may include comparison of tissue properties at different locations;
III. to monitor changes of electrical activity over periods of time not accessible with individual conventional electrodes (e.g., glass capillary or tungsten electrodes) in in vitro experiments.

Furthermore, the exceptional stability of the recording situation when MEAs are used allows analyses that would otherwise not be feasible, for example, to compare activity at a precise raster of recording sites or to record from contracting cardiac myocytes without interfering with the cells.

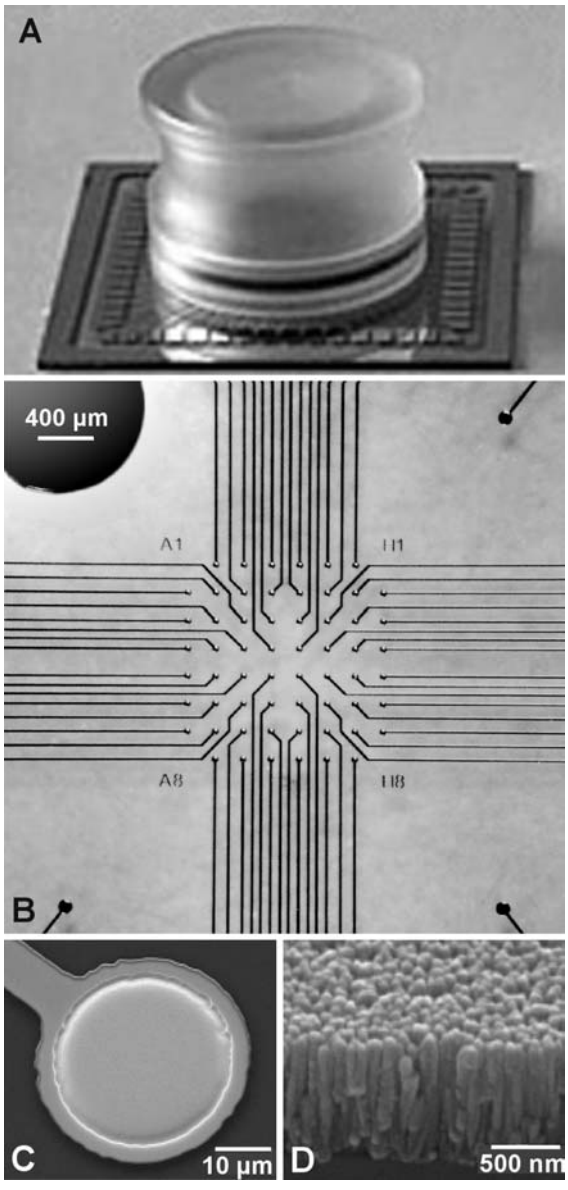

Fig. 2A–D The microelectrode array manufactured at the NMI Reutlingen. **A** MEA with closed culture chamber enabling long-term recording from organotypic cell and tissue cultures under sterile conditions. **B** Inner array of the MEA with 60 electrodes (diameter 10–50 µm, spacing 100 or 200 µm), a substrate-integrated reference electrode (*upper left corner*) and 3 additional stimulation electrodes (*other corners*). The connecting lanes are isolated by Si_3N_4. **C** SEM image of a TiN electrode. **D** The sputter-deposited TiN layer offers a nano-columnar structure providing low interface impedance

This contrasts with currently available cell-based assays that are not able to model complex interactions and connections between different types of cells or tissues. Moreover, other than conventional electrophysiological methods such as patch clamping and single-electrode recording that only allow activity to be recorded over minutes and hours, cells and tissues can be cultured directly on MEAs. This permits the investigation of system parameters in a natural network of excitable cells for up to months.

As the MEA technology can be applied to any electrogenic tissue, that is, central and peripheral neurons, heart cells, and muscle cells [12, 14, 18, 24, 25, 26, 27, 28, 29, 30, 31], the MEA biosensor is an ideal in vitro system to monitor both acute and chronic effects of drugs and toxins and to perform functional studies under physiological or induced pathophysiological conditions that mimic in vivo damage. By recording the electrical response of various locations on a tissue, a spatial map of drug effects at different sites can be generated which provides important clues about a drug's specificity.

In the following sections, three examples of MEA biosensor applications are described that have been developed for drug screening and discovery and safety pharmacology in the field of cardiac and neural research.

Field potential recording in cardiac myocyte culture

Pharmacological intervention, often for the purpose of treating syndromes unrelated to cardiac diseases, can increase the vulnerability of some patients to life-threatening rhythm disturbances. An important parameter that can cause cardiac arrhythmia is the prolongation of the QT interval of the surface electrocardiogram (ECG), which reflects the time from the beginning excitation of the ventricle to its peak repolarization. Directly corresponding to this parameter is the ventricular action potential duration, or as measured extracellular by MEAs, the duration of the field potential [23].

According to the latest guidelines from the "International Conference on Harmonisation", (ICH7A Document, www.ifpma.org/ich1.html) safety pharmacology for human pharmaceuticals is supposed to include in vitro assays assessing the potential of QT interval prolongation. Thus, screening for drug side effects on cardiac function has become crucial in pharmaceutical research and drug development.

There are a variety of in vitro cardiac assay systems including cellular approaches focusing on the analysis of ionic currents underlying the cardiac action potential, and organotypic approaches such as the Langendorff heart or isolated Purkinje fibers. However, most of these techniques are rather tedious, requiring highly trained staff performing the electrophysiological recordings and establishing sophisticated organotypic models. In contrast, the MEA cardiosensor described here is a fast, easy to handle, and efficient system for monitoring drug effects on cardiac action potential parameters (Figs. 3, 4).

For data analysis, the time from the initial to the repolarization peak was determined as the length of the ventricular field potential (Fig. 4A) that has been shown to correlate well with the QT interval in the ECG.

In order to assess drug effects on the ventricular field potential, different substances known to prolong the QT interval and cause cardiac arrhythmia were applied (Fig. 4B–D). Quinidine and Sotalol are clinically used as anti-arrhythmica; E4031 is an HERG channel inhibitor. Application of Quinidine resulted in a dose-dependent prolongation of

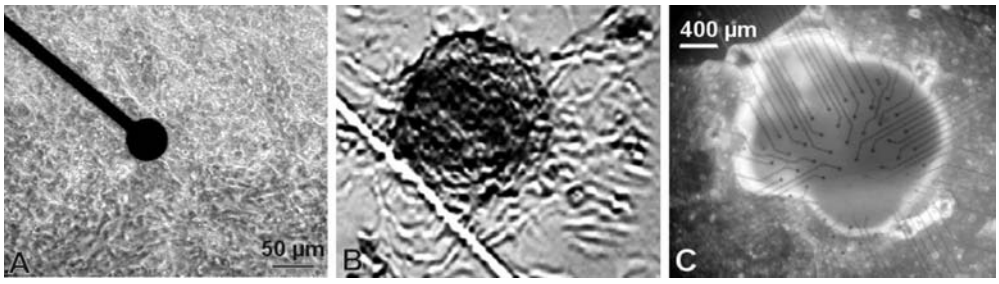

Fig. 3A–C MEA cardiosensor: for measurements of multisite cardiac field potentials, myocytes of embryonic chicken ventricles are placed on an MEA as **A** isolated cells that form a confluent monolayer after one day in vitro, or **B** cell aggregates in which myocytes were allowed to aggregate before placing them on an MEA. **C** Alternatively, also a whole embryonic heart can be kept on an MEA for a couple of days. Spontaneous contractions are observed in all conditions

the ventricular field potential (Fig. 4B) and a distortion in its rhythm (Fig. 4D). The effective ranges of Quinidine, Sotanol, and E4031 on the ventricular field potential duration are shown in Fig. 4C.

In conclusion, our data demonstrate that the MEA cardiosensor is a valuable tool to study drug actions on cardiac function.

Multisite electroretinogram of explanted retinas

The retina is a peripheral, easily accessible part of the central nervous system. Stimulation by light results in a complex signaling by neurons within the layers of the retina (cf. Fig. 5A). The retinal ganglion cells transmit retinal information to higher visual centers in the brain via their axons that form the optic nerve. Retinal function can be affected by acute injuries, intoxications or retinal diseases, either inherited or acquired, resulting in visual impairment or even blindness.

In clinical practice the so-called electroretinogram (ERG) is a widely used ocular electrophysiological test to diagnose impaired vision. Light impulses falling on the retina synchronously activate a large number of neurons and Müller glia cells, which regulate the extracellular potassium concentration. The resulting change in trans-retinal voltage is measured as the ERG. For an overview on structure and function of the retina, and the basis of the ERG see ref. [32].

The ERG has a multiphasic waveform. Its shape mainly depends on the stimulus conditions, the state of the retina's adaptation, and the species. The full-field ERG of a dark-adapted retina in response to a bright flash of white light consists of four major components: the fundamental a-, b-, and c-wave at light onset, and the d-wave at light offset. Each of the components can be attributed to the activity of certain retinal cells. Under pathophysiological conditions the shape and amplitude of these components is altered and can be influenced by pharmacological compounds.

A retina sensor, based on multisite recording of local ERGs in vitro, has been developed to easily and effectively assess effects of pharmacological compounds and putative therapeutica, drug side effects, and consequences of degeneration-related processes on retinal signaling.

For the recording of light-evoked activity, a retinal segment with the pigment epithelium, dissected from an explanted chicken retina, is placed ganglion cell site down on a MEA (Fig. 5A,B). Local ERGs (*micro*ERGs; Fig. 5C) with the typical components and ganglion cell spikes (Fig. 5D) can be recorded with the appropriate filter settings. The prominent components of the *micro*ERG can be pharmacologicaly identified as shown in Fig. 5E for the b-wave,

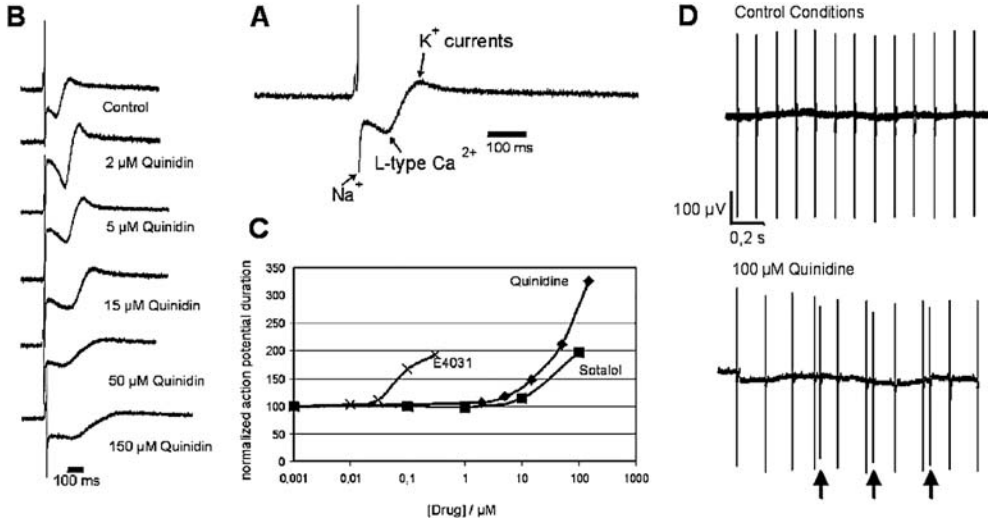

Fig. 4A–D Drug effects on ventricular field potential prolongation: **A** a typical field potential of ventricular myocytes under control conditions. The under-lying ionic conductances are indicated. Repolarization is mainly caused by potassium channels, of the I_{KR} type. **B** Influence of various Quinidine concentrations on the ventricular field potential of ventricular myocytes. **C** Effects of drugs known to prolong the QT interval in ECG and cause tachycardia. **D** Distortion in rhythm of ventricular field potential by Quinidine. *Arrows* point to early after potentials (EAD) induced by Quinidine application

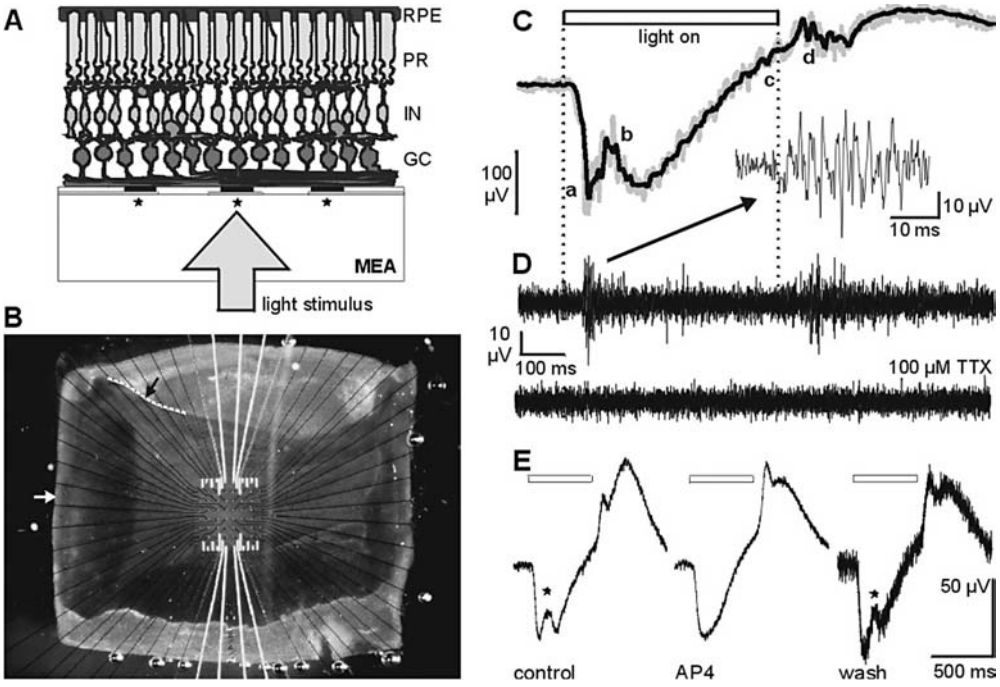

Fig. 5A–E MEA retinasensor. **A** An explanted retina is placed onto an MEA with the ganglion cell side (*RPE* retinal pigment epithelium, *PR* photoreceptor, *IN* interneurons, *GC* ganglion cells). The light stimulus is projected through the transparent MEA and retina onto the photoreceptors. Ganglion cell activity and retinal field potentials (*micro*ERG) are recorded by 60 substrate-integrated microelectrodes (*). **B** View through an MEA on a retinal segment (*white arrow*) from a chicken retina. The *broken line* marks the border of the pigment epithelium. **C** *micro*ERG with a-, b-, c-, and d-wave of a chicken retina, evoked by full-field white light, impulse duration 500 ms, 0.5 Hz. *Gray curve* single sweep, 0.5 Hz to 2.8 kHz. *Black line* 5 sweeps averaged, filtered 0.5–100 Hz. **D** Spike activity (*insert*), extracted from *gray curve* shown in *C* by off-line filtering at 200 Hz–2.8 kHz. The spikes were extinguished by 100 µM TTX. **E** Drug action on a light-evoked *micro*ERG. 2-Aminophosphonobutyric acid (AP4), a blocker of the on-signal pathway in the retina, resulted in the disappearance of the b-wave (*) that mainly reflects retinal Müller cell and bipolar cell activity. B-wave amplitude is restored after washing out the drug. Light pulses 500 ms, 0.5 Hz

which is smaller in recordings from isolated retinas than in recordings from intact eyes. During superfusion with drugs in defined concentrations, specific alteration of the ERGs can be monitored.

In its present form, the MEA retina sensor is suitable for drug testing over several hours, depending on the reversibility of tested drug effects. In order to monitor long-term drug effects, we currently are establishing an organotypic culture of a mammalian retina (mouse, rat).

Spatio-temporal structure of neuronal activity in brain slices

In principle, any part of the brain can be placed onto an MEA in order to study brain function and dysfunction, to assess potential drug side effects, or to screen for the effect of newly developed compounds. As an example for an MEA brain sensor application, an organotypic co-culture model is described in the following reflecting the perforant pathway projection into the hippocampus. It is suitable to study processes of neurite outgrowth, synaptogenesis, and regeneration.

Promoting repair and re-growth of neurons is an increasingly attractive approach to the treatment of neuronal injury caused by trauma, ischemia, or neurodegenerative diseases. Evaluation of treatments that may lead to neural regeneration require model systems that closely reflect in vivo conditions, enabling the evaluation of the potential of newly devised therapeutic strategies to be evaluated. In our MEA regeneration model [15, 16], slices of entorhinal cortex (EC) and dentate gyrus (DG) were placed on MEAs and cultured for up to 6 weeks. The functionality and specificity of the newly formed connections was shown by electrical stimulation and parallel recording of correlated activity in the target tissue (Fig. 6). The components of evoked signals in the DG can be isolated pharmacologically (Fig. 7).

For example, we evaluated the promotion of neuronal regeneration by Cd3, a synthetic agonist of the neural cell adhesion molecule NCAM [33], in a co-culture model of EC and DG on MEAs (Fig. 8.).

The approach was found to be a valid in vitro model for regeneration of the perforant pathway and a useful tool to investigate long-term pharmacological effects on synaptic activity and regenerative processes.

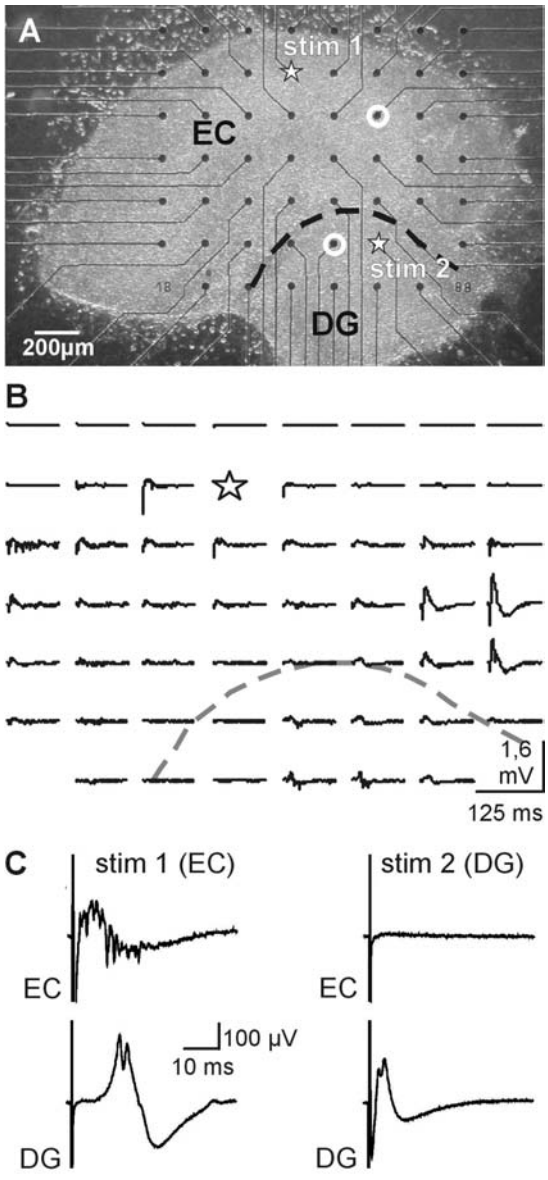

Fig. 6A–C MEA brainsensor. **A** Co-culture of entorhinal cortex (*EC*) and dentate gyrus (*DG*) on an MEA after 7 days in vitro. *Dividing line* between EC and DG, stimulation electrodes (*asterisks*) and exemplary recording electrodes in EC and DG (*rings*) are marked. **B** Evoked signals on MEA electrodes in EC and DG after electrical stimulation of the EC (*asterisk*, *stim 1*). Correlated signals in the DG suggest a functional connection between EC and DG. **C** Specificity of newly formed fibers between EC and DG. Electrical stimulation in the EC (*stim 1*) resulted in correlated activity in both the EC and DG, whereas stimulation in the DG (*stim 2*) only resulted in DG activity. This unidirectional propagation is in agreement with the perforant pathway projection in vivo

Analysis of field potential generation and data recorded with microelectrode arrays

Formation, spread, and recording of field potentials

With MEAs extracellular potentials of cellular sources are recorded at the two-dimensional surface of a conductive tissue sheet as sketched in Fig. 1A, where a tissue slice with electrogenic cells is brought into contact with the planar substrate. Such a system in principle consists of three main components: i) the tissue with the signal sources, ii) the interface between the tissue and the electrodes, and iii) the substrate with the embedded microelectrodes that are connected either to filter amplifiers and recording hardware or to stimulation sources.

The sources of the recorded signals are compartments of single cells, for example, dendrites or axon hillocks. Due to local alteration of ion channel conductance, they produce an extracellular field potential with a time course that is approximately equal to the transmembrane current of the active cellular compartments.

For fast events, the transmembrane current and therefore the extracellular recording is roughly equal to the first derivative of the transmembrane potential [34]. This can be easily illustrated by recordings from single cells contacting a planar electrode as outlined in Fig. 9. The principle of generation, extracellular spread, and recording of single-cell signals also holds for signals arising from sources in tissue slices. The MEA recording then may exhibit slow field potentials and fast spikes arising from action potentials. Differences in amplitude and shape of the recordings from single cells and tissue slices may occur due to the distance between source and electrode and due to the mechanisms of the coupling between membrane and electrode in single-cell contacts as shown in Fig. 9 and tissue and planar substrate, respectively.

The passive spread of cellular signals in tissue slices has been investigated by Egert et al. [35]. They could detect spike activity with MEA electrodes at distances of up to 100 µm from a neuron in an acute brain slice. Typically, signal sources are within a radius of 30 µm around the MEA electrode center. This is in agreement with theoretical and numerical calculations of idealized geometries [36].

By summation of the simultaneous activity of a larger population of cells in a tissue slice, a continuous potential field is generated. Its spatial modulation reflects the distribution, orientation, polarization, and density of signal sources. Recently, Fromherz published a model to describe the two-dimensional profile of field potentials in brain slices [37]. It adapts the fully fledged volume conductor theory used for current–source density analysis in three-dimensional tissue to the quite different boundary conditions of the tissue sheet. Besl and Fromherz showed that this model can be used to calculate the current–source density in the CA1 region of organotypic slices from rat hippocampus based on recordings obtained with a FET (field-effect transistor) array with sensor spacing of a few micrometers [29].

In Fig. 1, a signal chain from source to recorded data is shown that contains all components that may contribute to the actual shape of the recorded signals. These influences should be kept in mind when the recordings of field potentials are interpreted.

Despite the widespread use of MEAs, no complete theoretical descriptions of the interfacing of cells and tissue with planar metallic electrodes are available. The most

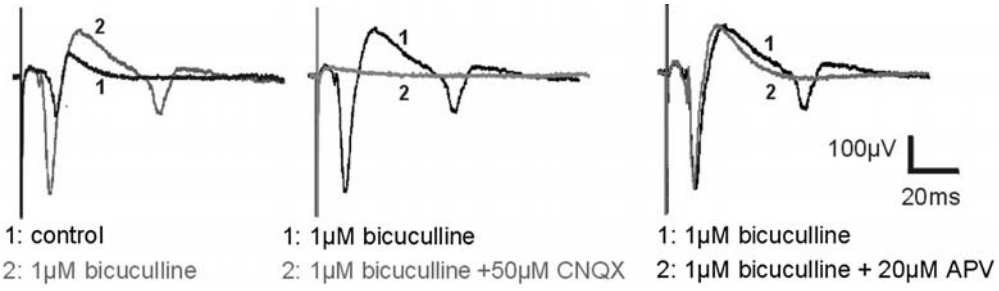

Fig. 7 Representative evoked signals from the DG after electrical stimulation of the EC under control conditions and after application of $GABA_A$ (bicuculline) and glutamate receptor antagonists (CNQX, APV). Individual excitatory signal components can be isolated pharmacologically

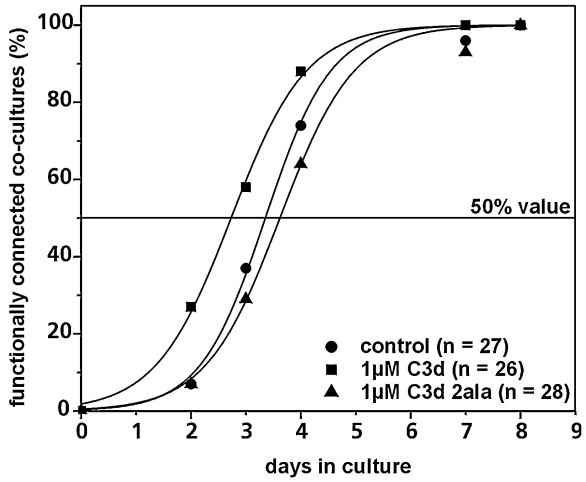

Fig. 8 Effect of synthetic NCAM agonist C3d on the formation of functional connections between EC and DG. The percentages of C3d-treated and untreated co-cultures that show signal transmission from EC to DG are plotted against days in culture. As an additional control, the non-effective peptide C3d 2ala was applied. Functional connections in presence of C3d are formed significantly faster (about one day) than under control conditions without affecting their specificity. The acceleration was blocked by antagonists to PLCγ and c-fyn, which are key mediators of the intracellular NCAM signaling pathways (data not shown)

advanced considerations on single-cell contacts are given by Buitenweg et al., who used a geometry-based finite-element model for studying the electrical properties of the contact between a passive membrane [38] and a membrane containing voltage-gated ion channels [39], respectively, and a planar electrode.

A complete theory of neuroelectronic interfacing by semiconductor chips with embedded field-effect transistors is given by Fromherz [40]. In principle, with slight modifications, his considerations and experimental results on the physics of the cell–insulator junction apply also for stimulation and recording with MEAs.

Interpretation of the recorded signal

Although the biophysical basis in principle is the same for any type of extracellular recording, the components of interest of the recorded voltage time series may differ.

The recorded signal may be analyzed for individual 'spikes', the extracellular correlates of an action potential generated by a neuron or a muscle cell (single-unit activity, up to approximately 400 μV_{pp} with neurons, a few millivolts with myocytes), or the spikes of small populations of cells 'seen' by one electrode (multi-unit activity, usually <100 μV_{pp}). The overlapping potentials of larger populations of cells create low-frequency components in the recording (local field potentials, LFP) that under certain conditions may reveal additional information not to be gained from spike data (e.g., the postsynaptic potentials (PSPs) of populations of neurons). Depending on the properties of the recording set-up and the electrodes, these components may occur as a mix, which is often separated

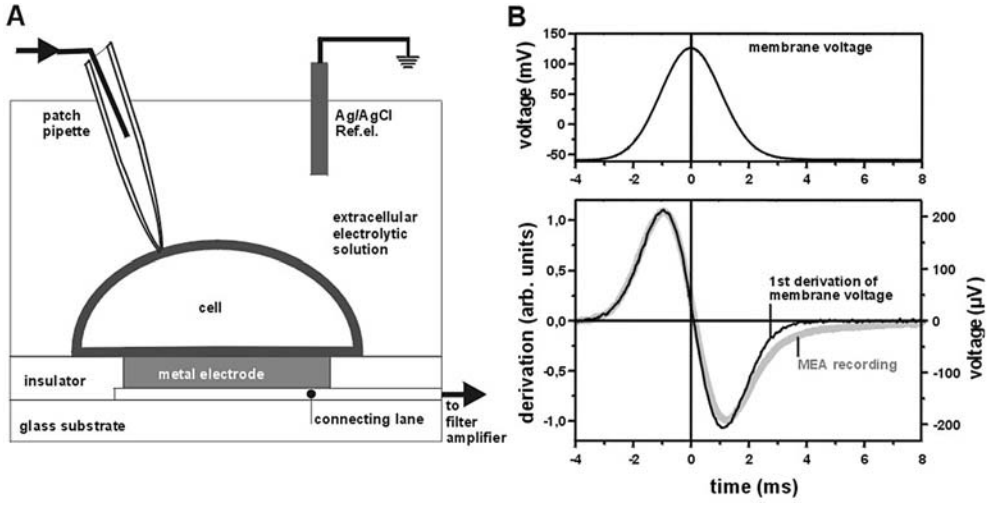

Fig. 9A,B Measurement of single-cell field potentials. **A** A single cell (CHO cell) cultured on a planar electrode is contacted by a patch pipette in order to control the membrane voltage in the whole-cell voltage clamp mode. **B** Comparison of intracellular and corresponding extracellular voltage. A Gaussian-like membrane voltage clamp (*top trace*) and MEA recording of the corresponding extracellular voltage (*bottom*, *bold gray line*) and first derivative of membrane potential (*black line*)

by appropriate filtering of the raw voltage trace as shown in Fig. 5.

Here, we will emphasize the background and approaches to the analysis of data collected with MEAs, somewhat neglecting standard tools used for single-electrode and multi-electrode recordings in vitro and in vivo. Further information on multi-electrode recording in vivo can be found in ref. [41].

Cardiac myocytes

Besides the difficulty to record from contracting cells, the electrical coupling of myocytes in the heart muscle leads to special conditions for extracellular recording and the interpretation of the results. Compared to neuronal tissue, the morphology and properties of the myocytes in cultures is relatively homogeneous with respect to cell morphology and cell type. Because of this, and in particular because of the coupling through gap junctions, the tissue within the recording horizon of an electrode can be linked to a very large cell of simple morphology, compared to which the recording electrode is very small. In myocyte cultures on MEAs (Fig. 3), the cells tightly adhere to the recording surface, contracting isometrically. As a result, motion artifacts do not occur and the signal-to-noise ratio (SNR) is generally quite high. Depending on the overall properties of the culture, the waveforms thus recorded closely match the first derivative of the transmembrane potential as shown in Fig. 9. Since the slopes in the action potential (AP) waveform reflects the currents flowing across the membrane, the time course of the LFP relates to the transmembrane currents. Changes, though not absolute values, of some of the properties of the AP shape can therefore be estimated from extracellular recordings alone [22, 23, 31], for example, duration and ascending slope of the AP, changes of Na^+ and Ca^{2+} currents. Furthermore, the propagation of the AP through the tissue can be observed [20, 42]. Together with the stability of the recording configuration, the analysis of the extracellular field potential thus facilitates the analysis of developmental processes, arrhythmia, and drug effects on cardiac myocytes in culture [23, 31, 43, 44].

Neuronal spike activity

Due to the complex geometry of the extracellular space and the neuron itself, little information can be extracted unambiguously from the waveform of the spike beyond the time of occurrence of an action potential. These properties and differences in the relative location of the electrode to the surrounding cells result in variable waveforms that are, however, reproducible across time for a given neuron. The differences of their waveforms can be used to separate populations of spike events originating from different neurons but detected at the same electrode ('spike sorting' of multi-unit recordings). When the SNR is sufficient (SNR>3, measured from peak to peak), spike sorting is a useful tool to isolate the response of one or a few neurons from the rest. A single MEA experiment can thus yield data from a large number of neurons.

Spike activity is then analyzed as a time series of events, the temporal structure of which yields information about the type and efficacy of an electrical or chemical stimulus. With MEAs, the result can give the distribution of the response in a population of cells with high temporal resolution (e.g., in firing rate analyses or peri-stimulus–time histograms (PSTHs)). If the activity of different neurons is independent from one another, MEA recordings will quickly compile a statistically relevant sample size and thus increase the experimental throughput [35, 45], though not the time needed to process the result. Although conventional programs for the analysis of extracellular recordings provide many of the tools necessary, it should be kept in mind that the sheer number of neurons that need to be analyzed demands some sort of batch processing, requiring suitable adaptation of such programs [21]. Similarly, even though not statistically independent, the collective response of a population of cells may yield information not available from a single neuron or the successive recording of several neurons [41, 46, 47, 48]. The generic purpose of multi-electrode recordings is, however, to identify interactions between neurons and their modulation in response to some stimulus [49, 50, 51, 52, 53, 54, 55, 56]. Although these reports refer to in vivo experiments, the analysis tools developed for this purpose are equally applicable to MEA recordings. The aggregate network response to a challenge by a drug, for example, in an organotypic brain slice, is expected to be more representative of the drugs in vivo action than studies on isolated cells. The statistical analysis of such network response can be demanding and is not yet fully established [57].

Multi-unit recordings (Fig. 5C,D; Fig. 6B,C) on the other hand, will yield information about the spike rate of neurons in a local population, 'averaging' across cell types, which can be compared for different recording sites [57, 58, 59, 60]. This type of data usually suffers from small SNRs, so that further analysis of individual spikes does not extract additional information; however, it requires considerably less pre-processing of the data.

Local field potentials (LFPs)

LFPs can be interpreted as the sum of the contributions of a larger population of cells, where the individual cells component depends on a number of factors. When the dendrite-soma axes of the cells in a population are aligned, for example, as for the electroretinogram (Fig. 5) and the pyramidal cells in the CA region of the hippocampus, synchronous activity can add to very large response amplitudes and SNRs (Figs. 6, 7). It is easy to see that for populations arranged less orderly or in a globular fashion (closed field), the fields may cancel if the neurons fire in synchrony. Due to this ambiguity, LFP waveforms can thus be quite difficult to interpret, unless the organization of the tissue studied is well known. Their advantage is,

however, that they are easily recorded and subthreshold responses (i.e., PSPs) are visible without penetrating the cell. LFP analysis has therefore been widely used to study synaptic plasticity or epileptic states, for example, see refs. [61, 62, 63, 64, 65, 66]. Depending on the experiment, LFP waveforms are analyzed for the magnitude, spatial extent, and dynamics of the responses, the slope of the early responses, the latencies of individual components within the response, spatial patterns [67], etc. In MEA recordings, LFPs were found to be useful to estimate the dynamics and the distribution of responses with respect to the anatomy of the tissue, because their spatial decay is in the range of the inter-electrode distances, allowing almost gapless coverage of the recording area [14, 19, 68, 69]. The diversity of these aspects requires flexible analysis tools [21], often custom programmed for a purpose, and careful comparison of different recordings sites.

Summary and outlook

We have shown that the MEA technology is a valuable tool to record data with a high information content with respect to drug action in an intact cellular environment of cardiac myocyte cultures, brain slices, either acute or maintained in organotypic culture, and explanted retinas. Additionally, a number of groups apply the MEA technology to study neural coding and drug interaction in dissociated neural cultures (for a review see ref. [6]). Microfabricated arrays, recording hardware, and software for data acquisition and analysis are now commercially available and enable continuous, stable recordings from 60 electrodes at sampling rates up to 50 kHz per channel. Due to this advance, the MEA technology now really bridges the gap between conventional in vitro methods and complex in vivo experiments.

For specific questions the arrays have been adapted to the needs in basic research and industrial development and will be available: MEAs with thin glass substrate allow for combining electrophysiological and optical recording techniques for high-content measurements at cellular and sub-cellular level; flexible, free-standing, perforated MEAs allow sandwich-like configurations for a combination of multi-site stimulation with multi-channel recording in tissue slices. Such arrays also enable an adaptation of the MEA to the curvature of anatomical structures in vivo. Special electrode designs and materials enable single-cell recordings with high SNR, and last but not least, improved fabrication methods will provide low-cost MEAs.

Furthermore, for future applications the development of sophisticated solutions have been brought into play: improved sensor designs and materials and cellular engineering will enable stable single-cell contacts that allow the recording of slow voltage-gated and ligand-gated ion currents through specific ion channels [70]. Arrays with numerous microelectrodes at high spatial density will allow simultaneous recordings from many dissociated cells without the need for controlling the positioning of cells on the electrodes by means of surface patterning and the mapping and analysis of neuronal activity in brain slices with high spatial and temporal resolution [29], [71]). Improved electronic circuits will provide artifact-reduced simultaneous stimulation and recording at many electrodes in parallel.

Due to the emerging demand for novel electrophysiological methods that allows for parallel and automated recording from cells and tissue it is expected that the MEA technology is now crossing the threshold to become a widely accepted and used standard tool in the field of drug discovery and basic research.

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