Influence of Temperature on Response Characteristics of Mouse Retinal Ganglion Cells

Malte T. Ahlers, Lars van Ahrens, Martin Greschner, Josef Ammermüller

Neurobiology, Carl von Ossietzky University Oldenburg, 26111 Oldenburg, Germany

Responses of neurons are strongly temperature dependent. To investigate a neural tissue under physiological conditions, it is therefore of high importance to precisely control the temperature during an experiment. Here we present a system which allowed us to study the temperature dependence of retinal ganglion cell responses of the mouse retina.

In addition, the temperature of the body surface and, therefore, the temperature of the eye and retina are affected by the ambient temperature. Thus, during seeing under natural conditions, temperature effects might play a certain role.

We used a 100 channel Utah-Multielectrode-Array for extracellular recordings of ganglion cell responses of the isolated mouse retina. In contrast to lithographic arrays this array requires a transparent rest (glass or lucite) for the retina through which the light stimulus can be applied. This brings about some difficulties for temperature control due to the heat sink of the rest. Tests showed, that a standard perfusion heating systems, which keeps the temperature of the superfusion medium constant, did not succeed in heating up the retina to a desired temperature. Other systems, like Peltier-elements could not be used because of their intransparency.

Our system solved this problems in an simple way. During recording the retina lies on a glass chamber through which water is pumped, and the light stimuli can pass through it. The water is heated up electrically, its temperature can therefore easily be regulated. On the surface of the chamber a sensor is placed which measures the actual temperature. A microcontroller unit performs a simple regulation-algorithm, which controls the heating of the water. This system achieves temperature-constancy within approximately +- 0,1 °C.

We recorded ganglion cell responses of isolated mouse retinas to full field light flashes, and determined the latency of the responses to the light onset under different temperatures between 26 °C and 42 °C.

As expected, the response latency of all recorded cells decreased with increasing temperature. In the low-temperature range the effect was stronger than in the higher range. There exists a significant effect in the physiologically relevant range of 34 °C – 38 °C on the response latency.
Organised Neural Networks In Culture

Allison Beattie

Centre For Cell Engineering, Joseph Black Building, The University of Glasgow, G12 8QQ. Tel: 0141 330 4756. email:a.beattie@bio.gla.ac.uk

Purpose: To growth and maintain primary spinal cord neurons in predetermined patterns of connectivity. Using multi-electrode recording devices, designed and fabricated within the Engineering branch of CCE, record and analysis network activity with regards to signal processing and pattern geometry.

Methods: Neonatal spinal cord neurones were chopped finely before being subjected to both enzymatic digestion and mechanical dissociation. Cells were collected and plated at 1x 10^4 cells/ml. Cultures were characterised using immunocytochemistry to determine neuron population (MAP2, β-tubulin) and synaptic activity (synaptophysin). Stamps were fabricated from silicon master die using various polymers available in the engineering sector. Stamp viability was tested with immunocytochemistry staining. After plating cells were maintained for up to 2 weeks in culture, with the media being changed every 3-4 days.

Results: The curing properties of MicroSet made it more versatile as a stamping material. Stamps were more uniform in distribution (as shown by SEM and Immuno staining) compared to stamps fabricated in conventional PDMS. Polycarbonate provided best results as a non-adhesive background substrate.

Conclusions: After 24 hrs in culture cells adhere to adhesive tracks of protein pattern, however only after 3 days do processes begin to shown. Although μCP of can control cell adhesion and contain neurite extension, neuron polarity and therefore signal direction cannot be predicted. Re-design of pattern geometry would further organise neural network and advance understanding of signal processing.
Developments of MEA devices for studying complex cellular networks dynamics in-vitro

L. Berdondini¹, P.D. van der Wal¹, N.F. de Rooij¹, M. Koudelka-Hep¹
S. Martinoia², M. Chiappalone², M. Tedesco², A. Garenne³, G. Le Masson³,
P. Wolters⁴, J. van Pelt⁴

(¹) Institute of Microtechnology (IMT), University of Neuchâtel, Neuchâtel, Switzerland;
(²) Departement of Biophysical and Electronic Eng. (DIBE), University of Genova, Genova, Italy.;
(³) INSERM EPI 9914, Institut François Magendie 1Bordeaux Cedex, France.
(⁴) Netherlands Inst. for Brain Research (NIBR), Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

Microelectrode arrays (MEAs) have become a valuable research tool for studying the in-vitro electrophysiological activity of excitable cells, e.g. neurons, cardiomyocytes on a simple network level. In this context, research on the complex network dynamic properties, e.g. learning and memory, could largely benefit from adapting and improving the current MEA technology. In this presentation, two ongoing technological developments contributing to this research field will be described.

In the European project NeuroBit [1], a MEA featuring 60 microelectrodes, on Pyrex substrate, has been completed with several SU-8 clustering structures in order to divide the network into interconnected sub-networks (Figure 1). The aim here is to enable the investigation of the sub-networks interactions, and network plasticity.

The clusters are fabricated with EPON SU-8, 350 µm in thickness, and have a diameter of 3 mm. They are interconnected by open-channels of 300 µm in width and 500 µm or 800 µm in length. The integrated microelectrodes, Pt or IrOₓ, have diameters of 30 µm or 10 µm, and their performances for recording and stimulation have been evaluated after fabrication and in culture conditions. In particular, their long-term stimulation stability has been tested by applying a biphasic potential in neurobasal solution (2 V p-p in amplitude and 500 µs in length). The injected RMS current was stable over 10'000 stimuli delivered each 2 s. The realised device is compatible with the MCS pre-amplifier and permitted recordings on rats’ dissociated cortical neurons.

In parallel to further improving the MEAs performances, e.g recording / stimulation characteristics, an emerging research topic, is the development of high density microelectrode arrays on large active areas. These devices would allow a high-resolution recording (imaging) of the network activity.

We have realized and tested a prototype based on the solid-state, active pixel sensor (APS) concept [2]. By modifying the pixel functionality, a 64 x 64 array of gold electrodes of 20 x 20 µm² was realized. It integrates in-pixel pre-amplifiers and on-chip addressing logic. The electrical characterization of the device in a phosphate solution showed a good stability of the electrodes for recording. Tests on neonatal rat cardiomyocytes cultures have demonstrated a good viability of the culture for several days allowing the recording of the electrical activity to be achieved.

References:
Modulation of rhythmic activity in networks of cortical neurons during development

Michela Chiappalone¹, Marco Bove², Alessandro Vato¹ and Sergio Martinoia¹

¹ Dept. of Biophysical and Electronic Engineering, University of Genoa, Genoa, Italy
²Dept. of Experimental Medicine, Section of Human Physiology, University of Genoa, Genoa, Italy

Purpose: In vitro cultured neuronal networks coupled to micro-electrode arrays (MEAs) constitute a valuable experimental model for studying changes in the neuronal dynamics at different stages of development. After a few days, neurons start to connect each other with functionally active synapses, forming a random network and displaying spontaneous electrophysiological activity. The shown patterns of collective rhythmic activity may change in time spontaneously, during the in-vitro development. Such activity-dependent modifications reflect changes in the synaptic efficacy, fact that is widely recognized as a cellular basis of learning, memory and developmental plasticity.

The aim of our study is to analyze the changes in dynamics of electrophysiological activity at different ages of the culture, trying to characterize the electrophysiological behavior of the cultured neurons identifying some repeatable patterns, each of them corresponding to a well-defined state of the network.

Methods: Neuronal cultures were taken from cerebral cortices of embryonic Wistar rats at embryonic day 18. Cells were then plated on Micro Electrode Arrays - MEAs (MultiChannelSystems) pre-coated with adhesion promoting molecules (Poly-D-Lysine and Laminin). Measurements from 10 preparations were carried out in physiological medium at five stages of the network maturation process: 7 DIV (i.e. Days In Vitro), 14 DIV, 21 DIV, 28 DIV and 35 DIV.

To investigate the network behavior at burst level, we developed an ad-hoc burst detection algorithm. For studying synaptic interactions within the monitored neuronal networks, also the spike train correlation analysis has been adopted.

Results: During the early stage of the in vitro development no organized, collective behavior can be noted: the only kind of activity is in form of random spiking, present in the majority of the recording channels. Starting from the second week an increase in the number of bursting sites can be noted, even if the bursting rate is low and the percentage of random (i.e. outside a burst) spikes reaches its minimum. After the second week, the bursting rate increases. This trend is maintained along with the development until the last observed week in vitro (28 DIV – 35 DIV), when the burst amplitude (i.e. number of spikes per burst) and the percentage of random spikes reach values not so different from the previous stages. Also the correlation among the channels is subject to changes during the development, reflecting an improvement of the correlation degree due to the stabilization of the synaptic connections among the monitored cells.

Conclusions: The obtained results show continuous changes in the spontaneous cortical network activity through the first three weeks in vitro and an approaching to a steady state with possible small fluctuations around a stable dynamics after four-five weeks in vitro. The involvement of synaptic mechanisms in the modification of the spontaneous activity of cultured cortical networks during development is presented and discussed.
Oxygen availability in acute brain slices on solid and perforated MEAs

Ulrich Egert¹, Maike Buchner¹, Samora Okujeni¹, Wilfried Nisch²

Neurobiology and Biophysics, Institute for Biology III, Albert-Ludwigs University Freiburg, Germany, ²Naturwissenschaftliches und Medizinisches Institut, Reutlingen, Germany

The conditions for extracellular recordings from acute brain slices with microelectrode arrays (MEA) differ from those under conventional recording conditions in that the slices are supplied with oxygen by perfusion across the top surface of the tissue but the recording takes places underneath the slice. The absolute availability of oxygen and sufficiency the supply with consumable components of the buffer, however, are not know for either case. Perforated MEAs (pMEA) have been shown to increase the vitality of slices under some conditions (Boppart et al. '92, IEEE BME 39:37-42), the causes for which are equally unknown. The perfusion conditions are critical not only for the supply of oxygen, but also for glucose supply and pH stability with changing metabolic activity.

We have measured the O₂-partial pressure (O₂PP) under various conditions and depth in acute cerebellar slices recorded with MEAs on solid and perforated substrates to assess the quality of the supply conditions in these slices. Perfusion with pMEAs consisted of a buffer flow through the perforation and a larger flow through a parallel perfusion pathway underneath the array, but not passing through the perforation. O₂PP was measured with a fiber-optic fluorescence sensor (30 μm Ø) inserted into the tissue at various depths.

O₂PP was approx. 800 hPa at the surface of the slice with a 6-8-fold exchange of the buffer per minute, decreased with depth in the tissue and at room temperature never fell below arterial O₂PP (120 hPa). For a given depth, temperature and perfusion rate O₂PP differed across the layers of the cerebellum, indicating differential metabolic requirements for molecular, Purkinje cell, granule cell, and white matter layers. At higher temperatures O₂PP decreased further and was insufficient above 30°C, resulting in a failure of spike activity. When perfusion was stopped O₂PP fell to 0 hPa close to the MEA surface within a few seconds.

pMEAs were tested with respect to potentially better O₂PP conditions and with respect to improving the signal-to-noise ratio (SNR) by suction through the MEA. With perfusion on both sides the minimal O₂PP found in the tissue was increased by about 100 hPa. Likewise, spike activity was more stable at higher temperatures.

The contribution of the perfusion through the pores of the MEA alone was not sufficient to account for the improvement but depended on the rate of perfusion underneath the slice.

Suction through the MEA could be expected to decrease the distance between the cells and the electrode, reduce the variability of this distance and thereby increase the spike amplitude. The SNR increased by up to 50% at low suction (approx. -100 hPa) already.

Our results quantitatively describe the supply situation in brain slices for recordings with MEAs but they are likewise valid for conventional recording configurations with submerged slices. pMEAs and perfusion of the slice through the perforation improved the O₂-availability, which was particularly critical at high temperatures. At the recording surface, O₂PP was comparable to in vivo levels. With the increased SNR pMEAs thus improve the overall recording conditions.

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Caged Neuron MEA

Jon Erickson*, Angela Tooker†, and Jerry Pine‡

Departments of Bioengineering*, Electrical Engineering†, and Physics‡
California Institute of Technology, Pasadena, CA 91125

We have designed and built neurocages on a silicon neurochip to enable detailed in vitro studies of live neural networks. This new design is less complex to fabricate than its predecessors and, moreover, supports normal neuronal outgrowth and network development while maintaining >50% long-term survival rate.

A central goal of neuroscience is to understand in detail how neural networks in the brain function and evolve. Conventional extracellular, patterned MEAs have been a useful tool for in vitro studies toward this goal [1, 2]. Results of such MEA experiments, however, can be difficult to interpret and their utility for more detailed studies is hindered by neuron mobility, effects of neural geometry, glial cell interference, and a lack of one-to-one correspondence between neurons and electrodes. To combat these issues, Maher et. al. [3] developed a neurochip with a 4x4 array of neurowells to mechanically trap an individual neuron near an electrode. These neurowells permitted normal neuronal development and networking, but fabrication and scaling difficulties, along with continued glial cell interference, motivated the design of a new structure, the neurocage [4, 5].

The neurocage is shown in Figure 1. It consists of a cylindrical chimney—30 µm in diameter and 4 µm high—to trap the neuron and prevent glial cell interference, with six tunnels radiating outward to create paths for axon and dendrite outgrowth. The tunnels are 1.5µm high, either 5 or 10µm wide, and extend for either 4 or 40 µm from the inner wall of the chimney. The chimney and tunnel structures are constructed by depositing a conformal layer of parylene over a photoresist sacrificial layer which is later dissolved away. An access hole—15 µm in diameter—is etched in the top of the chimney for loading neurons. DRIE etched anchors [6] secure the parylene neurocages to the silicon substrate. Currently, the neurochip consists of a 4x4 array of neurocages, as shown in Figure 2, but this design and fabrication process will be scaled up to produce larger arrays.

Figure 3 shows successful growth of neurons in the 4x4 array of neurocages; the culture is 5 days old. No significant differences in neuronal outgrowth or survival rate have been noted among the various combinations of tunnel widths and lengths. Once platinized gold electrodes are incorporated into the neurocages, it will be possible to record from and stimulate individual neurons in a one-to-one fashion, allowing for more detailed experiments and studies of live neural networks.

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References:
Figure 1: SEM (A) and Nomarski optical (B) images of a neurocage. The cage shown has a tunnel width of 10 m and tunnel length of 4 m.

Figure 2: 4x4 array of neurocages. The cages have 40 m long tunnels in (A) and 4 m in (B). The tunnel width is 10 m for both arrays shown.

Figure 3: Neurons in neurocages with outgrowths through tunnels and networked. The culture is 5 days old.
Irregular in vivo-Like Background Synaptic Activity Recreated In in vitro Neocortical Slices

Giugliano, M., Lüscher, H.-R.
Institute of Physiology, University of Bern, Switzerland

Estimates of the number of synaptic contacts of a neocortical cell range between 5000 and 60000, 70% of them originating from intracortical areas. Furthermore, neocortical neurons fire spontaneously at a frequency of 5–20Hz in awake animals. These considerations define a scenario in which neurons experience very large synaptic currents (i.e. hundreds of postsynaptic potentials over a ms-time scale). Such an intense background activity induces random-walk fluctuations in the postsynaptic membrane potentials and it is thought to have a profound impact on the neuronal integrative properties, on the response dynamics to external stimuli, as well as on the activity-dependent plasticities. These implications have been never systematically studied in vivo, because of the technical difficulties related to intracellular and patch-clamp recordings in behaving animals. On the other hand, acute neocortical slices are widely employed as a reduced in vitro model, but in spite of the many advantages they do not accurately represent the realistic cortical networks physiology. In particular, since deafferentation causes the lack of background synaptic activity, conclusions obtained in vitro may not transfer to in vivo situations. A substantial contribution at restoring a realistic network input drive may come from a novel application of substrate arrays of microelectrodes (MEAs). We developed an experimental set-up combining standard whole-cell patch-clamp and MEAs, in in vitro rat brain tissue slices (see the figure). By means of a asynchronous multi-site electrical stimulation, delivered via 60 microelectrodes, we could recreate in vivo-like sustained synaptic activity in the neuronal microcircuits, resulting in neuron membrane potential fluctuations and irregular spike emission, similar to those observed in vivo. We present an analysis of single-neuron recordings and discuss research directions, expected to fully exploit the potential of such a novel stimulation paradigm as an advanced tool for network-level neuroscience.

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Single-neuron discharge properties and network activity in dissociated cultures of neocortex


Institute of Physiology, University of Bern, Switzerland

Mature cultures of neurons dissociated from rat neocortex exhibit spontaneous, temporally patterned, electrical collective activity. Such an in vitro activity constitutes a possible framework for combining theoretical and experimental approaches, linking the single-neuron response properties to collective network phenomena. In this work, we aimed at closing a conceptual loop: from the identification of the single-cell discharge properties to the prediction of collective network phenomena and the comparison with those spontaneously emerging in vitro, extracellularly detected by means of substrate arrays of microelectrodes. Therefore, we experimentally characterized the single-cell discharge response properties to gauss-distributed noisy currents, under pharmacological blockade of the synaptic transmission. Such stochastic currents emulate a more realistic network input drive, compared to the conventional electrophysiological protocols. The neuronal response was evaluated in terms of the steady-state mean firing rate ($f$), as a function of the mean ($m$) and variance ($s^2$) of the injected stimuli. In each experiment, $m$ and $s^2$ were varied independently, reminiscent of the extended mean-field description of a large variety of possible presynaptic network organizations and mean activity levels. Experimental current-to-spike-rate responses $f(m, s^2)$ were similar to those of neurons in acute brain slices, and they could be quantitatively described as leaky Integrate-and-Fire (IF) point neurons. The identified model parameter set was finally employed in a numerical simulation of a network of IF excitatory neurons. Surprisingly, such a network spontaneously reproduced a collective patterned activity, similar to what we observed in cultured networks, under control conditions. This constitutes an indication that the IF model is an adequate minimal description of synaptic integration and neuronal excitability, when in vitro collective network patterned activities are considered. We suggest that such a collective activity can be interpreted in the framework of the extended mean-field theory, widely studied in the literature.

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Rat Brain Cortical and Striatal CryoCells on Microelectrode Arrays
Philipp Görtz, Wiebke Fleischer, Frauke Otto, Mario Siebler
Neurochip Laboratory, Department of Neurology, University of Düsseldorf

Background and Purpose

Neuronal networks on microelectrode arrays ("neurochips") may be used for screening of pharmacological substances and to elucidate complex neurophysiological interactions of metabolites in CNS diseases. Special neurological impairments – such as Parkinson’s or cognitive diseases – may be modeled by the use of cell types from different brain regions. To ensure reproducible measurements cell material is needed which is highly stable and easy to handle.

Methods

Cryopreserved cortical and striatal neurons ("CryoCells", QB M Cell Science, Ottawa, ON, Canada) of the rat as well as murine cortical neurons were plated on microelectrode arrays (MEAs, Multi Channel Systems, Reutlingen, Germany) in a density of 1.5–2 x 10⁵ cells / cm², obtaining 8 MEAs from 1 vial with 4x10⁶ cells. According to the protocol supplied by QB M, the cells were incubated in neurobasal media with B27 supplement (Invitrogen, Karlsruhe, Germany) in a humidified atmosphere (5% CO₂ / 95% air) at 37 °C. We did not use mitotic inhibitors to prevent proliferation of glial cells.

Results

In all cell types neurite outgrowth began several hours after plating and a neuritic network developed within a few days. The neuronal markers MAP2 (microtubule associated protein 2), neurofilament and GABA were detected in all cell types by immunocytochemical staining. Co-staining with antibodies against glial fibrillary acidic protein (GFAP) revealed an astrocyte ratio of about 50% after 4 weeks in vitro.

Spontaneous spike activity started in cortical neurons from rats and mice between day 14 and 21 after plating and lasted for up to 12 weeks even after repetitive semi-sterile measurements. Typically, the cells displayed correlated burst activity across the whole MEA. In contrast, even after several weeks in vitro only a very few “neurochips” with striatal cells showed sparse spontaneous spike activity.

Spike activity of the cortical neurons was modulated by classical neuroactive substances like TTX, glutamate, NMDA, APV, acetylcholine, bicuculline and magnesium resulting in changes in the number of bursts, burst duration, spike amplitude or synchrony between channels.

Conclusion

MEAs with cultured neurons are feasible for electrophysiological recordings. These “neurochips” provide the basis for screening of pharmaceutical substances as well as for building up models for CNS diseases.
Dose-dependent effects of the anaesthetic ketamine on the electrical activity of primary frontal cortex networks

Alexandra Gramowski¹, Claudia Richter¹, Frank Kletzin², Gabriele Nöldge-Schomburg², Dieter G. Weiss¹

¹Institut für Zellbiologie und Biosystemtechnik and ²Klinik für Anästhesiologie und Intensivtherapie, Universität Rostock, Rostock, Germany

Ketamine is an intravenous anaesthetic with hallucinogenic properties. This dissociative anaesthetic is used alone or in combination with other substances for sedation or anaesthesia. Lately it also abused as a modern drug called “Vitamin K” or “Special K”. The drug is very dose-specific in its physiological reaction. Although ketamine has been in clinical use for 3 decades, its neuropharmacology is still partially unsolved. Ketamine is known as NMDA receptor antagonist, but is also discussed to act at the GABA_A receptor and the opioid receptors.

We used primary murine frontal cortex networks on microelectrode arrays to study the electrophysiological behavior of ketamine in a dose dependent manner (1nM – 50µM). We investigated the effects of ketamine application to the spontaneous native activity and the NMDA only activity (after blockage of all receptors except that of NMDA).

On the native activity ketamine application revealed diverse effects. At 1nM ketamine increased the electrical activity up to 151 and 130 % for spike and burst rates, respectively, and significantly reduced the activity down to 9 and 15 % in a dose-dependent manner between 1µM and 50µM. This initial increase at lower concentrations was less pronounced under NMDA only conditions (109 and 110 % for spike and burst rate), whereas the drop of the activity at higher concentrations was more distinct (1.5 and 0.4 %). Under both conditions ketamine decreased the network burst synchronicity and rhythmicity, as well as the burst duration. The EC10, 50, and 90 values for ketamine under native conditions were 0.1, 0.9, 8.0µM and under NMDA only conditions 0.1, 0.5, 2.2, respectively.

These shifts in the EC values reveal the higher sensitivity for ketamine under NMDA only conditions than for native activity. The initial increase of the native activity after ketamine application and this shift in the concentration curve indicates a dose-dependent behavior with receptor-specific influences at different concentrations. At lower concentrations disinhibition, e.g. of the GABA_A receptor, seems to have more influence than inhibitory effects at the NMDA receptor at higher concentrations.

In conclusion this study will contribute to clarify the mechanism of main anaesthetic and negative side effects such as dysphoria, fear and nightmares. The findings might help in questions such as anaesthesia depth and proper dosage and administration of comedication.

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The temporal fine structure of retinal ganglion cell light responses improves intensity estimation.

Greschner Martin, Thiel Andreas, Ammermüller Josef

Neurobiology, Carl von Ossietzky University Oldenburg, 26111 Oldenburg, Germany

Retinal ganglion cells respond with distinct spiking patterns to changing light stimuli. These patterns have been reported as oscillatory or rhythmic bursting, both in mammalian and non-mammalian retinas [1,2]. In the turtle, ganglion cells respond to spatially homogeneous light flashes with a series of action potentials grouped into two to five bursts, occurring in the first 150 ms after an intensity change.

Extracellular recordings from ganglion cells were performed with a 100 channel multi-electrode system in isolated turtle retinas. Full field light stimuli, covering an intensity range of five log units, were applied to the retina using a LED.

In many cells, light flashes of increasing intensity evoked typical burst patterns. Number and timing of bursts changed in a systematic way as a function of intensity: latency of the first burst decreased with increasing intensity. Intermediate intensities generated more bursts, and the inter-burst-intervals decreased with decreasing intensities. The bursting pattern was independent from flash duration, and was only slightly altered by subsequent flashes. Within a given adaptation range, patterns are highly distinct with respect to the applied intensity. Therefore, these patterns could contribute to stimulus estimation. Using discriminant analysis, we found that the inter-burst-intervals became the second most important variable after initial latency, if ganglion cells with a clear burst pattern were used for estimation. The latency of the ON response represented best the final intensity reached after the light flash and was independent from the initial intensity before the light flash. The OFF response latency showed a converse behavior. The inter-burst-intervals depended on both the initial and final intensity, providing a relative latency useful for stimulus estimation.

To clarify the intraretinal mechanisms leading to this rhythmic ganglion cell activity, we designed a detailed computational model including all major retinal cell classes, described by a set of differential equations. To ensure a realistic input to the inner retinal cells, model photoreceptor and horizontal cell responses were fit to intracellular recordings obtained simultaneously to the extracellular spike data. Outer retinal cells already show main characteristics of the final ganglion cell responses, namely shortening latency, saturation, and longer overall responses at increasing intensity. This sustained outer retinal signal is transformed into a transient one with intensity dependent duration in both ON and OFF bipolar cells via delayed inhibition provided by an amacrine cell circuit. The final ganglion cell potential is formed from convergent antagonistic ON and OFF BC input, resulting in activity peaks that reproduce the recorded burst patterns characteristic intensity dependence. Furthermore, intracellular data from amacrine cells fits nicely with the postulated model amacrine cell types.


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Multichannel integrated circuits for recording neuronal signals from microelectrode array

P. Grybos, W. Dabrowski, T. Fiutowski, P. Hottowy

AGH University of Science and Technology, Faculty of Physics & Nuclear Techniques, al. Mickiewicza 30, 30-059 Krakow, POLAND

We present two low noise multichannel Application Specific Integrated Circuits (ASIC) called NEURO64 and NEUROPLAT64 for recording extracellular neuronal signals from microelectrode array. Both chips have been made in standard CMOS technology and can be used to build readout systems for simultaneous recording of signals from several hundreds of electrodes or more. These readout systems aim for understanding the mechanism of information processing in the correlated activity of cells building neuronal systems.

Each of the chips contains 64 readout channels. The single readout channel is built of low noise preamplifier and section of bandpass filters. In order to reduce the number of output lines, the 64 analogue signals from readout channels are multiplexed to single output by an analogue multiplexer. The main control parameters are gain and cut-off frequencies. The chips have been optimized for low noise, good matching performance and tolerance to the input offsets generated by neuronal cells.

The main parameters of NEURO64 and NEUROPLAT64 are shown in table below.

<table>
<thead>
<tr>
<th>Parameters / chip</th>
<th>NEURO64</th>
<th>NEUROPLAT64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functionality</td>
<td>Signal recording</td>
<td>Signal recording / platinization</td>
</tr>
<tr>
<td>Technology - area</td>
<td>CMOS 0.7µ - 6.4x4 mm²</td>
<td>CMOS 0.5µ - 6.4x3 mm²</td>
</tr>
<tr>
<td>Gain</td>
<td>40 dB - 80 dB</td>
<td>45 dB - 60 dB</td>
</tr>
<tr>
<td>Lower-cut off frequency</td>
<td>10 Hz - 130 Hz</td>
<td>12 Hz - 112 Hz</td>
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<tr>
<td>Higher-cut off frequency</td>
<td>400 Hz - 2.4 kHz</td>
<td>50 Hz - 4.5 kHz</td>
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<tr>
<td>Equivalent input noise</td>
<td>3 µV (30Hz - 1.4 kHz)</td>
<td>3 µV (85Hz - 2.5 kHz)</td>
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<tr>
<td>(measured in bandwidth)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Power per single channel</td>
<td>1.7 mV</td>
<td>2 mV</td>
</tr>
<tr>
<td>Tolerance to input offsets</td>
<td>mV range</td>
<td>AC coupling implemented</td>
</tr>
<tr>
<td>Spread of gain and cut-off frequencies (sd./mean)</td>
<td>1.5 - 2.0%</td>
<td>1.7%</td>
</tr>
</tbody>
</table>

Conclusions: Both presented ASIC due to their architecture and good parameters are adequate to simultaneous recording of extracellular electrical activity from hundreds of neurons. Nowadays the NEURO64 chips are working successfully in Retinal Readout System consisting of 512 readout channels (8 chips x 64 channels) for simultaneous recording of signals from alive retina stimulated by different light pattern.
CMOS Microchip for recording and stimulation of electrogenic cells

F. Heer¹, W. Franks¹, A. Blau², S. Hafizovic¹, F. Greve¹, S. Taschini¹, C. Ziegler², A. Hierlemann¹, H. Baltes¹

¹ ETH Zurich, Physical Electronics Laboratory (PEL), 8093 Zurich, Switzerland
² Kaiserslautern University of Technology (KTECH), Department of Physics, 67663 Kaiserslautern, Germany

Abstract:
A monolithic extra cellular recording system fabricated in an industrial CMOS-technology combined with post-CMOS processing is presented. The chip comprises 16 platinum electrodes in a 4 by 4 array, where each electrode is equipped with stimulation and signal-conditioning circuitry. The system architecture includes multiplexers and A/D converters for each row and a digital control unit.

Methods:
The 4.4x4.4 mm² CMOS chip presented here (see figure) consists of a 4 by 4 electrode array with an integrated reference electrode. Fabrication was performed using an industrial 0.6 µm CMOS process at Austriamicrosystems AG, Austria. The electrodes are 30x30 µm² with a 250 µm pitch.

Any electrode subset can be used for stimulation at any time; recording from all electrodes is possible throughout the measurement period. Stimulation and readout operations are controlled by the on-chip digital circuitry.

Filtering of the signals improves the signal to noise ratio. In our approach each electrode is equipped with a band pass filter with corner frequencies at 100 Hz and 50 kHz and a total gain of 1000. Implementing the filters at each electrode has several advantages. An in-pixel low pass filter allows a faster multiplexing and prevents aliasing when the signal is sampled. An in-pixel high pass filter cancels the offset, which allows the signal to be amplified before it is multiplexed.

The electrode is formed in a 2-mask post-processing procedure where the metal of the CMOS process (aluminum) is covered with biocompatible platinum. A 1.6 µm thick stack of alternating Si₃N₄ and SiO₂ layers protects the circuitry from the biological solution. The Pt-electrode has been electrically characterized and the experimental results have been fitted to an equivalent circuit model presented in [1].

Results:
The band-pass filter has been electrically characterized, using a gain-phase analyzer (HP-4194A). The equivalent input noise of the filter is below 9 µV_RMS (10 Hz – 100 kHz) and was measured with a spectrum analyzer (HP-4195A).

For biological tests, the chip has been mounted into a standard ceramic package (PLCC84) and covered with PDMS; bondwires were previously fixed with epoxy in order to make them insensitive against swelling of PDMS. First tests were performed using heart cells from chicken embryos, of which four typical spikes are overlaid in the figure.

Conclusions:
A microelectrode array capable of simultaneous recording and stimulation of electrogenic cells has been presented. The functionality of the circuitry components has been verified with regard to the initial specifications. First successful measurements were realized with heart cells from chicken embryos.

REFERENCES:

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Figures:

Contact information (first author)

Name: Heer Flavio
Address: ETH Hönggerberg HPT-H2, CH-8093 Zürich
Phone: +4116336577
Fax: +4116331054
Email: heer@iqe.phys.ethz.ch
Micro-fabrication technologies permit the development of multi-electrode array (MEA) biochips for the long-term stimulation and monitoring of cells/tissue in culture. The current fabrication costs and hence the price of most MEA biochips present one of the major limitations with regards to the widespread utilization and adoption of the technology. In order to overcome this limitation, MEA users typically reuse the MEA biochips as many times as possible. However, the quality of the electrodes progressively degrades with each repeated use of the devices, which may affect device performance and experimental reproducibility.

We have developed a cost-effective and versatile approach whose underlying concept is the reduction of MEA chip dimensions in order to get more devices per processed substrate. In order to make them compatible with standard MEA biochip amplifier interfaces, the MEA biochips are mounted onto printed circuit boards using quick, cheap and reliable manufacturing processes. A major advantage of this approach is that the MEA devices can be readily adapted to any amplifier interface, and also permits the development of multiple MEA device formats, such as microtiter plate formats for industrial needs.

Currently, we are extending our developments with microelectrode substrates in order to realize molecular biosensors for applications in medical diagnostics and drug discovery. We have adopted an approach that is based on self-assembled monolayers (SAMs) on gold electrodes using electrical impedance spectroscopy as a detection method. In medical diagnostics, proof of concept has been demonstrated for the detection of malaria antibodies and now intends to develop a rapid, sensitive and low-cost HIV test. In drug discovery, we are developing fluorescence-free cell-based assays via monitoring changes in extracellular concentrations of physiological ions such as H\(^+\), K\(^+\), Na\(^+\).

60 electrodes MEA biochip. High magnification pictures of planar and tip-shaped 3D electrode array workspace
Purpose:
Currently most MEAs are used for recording extracellular potentials. However MEAs also can be used as 2D stimulators. We have built a MEA system capable of simultaneous stimulation of 60 electrodes. The effect of electrical stimulation on cortical brain slices is recorded using multiple patch-clamp micropipette electrodes.

Methods:
A PC running MATLAB controls multiple digital-to-analog DAC cards (DAC) to produce high-resolution voltage sequences. Stimulation signals used include biphasic square voltage signals with amplitudes ranging from 0.5 to 1.3V and pulse durations of 1ms. Stimulation amplitude was limited to 1.3V to avoid electrochemical reactions at the electrode. The buffered DAC cards allow simultaneous stimulation of all electrodes with arbitrary pulse shapes. The stimulation sequence is triggered and synchronized from the patch-clamp recording system. A tight coupling of the brain slices to 3D MEA was obtained by coating the MEA with PEI (polyethylene imide).

Results:
EPSPs were recorded from neocortical rat brain slices. Stimulation at different electrode locations results in specific post-synaptic signals. The recorded signals can be expressed as synaptic conductance changes, which can be further decomposed in excitatory and inhibitory conductance changes.

Conclusions:
Simultaneous 2D multi-channel MEA stimulation used together with patch-clamp recording opens new possibilities for the study of network properties.
Learning in neuron culture via temperature control

Jacobi S., Moses E.

Physics of Complex Systems, Weizmann Inst., Rehovot, ISRAEL

Abstract:
Induced changes in behavior are related to learning in its most basic meaning, and have been shown to occur in neuron cultures in response to electrical stimulation. In this work, temperature control is proposed as a simple method to control the behavior of a neuron network.

We are using Multi-Electrode Arrays to probe the activity of neurons in a culture of hippocampal rat neurons on a long term basis. During the growth period, the neurons randomly connect to form a neural network, and they are then put into a specially designed temperature controlled incubator. The incubator allows feedback control of the temperature of the culture in response to the activity, which is simultaneously monitored by the electrode array.

A behavior parameter (BP) is defined based on the short-term correlations between the neurons, in a manner which is temperature invariant. The network activity is then measured in real time and the data used to extract the BP, which is continuously monitored. The temperature of the incubator is set to maximize the BP in the long term, thus changing the neuron network behavior.

The method will be demonstrated using several possible behavior parameters.

Purpose: Teach a neural network a desired behavior
Methods: Temperature controlled incubator is used to continuously change the temperature of a hippocampal rat neural culture. The neuron culture activity is sensed using multi-electrode array, and the action potential timings are processed in real-time in order to calculate a behavior parameter. The temperature is changed in order to change the network behavior in a way that will maximize the behavior parameter during the experiment.
Results: Results will be shown, in which various changes have been induced to the network behavior.
Conclusions: A new method for changing the behavior of neural cultures has been demonstrated.
MICROPATTERNED NEURONAL NETWORKS ON MICROELECTRODE ARRAYS
M. Jungblut, M. Pottek, C. Schwind, W. Knoll, C. Thielemann
Max Planck Institute for Polymer Research, Mainz, Germany

PURPOSE:
Multi-unit recording from neuronal networks cultured on microelectrode arrays (MEA) is a widely used approach to achieve basic understanding of network properties as well as the realization of cell-based biosensors. However, network formation is more or less random under primary culture conditions, and the cellular arrangement often performs a poor fit to the electrode positions resulting in successful recording of merely a small fraction of cells. An approach to overcome this shortcoming is to raise the number of cells on the MEA thereby accepting an increased complexity of the network.

In this study, we followed an alternative strategy to increase the portion of neurons located at the electrodes. Appropriate settlement of the neurons is biased by taking control over the adhesive properties of the MEA surface by two-dimensional patterning of the protein layer generally used to promote cell adhesion and growth. This combination of surface structuring and multi-unit recording may offer a promising means to characterize neuronal activity in networks of limited complexity.

METHODS:
The microcontact printing technique was employed to adsorb various patterns of poly-D-lysine (PDL) to the MEA chips (MCS, Reutlingen, and self-made). PDMS (polydimethylsiloxane) stamps were soaked in a solution of sterile PDL (0.1 mg/ml in PBS) and brought into tight contact with the chip surface for 15 minutes. The PDL stamp was adjusted onto the electrode geometry by an optical setup. To find optimal conditions for the two-dimensional cell arrangement, rectangular, circular, and triangular patterns were utilized (line width 5 µm). The pattern geometries were chosen to offer appropriate adhesion points for the cell bodies at the nodes of the meshwork while the lines should guide the outgrowth of neurites.

Neocortical cells were obtained from 19-day old rat fetuses (E19), seeded on the MEA with a density of 100-300 cells/mm², and were cultured at 37 °C and 5% CO₂ in serum free B27/Neurobasal medium supplemented with 0.5 mM glutamine.

RESULTS:
Cortical cells were found to grow in patterns showing good compliance with the predetermined PDL pattern.

After three weeks in vitro electrical activity consisting of single spikes and bursts was observed. Chemical stimulation with well-known neuroactive drugs demonstrated that the network’s reaction is physiologically reasonable. Furthermore, immunocytochemical assays illustrated that synaptogenesis occurred in these cultures accounting for a reliable network activity.

CONCLUSIONS:
In this work, we present extracellular signal recordings of a patterned neuronal network on a microelectrode array chip. We found that neuronal cells can form active networks with synapses on bio-patterned MEA’s, if the chip preparation and the cell culture conditions are chosen carefully. Network activity could be measured for several weeks. Further work will focus on long-term recording of sparse networks to gain fundamental understanding of neural network behaviour, which is of interest not only for basic neuroscience studies but also for cell-based biosensor technology and tissue engineering.
A microfluidic dispensing system for localized stimulation of cellular networks

S.Koster¹, T.Kraus¹, E.Verpoorte¹,², N.F. de Rooij¹

1 University of Neuchâtel, Institute of Microtechnology, Neuchatel, Switzerland, 2 Groningen Research Inst. of Pharmacy, Univ. of Groningen, the Netherlands

We present a 3-D microfluidic device that enables localized drug delivery to living cells growing on an active electrode array fabricated using CMOS technology with independently addressable electrodes. More details on the electrode array can be found in another contribution at this meeting. The device features a flowcell in which the electrode array will be integrated using a flip-chip approach. Drugs can be introduced at several positions, allowing drugs to be delivered to the cells growing on the electrodes at eight different locations. The functioning of the drug delivery device was tested, without electrode array, using an organism that is fairly simple to cultivate, slime mould. The device allowed us to deliver drugs to the cell to evoke a measurable change in its oscillation behaviour.

A multilayer device, containing a silicon-based flowcell, is used for the experiments (see figure). The flowcell is closed by a glass coverplate in which 40-µm drug-delivery holes have been drilled using an excimer laser. A rectangular piece of PDMS, in which microchannels are moulded, is bonded to the glass coverplate to connect the laser-drilled holes with external fluidics. Nutrients are introduced into the cell cultivation area through a separate inlet. Drugs can be injected through one of the eight laser-drilled holes into the nutrient flow a few millimetres upstream from the cell cultivation area, as shown by the red ‘drug’ line in the figure. The drugs then enter the cell cultivation area as a thin stream to stimulate each cell or cell section that comes into contact with it. Slime mould, a multi-nucleated single cell, was grown in the flow cell on top of an agar layer that was poured on the bottom of the cultivation chamber. Slime mould is ubiquitous and a good testing organism for our drug delivery system since the slime mould is robust and easy to cultivate. Mineral water was used as a nutrient (75 µL/min). Ethanol (1 µL/min), 60% with red dye for visualisation was introduced through one of the laser-drilled holes into the flow cell. Ethanol is toxic to the slime and is expected to kill only the part of the slime that is exposed to ethanol.

The drug delivery experiment was accompanied by measuring the thickness oscillations of the slime mold (recording light intensity with a CCD camera) caused by protoplasm streams inside the slime. Frequencies are typically on the order of 0.01 Hz. From the oscillations it could clearly be seen that the slime that is exposed to ethanol stops oscillating whereas the oscillations of the unexposed slime was unaffected.

At present, the HL-1 tumor cell line of mouse cardiac cells (together with W. Franks, ETH Zurich) and nerve cells of chicken embryos (together with A. Blau, Kaiserslautern University of Technology) were successfully cultivated in our device. Drug delivery experiments to these cells, grown on top of an electrode array, are planned.

Figure Schematic overview of the flow cell. The silicon flow cell was reversibly closed with a glass coverplate using grease. The PDMS layer was bonded to the glass coverplate using an oxygen plasma. Drugs can be introduced in the cell cultivation area through one of the microfluidic channels connected to laser drilled holes, represented by the red line.

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Mapping low frequency-induced synaptic plasticity in the rat hippocampal formation with a microelectrode array.

Fabien Lanté, Max Récasens and Michel Vignes

FRE 2693 CNRS ‘Plasticité Cérébrale’ Université Montpellier II, Montpellier cedex 05, France

Purpose:
Activity-dependent alterations of synaptic efficacy, such as long term potentiation (LTP) are the most likely mechanisms underlying synaptic plasticity required for memory storage and retrieval. These modifications mediated by afferent stimulations have specific properties according to the pathway considered. For instance, LTP in the CA1 area of the hippocampus has distinct properties from those of LTP in the CA3 area. Previous studies have demonstrated that in the CA3 area short lasting low frequency-mediated synaptic modifications were also synapse-specific.

Methods:
In order to get an accurate insight into the regionalisation of low frequency-mediated alterations of synaptic efficacy in the hippocampus, we have recorded synaptic transmission evoked by electrical stimulation in rat acute hippocampal slices with a 60-microelectrode array. Experiments were carried out at 37°C and the preparation continuously perfused with extracellular physiological medium (flow rate~1-2mL.min⁻¹). Perfusate included GABAₐ antagonist picrotoxin (50µM).

Results:
When low frequency stimuli (1Hz; 5 minutes) were delivered in the CA3 area, fEPSP amplitude was transiently and reversibly enhanced to 148 ± 8 % (n = 5) of control (obtained by stimulating at 0.1 Hz). This was observed by stimulation of association/commissural fibres or mossy fibres. When such a protocol was applied in the CA1 area, a transient enhancement of fEPSP (138 ± 2 of control, n = 6) was recorded but also a persistent enhancement of synaptic efficacy, characterised by a slow onset. On average, fEPSP amplitude was 148 ± 7 % of control (n = 8) after 40 minutes. This form of synaptic plasticity was insensitive to N-methyl-D-aspartate receptors blockade. By contrast, when the protocol was applied to dentate cells, a reversible depression was observed in the hilar area of the dentate gyrus. This depression was reversed by 83 ± 3 % (n = 4) when A1 receptor antagonist DPCPX was applied. To some extent, GABAₐ receptors and group II metabotropic glutamate receptors were also involved.

Conclusions:
Therefore, it appears that low frequency-mediated synaptic plasticity is highly regionalised in the hippocampus. This reinforces the specificity of hippocampal pathways in plastic processes.
A detailed investigation of neuronal networks requires a defined topology of the synaptic connections and a stimulation and recording technique that allows long-term supervision of the neurons involved. In previous studies, field-effect transistors and capacitive stimulation areas on silicon chips were shown to be suited for non-invasive electrical interfacing of mollusc neurons [1]. Recently, we obtained small networks of defined geometry using topographical guidance of snail neurons [2]. Here we present a combination of these two methods.

We fabricated silicon chips with arrays of bidirectional contacts of capacitive stimulators and field-effect transistors. On top of these chips, topographic polyester structures were processed consisting of pits that were aligned with the bidirectional contacts and of narrow connecting grooves. Neuronal somata were dissociated from Lymnaea stagnalis and placed into the pits. The grooves guided the outgrowth of neurites and held them in their grown geometry with the cell bodies being immobilized by the pits. Electrical synapses formed when the growing neurites encountered in the grooves. Individual neurons of small nets were capacitively stimulated by voltage pulses applied to the chip. Signals propagated along the neurites passed the synapses and triggered action potentials in postsynaptic neurons which were recorded by the respective transistors.

Floating Gate Transistors for Cell Coupling

S. Meyburg¹, M. Goryll², J. Moers², H. Lüth², S. Ingebrandt¹, A. Offenhäusser¹

Institute of Thin Films and Interfaces (ISG), ¹Bio and Chemo Sensors (ISG2) ²Semiconductors Thin Films and Devices (ISG1), Forschungszentrum Jülich GmbH, Jülich Germany

Purpose:
A new type of field effect transistor (FET) for extracellular recordings from electrically active cells is presented. The floating gate architecture combines a CMOS-type n-channel transistor with a sensing area of varying size. The concept allows an independent optimisation of the transistor design towards low noise and an optimal size of the cell-sensing area.

Methods:
Arrays of 4x4 FETs were fabricated in our institute's cleanroom facilities. The process contains those steps of a standard CMOS process that were necessary to build up n-channel transistors. The devices were passivated with a stack of silicon oxide, silicon nitride and silicon oxide (ONO). The sensing areas were opened by reactive ion etching and thermally oxidized to form 10nm of silicon oxide. The devices were encapsulated and electrically characterized in water with an Ag/AgCl reference electrode. For the extracellular signal recordings with embryonic rat cardiac myocytes the chips were coated with fibronectin.

Results:
The new sensors had transconductances comparable to previous ISFET designs of our group. The peak-to-peak noise was less than 300µV. The free floating gate potentials induced a drift of the output signals in addition to the usual drift of FET devices, that has to be controlled for long-term measurements in future. Action potentials of embryonic rat cardiac myocytes cultured on chips were recorded before and after the addition of positive chronotropic isoproterenol. The signal shapes of the measured action potentials were comparable to previous measurements of our group using ISFET devices without floating gates. The encapsulated chips were robust and reusable for many times.

Conclusions:
The electrical properties of the new sensors are comparable to former ISFET designs of our group. Moreover the devices are robust and allow to optimize the sensing area and the transistor independently. The fabrication process was close to standardized CMOS. An extention of the process towards complete CMOS allows to realize additional features such as pre-processing of signals on chip, a higher number of sensor spots by addressing individual FETs and stimulation and recording of cells simultaneously.
Interfacing neuronal microcircuits cultured on microelectrode arrays with pulsed artificial neural networks

Fabrice Olivier Morin

Japan Advanced Institute of Science and Technology, School of Chemical Materials Science, Tamiya Laboratory, 1-1 Asahidai, Tatsunokuchi Machi, Nomi Gun, Ishikawa Ken, Japan,

Neuronal cells cultured on the surface of planar microelectrode arrays can be used as biosensors, as a tool for neurosciences or as a platform for the development of neuroprosthetic devices. In any case, the interpretation of the signals recorded from the electrodes is a tricky exercise. Under the right culture and recording conditions, however, it is possible to record over several minutes, or even hours, several thousand spikes distributed across one or more channels (see for example [1]). This feature offers interesting possibilities for the development of a new kind of processing paradigm for spike signals from microelectrode arrays.

Our approach is to use artificial neural networks (ANNs) as processing units for the signals recorded by the microelectrode array. Indeed recent theoretical works have shown that networks of spiking neurons driven by a time-varying signal can actually present information contained in the latter in such a way that simple linear readout devices can efficiently process and/or retrieve it [2,3]. Attractive as this approach may sound for multi-electrode data processing, adapting the ANN to the inputs remains a major obstacle. Taking the Panasonic Med64 system as a starting point, we have thus designed a software interface that tackles that problem. Based on LabView 7, it enables us to record time stamps of multi-channel neuronal activity, and then passes the data (through an ActiveX object) on to Matlab 6.1. The latter is first filtered for noise removal and spikes are subsequently isolated by an ad-hoc algorithm. The timing of these spikes is the parameter that is passed on to ANNs for further processing.

So far, we have shown that a simulated neuronal microcircuit can adapt its activity to the activity present in the neurons cultured onto the microelectrodes. It does so by implementing a hebbian-learning paradigm where learning epochs are given by the time stamps recorded via LabView. However, we have found that if the learning algorithm is allowed to proceed without any limitation, it leads to the synchronization of the bursts in the artificial neural network. Thus we have devised a mathematical measure of the synchrony of the spiking activity within a network that provides us with a criterion for stopping learning when the ANN exhibits complex dynamics, without “over-synchronization” compared to the driving inputs. We intend to test the validity of our approach by using the current set-up to carry out short-term prediction of network activity.

Using artificial neural networks as kernels for projections in high-dimensional spaces in conjunction to the long-term recording ability of microelectrode arrays enables one to generate data sets of a size that is sufficient to apply mathematical tools from information theory. We believe that this should provide an opportunity not only for confronting theory and experiment, but also for very practical applications. The approach described here, for example, will definitely be useful in neuroprosthetics since it can implement real-time computations on complex multi-channel signals.

REFERENCES:


PURPOSE
Cell-based assays are used in pharmaceutical research to screen large amounts of substances in order to analyze their pharmaceutical relevance. Thereby, information about drug specificity and cell viability is usually achieved by optical methods. Besides this, a highly analytical and parallel detection method is offered by functional coupling of cell-based assays with a microelectrode array (MEA).

To perform such extracellular recordings from highly sensitive electrogenic cells like cardiac myocytes, we combined the MEA biosensor with a microfluidic system. As a result, a fully integrated but modular cell culture “lab-on-a-chip” providing optimal cell culture conditions is presented. Low substance volumes, reduced evaporation, and precise control of drug concentration are benefits that make this approach attractive for drug screening applications.

METHODS
A MEA chip was designed for the integration into the microfluidic system. Sixteen electrodes (40 \( \times \) 40 \( \mu \)m\(^2\)) and an additional internal reference electrode were fabricated on a glass substrate. An integrated platinum sensor allows to control the temperature on the chip. Signals recorded by the MEA are amplified and bandpass filtered by a 64-channel filter amplifier (Multi Channel Systems, Reutlingen, Germany).

The flow-through chip developed for this purpose, is a multi-layer structure of 150 \( \mu \)m thin optical glass at the bottom and a three-dimensionally structured silicon wafer of 400 \( \mu \)m thickness on the top. Glass and silicon are assembled by an anodic bonding process. The fluidic channel of 200 \( \mu \)m height is realized by an advanced silicon etching step applied to the backside of the chip (see Figure above).

RESULTS and CONCLUSION
Below, the mean spike rate of the myocyte culture during the course of an Isoproterenol treatment is shown. A dose-dependent increase in spike activity could be observed. Original spike rate values recovered during rinsing.

This setup permits the extraction of parameters like spike rate, spike waveform, and spreading velocity as a function of the drug concentration added to the cell culture. The dynamics and reversibility of the cell response can be investigated by repeated application of different drug concentrations and washing steps.
Electrical stimulation of neuronal networks by a planar Au-Ti-electrode interface

A. Reiher¹, A. Krtschil¹, S. Günther¹, H. Witte¹, A. Krostit¹, A. de Lima², T. Opitz², T. Voigt², K. Kubë³, V. Spravedlyvyv³, A. Herzog³, B. Michaelis³

¹ Institute of Experimental Physics, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany, ² Institute of Physiology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany, ³ Institute of Electronics, Signal Processing, and communication technology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany

Neuronal networks are subject of a wide variety of intensive research activities like the development of artificial neuronal networks with application potential for information technology. Another aspect is the exploration of processes within natural networks, e.g., the CNS, in order to optimize learning mechanisms or to heal neuronal disorders. The aim of our work is to create an interface between networks in vitro formed by neurons from cortices of embryonic rats and semiconductor devices or electrode arrays to communicate with the cells and to electrically modify the plasticity of the network.

Our interface is prepared by electron beam evaporation and consists of a planar finger structure of Gold on a Ti-undercoating with a gap of 300 µm between the electrodes exhibiting an overall electrode thickness of 55 nm. The glass with the electrodes is integrated on the bottom of the Petri dish and the cell culture is cultivated directly on top of it. To verify its functionality for electrical network stimulation, the parameters of the stimulation pulses, i.e. number of pulses, shape, amplitude, and length, are systematically varied and there influence on the network activity is analysed for a part of the network by Ca²⁺-fluorescence imaging and for single neurons by patch-clamp measurements.

As a result, stimulated network activity, i.e. an induced generation of action potentials, is observed and the threshold voltage amplitude is reproducibly determined to be 1.8-2.2 V. This pulse height is sufficient to provide synchronous network activity, but is small enough to avoid electrolytical effects. The influence of the other stimulation parameters as well as fatigue effects which are observed within the network after excitation are presented in detail and compared with simulation calculations on biological realistic neuronal networks. Furthermore, impedance measurements at the interface with and without cells are performed and will be discussed, too.

These results demonstrate the principal ability of our system for electrical network stimulation and provide information on the corresponding threshold values. We are currently testing the next generation of electrodes containing arrays of 30 µm sized electrodes for detection of neuronal responses and more complex geometries for excitation of multiple compartments.

Fig.: DIC (a) and Fluo-3 fluorescence (b, c) images of a part of the network located between the electrodes showing stimulated neuronal network activity as a consequence of our electrical pulses. The white spots are due to enhanced fluorescence and indicate the firing neurons by the increase of the intracellular Ca²⁺-concentration. Image (b) was taken directly after stimulation. In contrast, without stimulation pulses (c) only spontaneous activity was observed.
MULTIPARAMETRIC MICROCHIPS


*Biophysics Dept., Rostock University, Rostock, Germany; **Bionas GmbH, Rostock, Germany; ***Micronas GmbH, Freiburg, Germany

Would you agree to the following statement?
*The more cellular parameters we can study in parallel at living cells on a sensorchip the better we can understand for example cellular reactions to an applied test substance.*

A better understanding of the multifunctional cellular processing of input- and output-signals is fundamental for basic research as well as for various fields of biomedical applications. As a first approach for on-line monitoring of cellular reactions under well-controlled experimental conditions we develop(ed) different so called Cell Monitoring Systems (CMS®). They allow the parallel and non-invasive measurement of different parameters of cellular systems by the use of CMOS silicon microsensors. In cooperation with the semiconductor company Micronas we realised different silicon based microsensor chips.

The cells are directly cultured on the sensorchip. We can measure metabolic parameters such as acidification and respiration as well as the adherence/morphology of cells. Additionally we focus on the development of CMSs to measure electrical signals of neuronal networks cultured on our CMOS neurosensor microchip. The results are comparable to those obtained with previously established multi-electrode array (MEA) methods.

With our present sensor chip system we can measure metabolic and electrical signals on the same chip. Measurements of acidification rate and oxygen consumption as well as electrical activity of neuronal networks from murine fetal spinal cord and frontal cortex tissues have been successful.

Currently the next generation of a CMOS neurosensor chip with integrated electronics (filters, preamplifiers, multiplexer, I²C-bus, ...) is tested. Therewith more sensors could be integrated using the same chip carrier and additional features like PC controlled switching between stimulation and recording could be realized.

To manage the high amount of data we also develop algorithms for automated spike detection and unit separation. For the interpretation of the complex neuronal data sets we use pattern recognition methods.

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A New Platform for Studying Information Processing by Natural Neural Networks

Henry Baltes\textsuperscript{2}, Axel Blau\textsuperscript{1}, Paolo Bonifazi\textsuperscript{4}, Karl Brander\textsuperscript{5}, Olivier Dubochet\textsuperscript{6}, Wendy Franks\textsuperscript{2}, Frauke Greve\textsuperscript{2}, Sadik Hafizovic\textsuperscript{2}, Flavio Heer\textsuperscript{2}, Thomas Hessler\textsuperscript{5}, Andreas Hierlemann\textsuperscript{2}, Barbara Kochte-Clemens\textsuperscript{1}, Sander Koster\textsuperscript{3}, Tobias Kraus\textsuperscript{3}, Christiane Leister\textsuperscript{5}, Nicolaas F. de Rooij\textsuperscript{3}, Elisabetta Ruaro\textsuperscript{4}, Vincent Torre\textsuperscript{4}, Christiane Ziegler\textsuperscript{1*}

\textsuperscript{1} Kaiserslautern University of Technology (KTECH), Department of Physics, Kaiserslautern, Germany; \textsuperscript{2} ETH Zurich, Physical Electronics Laboratory (PEL), Zurich, Switzerland; \textsuperscript{3} University of Neuchâtel, Institute of Microtechnology-Sensors, Actuators, & Microsystems, Laboratory, Neuchatel, Switzerland; \textsuperscript{4} Scuola Internazionale di Studi Superiori Avanzati, Trieste, Italy; \textsuperscript{5} Leister Process Technologies, Kaegiswil, Switzerland

Extracellular multielectrode array recording systems have emerged over the past three decades to complement patch-clamping and optical recording techniques in basic and applied cardiac and neuroscience research. Such systems combine two important aspects for studying metabolic or information processing events in electrogenic tissue. These are, firstly, rather high lateral and temporal sampling densities and, secondly, electrical stimulation channels for modulating electrogenic activity arbitrarily. Yet, higher electrode count and strategies for enhancing longevity as well as automatic and localized drug application would considerably help in studying and exploiting long-term phenomena in brain slices, cultures of cardiomyocytes, or cultured neural networks.

We are proposing a prototype of an integrated in-vitro platform for long-term recording and stimulation of natural neural networks to study their information processing pathways, and to use them for parallel processing applications (e.g. pattern recognition). It consists of an active electrode array in CMOS technology with currently 16 independently addressable 40 µm x 40 µm electrodes with on-chip signal amplification and pre-processing capabilities. Details on its features and performance are presented in a complementary contribution at this meeting. The chip will be flip-chip bonded to a dedicated microfluidic chamber with volumes below 50 µl for nutrient and localized drug delivery. Currently, neural networks from the brain of embryonic chicken or rats, or their respective neural progenitors are grown on thin artificial and structured adhesion layers to ensure tight contact to the recording and stimulation electrodes. The feasibility of using such networks for discriminating patterns is demonstrated exemplarily in basic conditioning experiments relying on electrical stimulation on subsets of available electrodes.

The envisioned final version of such disposable recording and stimulation device with 1024 electrodes and integrated microfluidics will allow researchers to study and elicit electrical activity within cardiac tissue or complex neural networks in much higher spatial and temporal detail than currently possible. Besides investigating connectivity and parallel processing in neural systems, the bidirectional design of the electronics allows for the development of biohybrid devices for a large variety of sensing, perception and drug development applications.

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Joining Ionics and Electronics:  
Semiconductor Chips with Ion Channels, Nerve Cells and Brain Tissue

Peter Fromherz  
Department of Membrane and Neurophysics  
Max Planck Institute for Biochemistry  
Martinsried / Munich, Germany

Electrical information processing in brains and computers relies on different charge carriers – slow ions in water and fast electrons in silicon. It is a challenge to join neuronal networks and computer chips on a microscopic level, to get better insight of brain dynamics, to develop pharmaceutical sensors and to develop hybrid processors for medical and technological applications. The research has two aspects: (i) Elucidation of the local electrical dynamics at the cell-silicon interface. (ii) Assembly of hybrid neuron-semiconductor devices.

In the first area we analyze the neuron-silicon contact using luminescent dyes. On the basis of the optical microcavity effect on silicon we determine a separation of 50 nm between cells and chips. Taking advantage of the molecular Stark effect we measure a sheet resistance of 10 MOhm in the junction. In that junction, ionic-electronic communication is achieved by a current-voltage-current mechanism: ionic and capacitive currents through the cell membrane give rise to a voltage in the junction that affects electronic current in silicon. Capacitive current through the silicon/electrolyte interface gives rise to a voltage in the junction that affects ionic current in the cell. Both directions of interfacing are studied in detail with recombinant ion channels on transistors and capacitors.

In the second area we proceed in two directions: (i) Using large neurons from snails we build well defined small neuroelectronic devices. E.g. an elementary neuronal memory with a chemical synapse is connected to a chip by capacitor stimulation of the presynaptic and transistor recording of the postsynaptic neuron. (ii) Using thin slices from rat brain we study the interfacing of neuronal tissue to silicon chips. With capacitors for stimulation and transistors for recording a signal loop silicon-synapse-silicon was assembled.

Simple neuron-silicon technology is transferred to chips that are fabricated by an extended CMOS process. A chip with a multi transistor array of 16000 recording sites is able to map neuronal activity at resolution of 8 micrometer and 2 kHz. At present it is being used to study the dynamics of individual snail neurons, of grown neuronal networks and of brain slices.
Mapping Neuronal Activity with a CMOS Chip at a Resolution of 8 Micrometer

A. Lambacher\textsuperscript{1)}, M. Jenkner\textsuperscript{2)}, B. Eversmann\textsuperscript{2)}, M. Merz\textsuperscript{1)}, A. Kaul\textsuperscript{1)}, F. Hoffmann\textsuperscript{2)}, R. Thewes\textsuperscript{2)}, and P. Fromherz\textsuperscript{1)}

\textsuperscript{1)} Max Planck Institute for Biochemistry, Department of Membrane and Neurophysics, Martinsried/München, Germany, and \textsuperscript{2)} Infineon Technologies, Corporate Research, München, Germany

Transistors on silicon are able to record the extracellular voltage beneath individual cultured neurons and cultured brain slices \cite{1-3}. Mapping electrical voltage has been achieved in one dimension up to a spatial resolution of 4 micrometer using linear transistor arrays. Twodimensional mapping at high spatial resolution with directly contacted transistors is prevented by the numerous contact lanes of the transistors on the chip. To overcome that problem, a CMOS chip was developed with 128x128 sensors at 8 micrometer spatial resolution which can be read out by integrated multiplexing electronics \cite{4,5}. The sensors can be individually addressed and therefore freely defined regions on the chip can be measured. The resulting frame rate extends from 2kHz (full frame) to 4MHz (single transistor). Here we report on first test experiments of electrical mapping using individual neurons from Lymnaea stagnalis. We show that extracellular voltage can be recorded with a spatial resolution of 8 micrometer and a time resolution of at least 2 kHz. Various waveforms at various places are assigned to an inhomogeneous distribution of ion conductances in the attached cell membrane.

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Neural recordings of dissociated hippocampal cultures using a three dimensional tip shaped microelectrode array

Yoonkey Nam1), Bruce C. Wheeler2)

1) Department of Electrical and Computer Engineering, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA; Department of Bioengineering, Department of Electrical and Computer Engineering, Neuroscience Program, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Here we report the feasibility of neural recording and stimulation using a commercially available multielectrode array with three dimensional tips. Hippocampal neurons were cultured for more than 4 weeks and extracellular spikes were readily recordable from 10 days after the culture. The number of electrodes that could detect spikes increased as cultures get matured.

Purpose

Multielectrode arrays with three dimensional tips (3D MEA) were developed to be used in acute slice cultures and are available commercially. This electrode is potentially attractive for recording from dissociated neurons since the background noise level is low due to large surface area of each electrode. Also, the large smooth electrode surface makes it possible for neurons to attach and grow, possibly leading to good neuron/electrode coupling. However, the size and the shape of the electrode might lead to weakened neuronal signals as well as discourage neuron growth. To resolve the issues we explored the use of these arrays with low density dissociated cultures of hippocampal neurons.

Methods

3D MEAs were purchased from Ayanda Biosystems SA (CH-1015 Lausanne, Switzerland) and treated with poly-D-lysine (0.1 mg/mL in distilled water, Sigma-Aldrich). Hippocampal neurons are taken from embryonic day 18 rats, mechanically dissociated and delivered in Hibernate E from BrainBits TM (www.siumed.edu/brainbits). Hippocampal tissue was triturated and plated in Neurobasal/B27 medium (Invitrogen, Carlsbad, CA) containing 25 µM glutamate and 0.5 mM glutamine, incubated at 37 ºC in 9% O2 and 5% CO2. Cell plating density was 100 cells/mm². The MEA 60 system (MultiChannel Systems, Germany) was used to acquire data from 3D MEA. The thresholds for spike detection were set at ± 6 STD (standard deviation) which was calculated automatically in MC Rack software.

Results

Three cultures were maintained more than 4 weeks for recording and stimulation. Until 10 days after the culture, no electrodes detected spikes. At 14 DIV, 10 (n = 2) electrodes out of 60 detected spikes and 41 ± 4 electrodes (s.d., n = 2) detected spikes at 27 DIV. The amplitude of recorded spikes ranged from 40 – 700 µVpp, while background noise levels were 22 ± 1 µVpp (s.d., n = 60). The amplitude of the spikes generally increased as the cultures mature. Waveforms were largely biphasic with larger positive peaks resembling the first derivative of action potential.

Conclusions

We have demonstrated the use of 3D MEAs for monitoring neural activity of dissociated hippocampal cultures in vitro for more than a month. The quality of signals recorded from these large electrodes is quite good suggesting that these devices will be useful for a variety of studies using dissociated neurons.

Acknowledgement

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Perforated MEAs and suction electrodes for improved cell and tissue recording

B. Stein¹, W. Nisch¹, U. Egert², S. Bauerdick¹, R. Rudorf¹, O. Klink¹, K. H. Boven³, A. Stett¹

¹ NMI Natural and Medical Sciences Institute, Markwiesenstr. 55, 72770 Reutlingen, Germany
² Albert-Ludwigs University Freiburg, Schaenzlestr. 1, 79104 Freiburg, Germany
³ Multi Channel Systems MCS GmbH, Aspenhaeustrasse 21, 72770 Reutlingen, Germany

In the field of drug discovery and safety pharmacology there is a great demand after methods that offer highly parallel and automated monitoring of electrophysiological activity of intact cells and tissues to determine the effect of pharmacological compounds. For this purpose we develop new microelectrode arrays (MEA) and electrode designs to record extracellular signals of single cells and tissues with enhanced signal-to-noise-ratio.

Perforated MEAs for tissue recording. For recordings with brain slices we developed new MEAs with numerous openings in the substrate (Fig. 1). By applying negative pressure to these openings it is possible to position and fixate the slices and to perfuse them from both sides. With negative pressure also the contact between tissue and electrodes is intensified and therefore the magnitude of extracellular recorded signals enhanced. The evoked potentials from acute brain slices recorded with this new MEA reaches up to 3 mVp-p.

Suction electrodes for single cell recording. A prerequisite for recording of dose-response curves with MEAs from intact single cells expressing ligand-gated ion channels is a reproducible and stable contact between cell membrane and planar electrodes. Since the magnitude of recorded signals is directly proportional to the area of the electrode that is covered by them and strongly depends on the cell-electrode distance, we investigated a new electrode design (Fig. 2a). An annular opening surrounds the planar electrode. Application of a small suction to the opening allows precise positioning of a single cell out of a freshly prepared cell suspension on the electrode (Fig. 2b). The negative pressure should also decrease the cell-surface distance and therefore improve the seal resistance. In combination with improved filter amplifier this suction electrode offers the opportunity for recording slow extracellular potentials originating from ligand-gated ion channels in the context of intact cells.

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Real-time bidirectional communication with neuronal cultures

D. A. Wagenaar (1), S. M. Potter (2) and J. Pine (1)

(1) Department of Physics, California Institute of Technology,  
(2) Department of Biomedical Engineering, Georgia Institute of Technology

Multi-electrode arrays (MEAs) enable researchers to electrically communicate with large numbers of cells in a neuronal culture. Technology to record from 60 electrodes simultaneously has been commercially available for many years. By contrast, stimulation studies have mostly been limited to small numbers of electrodes. In this talk I will describe a device that allows stimulation of any of 60 electrodes with real-time software control over channel selection [1]. The device can be directly plugged into MultiChannel Systems MEA preamplifiers, is inexpensive to produce, and can easily be modified for use with other recording hardware.

Using this device to stimulate high-density cultures of cortical neurons, we found that any electrode that is in sufficiently close contact with the culture to record activity, can also be used to evoke activity. We quantified the efficacy of a range of stimulus pulse shapes, both under voltage- and current-control, and found that voltage-controlled, biphasic, positive-phase-first pulses are the most effective stimuli for any given peak-voltage quotient [2]. This is good news, because voltage-controlled stimuli have the added advantage that electrochemistry can be explicitly controlled, make their use considerably safer than current-controlled stimuli.

By connecting this stimulator to our freely available data acquisition software, MeaBench [3], we close the feedback loop between cell culture and computer. The system can generate stimuli in response to recorded action potentials within 15 ms, a timescale that corresponds to only a few typical cortical neuron-to-neuron propagation delays, and similar to the time constant of NMDA channels. Thus, we can now communicate bidirectionally through a 60-channel-wide channel with cortical cultures, at a tempo that matches the neurons' own.


Studies of cortical network function on the basis of single- and multiple single-neuron recordings have revealed neuronal interactions which depend on stimulus and behavioral context. These interactions exhibit dynamics on several different time scales, with time constants down to the millisecond range. Mechanisms underlying such dynamic network organization are investigated by experimental and theoretical approaches. Our current research focuses on two interrelated aspects: precision and variability of cortical network activity. Starting from previous model work in which we investigated conditions for the occurrence of precise joint-spiking events in cortical network activity, I will present recent findings from ongoing experimental and theoretical work in our laboratory, undertaken to test and expand some of the model predictions. Specifically, I will discuss new findings regarding the feasibility and constraints of precise synchronization dynamics in cortical networks, resulting from a critical evaluation of biological constraints from cortical connectivity and in vivo physiology, and dynamical constraints from large-scale network simulations.

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The ability of the brain to retain information is thought to involve input-induced changes in the collective behaviour of neuronal assemblies that is sustained in the absence of external stimuli. Thus, the induction of stable periods of slow rhythm, recurrent neural activity in cortical circuits may be important for memory. However, the molecular mechanisms and intracellular signaling pathways through which synaptic inputs can switch on and switch off particular firing patterns are unknown. The aim of our study was to investigate biochemical mechanisms underlying the induction and maintenance of stimulus-induced persistent activity states.

We used hippocampal neurons that, after a culturing period of 10 to 14 days, form an elaborate and highly interconnected network consisting of about 90% excitatory neurons and about 10% inhibitory interneurons. A brief exposure of hippocampal cultures to the GABA<sub>A</sub> antagonist bicuculline resulted in a change in network behaviour. Repeated multi-site electrical recordings on Microelectrode Arrays (MEA) revealed that, after stimulation, the initial random spike pattern is replaced by rhythmic spike bursts. Recurrent activity was synchronous, self-sustaining and could persist for several days after washout of bicuculline. We could show that this recurrent activity was induced by calcium flux through synaptic NMDA receptors, appears to require activation of MAP kinases (ERK1/2), and was associated with an increase in AMPA receptor surface expression. Recurrent activity was readily switched off after temporary blockade of glutamatergic synaptic transmission leading to AMPA receptor endocytosis. The late phase (> 4 hours) of this form of network plasticity was dependent on gene transcription taking place in a critical period of 120 minutes following induction.

Thus, MAP kinase (ERK1/2) signaling and AMPA receptor trafficking may be involved in the induction of sustained recurrent activity that could play a role in building and consolidating neuronal circuits. Our results also indicate that the dialogue between synapses and the nucleus is important to uphold recurrent activity pattern.
Contributions to in vitro network neurosciences by MEAs and novel electrophysiological protocols


Institute of Physiology, University of Bern, Switzerland

Estimates of the number of synaptic contacts of a neocortical cell range between 5000 and 60000, 70% of them originating from intracortical areas. Furthermore, neocortical neurons fire spontaneously at a frequency of 5–20Hz in awake animals. These considerations define a scenario in which neurons experience large synaptic currents (i.e. hundreds of postsynaptic potentials over a ms-time scale). Such an intense background activity induces random-walk fluctuations in the postsynaptic membrane potentials and it is thought to have a profound impact on the neuronal integrative properties, on the response dynamics to external stimuli, as well as on the activity-dependent plasticities. These implications have been never systematically studied in vivo, because of the technical difficulties related to intracellular and patch-clamp recordings in behaving animals. On the other hand, in vitro preparations are widely employed as reduced models, but in spite of the many advantages they do not accurately represent the realistic cortical networks physiology. Similarly, conventional electrophysiological protocols are used to investigate single-neuron and network properties, under unaccurate and artificial conditions.

In this talk, I will present the results from two different research projects. These projects are linked as they have been inspired by a common theoretical framework, related to the Extended Mean Field Theory of synaptic interactions in network models. They both deal with an attempt at recreating more realistic conditions for the investigation of the electrophysiological properties of individual neocortical neurons as well as the collective activity, emerging at the network level.

The first part of the talk will summarize the contributions at restoring a realistic network input drive in acute cortical slices, by a novel application of substrate arrays of three-dimensional microelectrodes (MEAs), employed for distributed noisy electrical stimulation.

The second part will focus on mature cultures of neurons dissociated from rat neocortex and cultured over planar MEAs: they exhibit spontaneous, temporally patterned, electrical collective activity. Such an in vitro activity constitutes an ideal example for combining the theoretical and experimental approaches previously mentioned, linking the single-neuron response properties to collective network phenomena. A computer model of a network of integrate-and-fire (IF) excitatory neurons is finally presented. Surprisingly, such a network spontaneously reproduced a collective patterned activity, similar to what is observed in cultured networks. This constitutes an indication that the IF model, experimentally identified by the novel paradigm, is an adequate minimal description of synaptic integration and neuronal excitability, when in vitro collective network patterned activities are considered.

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Adaptation and learning are basic and general phenomena. My assumptions are that (1) these phenomena are "beyond anatomy" in the sense that they are realized in a wide range of neural systems that are different from each other at the cellular and tissue organization levels. (2) To be manifested at the behavioral level, the mechanisms underlying adaptation and learning need be considered from the neuronal population point of view; for, as much as we know, no behavior is dependent on the activity of a single neuron, let alone single spike or single synapse. With these two assumptions in mind, we are attempting an experimental approach to uncover the principles underlying adaptation and learning in population terms. The properties of the experimental model in use, a large random network of cortical neurons, will be presented. As a prototype of network adaptation I will show how selective adaptation to input is realized by these "structureless" networks, where interactions between different activation paths, as well as the balance between excitatory/inhibitory sub-networks, play a key role. I will proceed by commenting about two general types of learning environments, one in which a rewarding entity is required (e.g. Schultz, 2002; ) and the other in which learning follows the classic drive-reduction principle (Freud, 1985; Hull, 1943; Guthrie, 1946). Based on our recent results from dopamine application experiments I will suggest that the two types of learning environments are in fact closely related to each other. I will close the presentation by listing the required technological developments that will enable experimentally addressing key aspects of network neurophysiology, and their impacts on future brain-machine interfacing.
Circadian activity rhythms in acute and organotypic explants of the hypothalamus of the mouse

Ehab Tousson*, Sabine Mahr, Christopher Klisch, Hilmar Meissl

Max Planck Institute for Brain Research, Neuroanatomical Dept., 60528 Frankfurt/Main, Germany

Purpose: The suprachiasmatic nucleus (SCN) in the hypothalamus controls in mammals the circadian rhythm of many physiological and behavioral events by an orchestrated output of the electrical activity of SCN neurons. Light can phase-shift the endogenous oscillator in the SCN by retinal photoreception, synchronizing it with the prevailing environmental day-night cycle. The circadian information is then transmitted to different neuronal targets: neuroendocrine and autonomic neurons in the hypothalamus, as well as in areas outside of the hypothalamus. We thought to identify the brain targets of SCN neurons which might indicate a close relationship to neuroendocrine and autonomic brain centres.

Methods: In the present study we cultured hypothalamic brain slices of CD1 mice (3 weeks old for acute slices, 2 days old for organotypic slices) on multi-microelectrode arrays (MEAs) to study simultaneously the electrical activity of SCN neurons of mice and their possible target neurons in the hypothalamus.

Results: Clear circadian rhythms in spontaneous firing rate with periods near 24 hours (acute slices: 24.0±0.2; n=21 and organotypic slices 23.7±0.2; n=18) were observed not only in the region of the SCN and in directly adjacent hypothalamic areas, but also in the dorsal hypothalamus, in the subparaventricular zone, the paraventricular nucleus of the hypothalamus (PVN) and in regions where descending paraventricular pathways have been described. Recordings from acute and organotypic brain slices from the SCN and PVN gave essentially similar results concerning the distribution of circadian signals and the phase of the rhythms. In acute slice preparations the mean activity showed a peak near midday, at CT 7.0, whereas in organotypic slice cultures the time of peak activity was considerably shifted, due to the absence of a retinal input and the lack of a synchronizing stimulus that is able to adjust the rhythm. The time of peak activity was stable across several cycles in both preparations. The rhythmic activity was clearly, and reversibly, reduced or completely inhibited by application of GABA (100 µM) or by tetrodotoxin (200 nM). The circadian rhythms recorded outside of the SCN disappeared after ablation of the nucleus showing that the rhythm is generated by SCN neurons, but could be restored by SCN grafts indicating that a humoral factor is responsible for the restoration of circadian rhythmicity in the absence of neural connections. Periodic application of arginine-vasopressin (AVP) in SCN-lesioned animals provided evidence that AVP can induce rhythmicity in the hypothalamus.

Conclusions: These data indicate that the SCN uses a dual, neuronal and humoral, mechanism for communication with its targets in the brain.

*present address: Dept. of Zoology, Faculty of Science, Tanta University, Egypt
Coding of time-varying signals in a population of cultured neurons

V. Sanguineti¹, L. Cozzi¹, P. D’Angelo¹, S. Martinotia²

¹ Department of Informatics, Systems and Telematics (DIST), University of Genova, Genova, Italy, ² Department of Biophysical and Electronic Engineering (DIBE), University of Genova, Genova, Italy

Purpose: As part of the development of a neural interface, consisting of a population of cultured neurons connected bi-directionally to a small mobile robot, we explore the issue of how time-varying ‘sensory’ signals can be coded, as trains of stimuli, in a population of cultured neurons.

In particular, we ask (i) how much of the original signal is lost during translation into patterns of stimulation; (ii) whether and to what extent the original signals can be reconstructed from observation of the firing patterns at different recording sites, and (iii) whether and how much such reconstruction can benefit from using multi-site recordings.

Methods: We used cultured cortical neurons, extracted from rat embryos (17-18 days) and plated on a micro-electrode array (MEA60System, Multichannel Systems). Experiments were performed after 18-34 days in-vitro, when these preparations display a good degree of spontaneous activity. This neural preparation was connected to a miniature mobile robot (Khepera II, K-team), equipped with two wheels and eight infrared proximity sensors, that provide information about the distance from obstacles. The time-varying signals generated by the robot sensors while moving were averaged into global 'left' and 'right' signals, and translated into two separate patterns of electrical stimuli (bipolar, monophasic, peak-to-peak amplitude 1.5 V, duration 250 µs) so that at each time left and right sensor activity specifies the next inter-stimulus interval. We set the maximum rate of stimulation, corresponding to hitting an obstacle, to 2-5 Hz. Stimuli were delivered to the neural population through two selected microelectrodes. The firing patterns evoked by electrical stimulation are detected in real-time from the neural activity, recorded from two separate subsets of eight microelectrodes each. The estimated instantaneous firing rates are then averaged over the two subsets, and used to separately control the two wheels of the robot. Experiments lasted 600 s each, during which the robot moved randomly inside a circular arena. To quantify the accuracy of coding and of reconstruction of the sensory stimuli from the recorded neural activity, we used the patterns of stimulation and the recorded patterns of firing, respectively, to estimate the original time-varying sensory signal by using the Wiener-Kolmogorov optimal filtering approach. We took the squared correlation coefficient between original and reconstructed signal as a measure of accuracy of, respectively, coding (hereafter referred as the Coding Fraction, CF) and reconstruction (Decoding Fraction, DF). These quantities can be interpreted as the portion of the variance of the signal which can be recovered from either the stimuli and the firing patterns. To assess how reconstruction benefits from multi-site recordings, we computed the DF for 1, 2,...,8 recording sites.

Results: For each experiment, the estimated CF establishes an upper bound for the maximum reconstruction that can be achieved. There is a simple relation, similar to the sampling theorem, between the average rate of stimulation and the bandwidth of the sensory signal. The estimated DF for reconstruction from one single recording site varies greatly from site to site, between nearly 0 to a maximum of 29% of the variance of the sensory input. Moreover, in spite of the high correlation among firing patterns at different sites, DF improves as more sites are used for reconstruction. However, DF improvement is highly dependent on the choice of the recording sites.

Conclusions: Our results show that a substantial part of the input can be recovered from the recorded spike trains, thus suggesting that a neural interface which codes a time-varying sensory signal is indeed feasible. The high variability in reconstruction performance suggests that selection of the outputs is crucial in applications, like bi-directional neural interfaces, where one wants to maximize the sensitivity of neural activity on the sensory inputs.
Dynamics and plasticity in developing neuronal networks in vitro

Jaap van Pelt, Pieter S. Wolters, Michael A. Corner, Ildiko Vajda, Wim L.C. Rutten, Ger J.A. Ramakers

Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands

Extracellular action potentials were recorded from developing dissociated rat neocortical networks continuously for up to 49 days in vitro using planar multi-electrode arrays. Spontaneous neuronal activity emerged towards the end of the first week in vitro, and from then on exhibited periods of elevated firing rates, lasting for a few days up to weeks, which were largely uncorrelated among different recording sites. On a time scale of seconds to minutes, network activity typically displayed an ongoing repetition of short episodes of synchronous firing at many sites (network bursts). Network bursts were highly variable in their individual spatio-temporal firing patterns but showed a remarkably stable underlying probabilistic structure (obtained by summing consecutive bursts) on a time scale of hours. On still longer time scales, network bursts evolved gradually, with a significant broadening (to about 2 sec) in the 3rd week in vitro, followed by a drastic shortening after about one month in vitro. Bursts at this age were characterized by highly synchronized onsets reaching peak firing levels within less than ca. 60 msec. This pattern persisted for the rest of the culture period. Throughout the recording period, active sites showed highly persistent temporal relationships within network bursts. These longitudinal recordings of network firing have brought to light a reproducible pattern of complex changes in spontaneous firing dynamics of bursts during the development of isolated cortical neurons into synaptically interconnected networks.
Keynote Address
Capitalizing on the unique recording capabilities of silicon electrode arrays

Tim Blanche
Dept of Ophthalmology & Visual Sciences, Univ. of British Columbia, Vancouver, Canada

Single unit electrodes have for decades been the principal technology for in vivo neuro-physiological studies. The desire to understand the function of biological neuronal networks has more recently motivated the development of electrodes designed to record simultaneously from multiple neurons. Silicon multichannel electrode arrays, or polystrodes, are one such technology. In collaboration with the University of Michigan’s Center for Neural Communication Technology, we developed a variety of 54-channel polystrodes for multiunit in vivo studies of cat visual cortex. High-density, multichannel electrodes such as these present significant challenges for conventional data acquisition, spike detection and sorting methods. However, precisely defined electrode site geometries makes possible innovative applications such as 3D spatial neuron localization and classification of cell type.

There are two parts to my presentation. The first deals with signal pre-processing of continuously acquired spike waveforms. Algorithms for reliable spike detection and sorting from large contiguous electrode arrays will also be discussed. In the second half, I will present a model for estimating neuron location and type based solely on extracellular multiunit potentials recorded with polystrodes.

Polytrodes have the potential to record simultaneously from hundreds of neurons. In practice, neuron yield is limited by spike waveform fidelity, and the efficacy of spike detection and sorting techniques. In addition, what are relatively infrequent problems for tetrodes, for example detection of coincident spikes, become major problems for polystrodes. Judicious application of existing signal processing methods, in combination with new spike detection algorithms, can help realise the large-scale recording potential of polystrodes. Windowed-sinc interpolation of waveforms with sample-and-hold delay correction is shown to accurately reconstruct spike shapes, improve the reliability of threshold-based event detection, and facilitate accurate spike sorting by reducing waveform variability. Interpolation also obviates the need to oversample, resulting in a large reduction in acquisition bandwidth and storage requirements. A spatiotemporal spike detection algorithm assesses each spike's size and location to determine which channels to 'lock-out' and for how long. This allows detection of synchronous spikes on disparate polystrode sites, and minimizes the chance of missing near-synchronous spikes on adjacent sites. On tests with real and simulated data this method performed as well or better than more sophisticated, computationally intensive algorithms.

Historically, extracellular electrodes do not provide any information about cell type (hence the terms 'unit' and 'multiunit'), nor anything about sub-threshold intracellular events. The ability to broadly classify cell type (ie. pyramidal vs. inhibitory interneuron), currently the exclusive domain of intracellular recording, offers the prospect of studying the interactions between different neuronal classes thought to play specific roles in cortical circuits. In many cases it is also difficult to ascertain the exact location of recorded cells from histological electrode tracks. Imprecise knowledge of the spatial relations between recorded neurons is particularly problematic for cortical receptive field mapping studies and, for example, in studies of cortical circuits where the lamina location may be important.

To this end, a mixed monopole/dipole model of extracellular spike potential distributions was developed. To allow for tissue anisotropies and the elongated fields of pyramidal cells, isopotential surfaces are modeled as prolate ellipsoids with principal axes that can have any orientation relative to the polystrode. Independent parameters describe the radial dependence of the potentials in each spatial dimension. Recorded neurons are assumed to lie in front of the polystrode. Levenberg-Marquardt optimization is used to fit the spike waveforms to model functions. Estimated neuron locations emerge as tight, non-overlapping spherical clusters within 150µm of the polystrode. Cluster locations move concordantly with polystrode movements. Model-derived anisotropies are similar to known tissue conductivity tensors for cat cortex. Field potential spreads are bimodally distributed, consistent with the spike shapes and firing patterns of pyramidal cells and interneurons. Some spikes
have current sources that move 100s of microns, with a direction and velocity consistent with back-propagating action potentials (BPAPs).

Polytrodes, when combined with novel signal processing techniques, can be used to study detailed receptive-field maps, structure-function organisation of cortical columns, and in vivo BPAPs.
Extracellular recordings with microelectrode arrays (MEAs) open unique opportunities and a comparatively new window on the electrical activity of cellular networks. Beside the information extracted from this activity with tools available for the analysis of extracellular recordings in general, MEA recordings promise to yield more insights on the dynamics and meaning of network activity. These opportunities, however, are accompanied by quite a number of challenges and some pitfalls during data analysis. The tutorial will give a short introduction of the implicit ideas and techniques underlying some of the more conventional tools for the analyses of spike time series and local field potentials, and address the possibilities and challenges to be met during the analysis of the spatio-temporal structure of activity detected with MEAs.
Towards a new model for the cell-sensor contact

S. Ingebrandt, G. Wrobel, M. Pabst, S. Meyburg, A. Offenhäusser

Forschungszentrum Jülich, Institut für Schichten und Grenzflächen (ISG-2), Jülich, Germany

The theoretical description of the electrical contact of single cells to single sensor spots is still not fully understood. Attempts to describe the contact by an equivalent, fully electronic circuit model – the Point Contact Model [1] – lead to much too small signal amplitudes of the extracellular recorded signals. In recent works this effect was explained as increased conductivity of the attached part of the cellular membrane. Further improvements of the Point Contact Model as a Plate Contact Model taking cable theory for a two-dimensional cable formed by cell and sensor surface into account [2], still don’t explain the observed effects.

We focus in our work on the dynamic description of the cell-transistor contact. We examined the electrical coupling of HEK293 cells, which were stably transfected with the voltage-gated ether-à-go-go potassium channel (beag1). The kinetics of the beag1-channels can be modulated by patch-clamp protocols and different divalent cations in the extracellular medium. Cells were cultured on n- and on p-channel field-effect transistor (FET) devices to compare the influence of the applied bias voltage on the signal shapes. As second cellular system we used hippocampal neurons from embryonic rats coupled to n-channel transistors. Potassium currents of these cells are composed of fast A-type potassium currents and slower K-type potassium currents. By comparing signal shapes from p- and n-channel transistors we find huge differences in amplitude and time-dependence, which strongly suggests that the bias voltage applied over the seal resistor in the cell-sensor junction influence the extracellular recorded signal. In all recordings on both chip types quick potassium currents (in the range of few ms) cannot be recorded [3]. The effect was observed in previous works using hippocampal neurons and was explained as depletion of specific potassium channel types (A-type) in the cell-sensor junction [4]. With our n-channel devices it was in addition possible to record a clear contribution of the fast A-type potassium currents of hippocampal neurons to the extracellular recorded signal shape in contrast to previous observations. Our results with the controlled kinetic of the potassium activity in the cell-sensor junction strongly suggest that electrodiffusion of potassium ions in the small cleft is responsible for the observed effects. We introduce first attempts to model the electrodiffusion of potassium ions in a Monte Carlo Simulation to explain signal shapes.

References:

Pattern Recognition Approach to Classification of Hepatic Encephalopathy Grades based on Specific Spike Train Changes of Frontal Cortex Networks on Microelectrode Arrays

Olaf Schröder1, Christiane Teichmann1, Alexandra Gramowski2, Jan Loock3, Steffen Mitzner3 and Dieter G. Weiss2

1Pattern Expert, Borsdorf, Germany 2 Institut für Zellbiologie und Biosystemtechnik, 3 Klinik für Innere Medizin, Universität Rostock, Rostock, Germany

Purpose: Hepatic encephalopathy (HE) and its grades are difficult to diagnose by physiological and biochemical parameters. Therefore, in this project we develop a diagnostic tool for HE grading based on neuronal sensor chips. Because of the biological diversity in the response of neuronal network activity induced by ultrafiltrate blood samples from patients with different degrees of HE, the activity for the various coma stages cannot be readily distinguished. Consequently we tried to analyse these data with algorithms from neuroinformatics.

The first question was to find out which experimental setup provides the best results? And also which concentration (vol% of ultrafiltrate in the medium) yields the best results? How many experiments are needed?

Methods: Blood serum was obtained from patients in different stages of HE (Grade 0-IV). Healthy subjects served as controls. Neuronal networks were cultivated from primary murine frontal cortex seeded on 64-microelectrode arrays. Cultures were used at the age of 4-6 weeks in vitro. The filtrate content in the culture medium was varied in 4 concentration steps up to 40 vol%. After applying the probes spike trains were recorded for 90 minutes after reaching a stable phase of at least 30 minutes. Recorded data were analysed with Pattern Expert Squid (38 experiments, 190 segments). We computed 104 activity features such as spike rates, burst rates etc.. Afterwards we analyzed these data with machine learning algorithms as multi layer perceptron and with support vector machines.

The most important step is the selection of features. It is clear that not all of 104 computed parameters have the same influence on decision making. To find the best 10 features of 104 features one has to find them out from (104 over 10) combinations.

Results: The combinations of the added probe and neuronal network culture have a unique fingerprint. For 38 classes we obtained a total correct prediction rate of 85.5%. It was not possible to classify probe concentrations by the spike train, like it was possible with several former tested single substances.

Scoring methods to describe influences of parameters show that 20% concentration delivers best results to differentiate between HE stages. Classification of data records for healthy subjects and patients with degree 4 is possible with a correct prediction rate of 89.5%. Classification of healthy subjects and patients with degrees 3 and 4 in one class is also possible.

Conclusions: Classification of hepatic encephalopathy with neuronal biosensors chips is possible. For the classification of different coma stages more experiments are needed. It is expected that optimizing of spike train analysis and classification algorithms will yield better results. Phenomena of individual fingerprints of the blood probes should be investigated in the future.

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MEA Data Analysis for Substance Screening on Neuronal Networks

Martin Konieczny, Christian Mayer, Stephan Theiss

RESULT GmbH, Tönisvorst, and Department of Neurology, Heinrich-Heine-University, Düsseldorf, Germany

Introduction:
Meaningful analysis of electrophysiological data from microelectrode arrays (MEA) is highly application-dependent. For screening of potentially neuroactive agents, drugs or toxins on dissociated neuronal cell cultures, a detailed analysis of the firing pattern across the network may give important additional insight beyond particular spike waveforms, single-channel or population data like spike- or burst-properties. To date, no standardized parameter has been defined for quantitative measurement of synchrony on MEA.

Purpose:
To develop parameters characterizing neuronal firing patterns by synchrony and correlation “across networks”, and test their power to detect subtle drug-induced changes.

Methods:
In a stepwise procedure data complexity was reduced. Raw data sampled at a rate of 25 kHz were high-pass filtered to suppress slow artifacts, and spikes were detected by a noise-level adjusted threshold. The subsequent burst analysis employed an entropy-based statistical definition suppressing additional spurious bursts by the introduction of an entropy tolerance at the burst end.

For assessment of synchrony and correlation between channels spike time stamps were binned on a range of time scales, and bins were dichotomized. Several parameter definitions for synchrony were compared.

Two correlation measures were applied: Pearson’s correlation coefficient and Cohen’s kappa statistic. Their dependence on bin width was examined both by simulation of artificial spike trains according to various gamma-distributions and on experimental data.

Correlation, kappa statistic and synchrony were evaluated on exemplary recordings of cryopreserved dissociated primary cortex and striatal neurons from Sprague-Dawley rats (QBM Cell Science), as well as on hNT2-neurons cultured on 60-channel MEA (Multichannel Systems).

Results:
Automated detection reliably yielded spike- and burst-data validated by human observers. The three cell types exhibited different firing behavior in terms of the proposed parameters synchrony, inter-channel correlation and kappa statistic. The parameters correlate well with the visual inspection of the firing patterns. Exemplarily, dose-response curves were obtained for cryopreserved cortex neurons under application of NMDA and acetylcholine.

Conclusions:
Subtle changes in firing patterns of neuronal networks on MEA may be revealed by analyzing correlation and synchrony over a range of time scales. These parameters may allow both discrimination between different neuronal cell types and quantification of drug effects on networks of dissociated neurons.
The Learning Retina Implant System: First steps towards a clinical application

Holger Becker

IIP-Technologies GmbH, Bonn Germany

Keywords: stimulation electrodes, retina stimulation

Purpose:
to explore the visual perceptions evoked by acute electrical stimulation of the human retina in subjects suffering from retinitis pigmentosa.

Methods:
A multi-center study has been initiated in accordance to the German medical device law and GCP guidelines. Various Ethic committees had authorized the study protocol.
The subjects are examined extensively before inclusion into the study and also after operation and during the follow up. Vitrectomy is performed for a complete removal of the vitreous, local anesthesia is applied subconjuctival to the eye muscles. A newly developed instrument is held by the surgeon to insert, position and keep a micro-contact film onto the subject's macula for ganglion cell stimulation. The micro-contact film utilizes IrOx-electrodes to apply the stimulation currents provided by a current generator. Both, contact film and generator are manufactured by IIP-technologies, Bonn, Germany. Stimulation is performed over a maximum duration of 45 minutes, afterwards the film is removed and the subjects are extensively interviewed.

Results:
More than 80% of the treated subjects reported visual perceptions. Even subject who had no perception at all over years did have a light perception. Yellow and blue are the most reported colors; brightness was described comparable to the light of a candle.

Conclusion:
Electrical stimulation of the human retina does result in visual perceptions. This could be a first step towards a clinical application.
Towards an implantable brain-machine interface based on epicortical field potentials

Carsten Mehring

Neurobiology and Animal Physiology
Institute of Biology I, Albert-Ludwigs-University Freiburg
Hauptstr. 1, D-79104 Freiburg, Germany
mehring@biologie.uni-freiburg.de

Today, a major challenge in neural engineering is to develop brain-machine interfaces (BMIs) for the restoration of motor control and communication in paralyzed patients. One fundamental and unresolved question is which neuronal signal type should be utilized for this purpose. Recent studies demonstrated that an external actuator (e.g. a computer cursor or a robotic arm) can be controlled by the spiking activity of tens to hundreds single neurons recorded from monkey motor cortex. Here, we investigate whether signals reflecting the ensemble activity of local groups of neurons might also be a suitable control signal for BMIs.

At first, we explored to which degree local field potentials (LFPs) recorded from the motor cortex of monkeys performing center-out arm movements can serve as an alternative to single-unit spike trains. We found that LFPs carried essentially the same amount of information about the direction and trajectories of arm movements as the spike trains of single cells, demonstrating the feasibility of using LFPs for the reconstruction of arm movements, e.g. for the control of neuronal motor prostheses.

Secondly we tested whether these findings can be extended to humans by analyzing neuronal activity of the human frontal cortex measured intracranially by means of electrocorticography, using a high-resolution grid (up to 112 electrodes) implanted subdurally in patients for epilepsy diagnosis. We could show that: (1) the direction of arm reaching movements can be accurately inferred from neuronal population activity of the human frontal lobe. (2) Population signals from primary motor cortex carry most directional information within frontal cortex. (3) Single-trial movement inference can be achieved by decoding signals measured with electrodes placed directly on the brain surface, presenting an alternative approach to previous animal models of BMIs using electrodes penetrating, and thus disrupting brain tissue.

Taken together, our findings are a significant first step for the development of new solutions for controlling neuronal motor prostheses.

This presentation summarizes results from a number of ongoing collaborations – contributions by Ad Aertsen, Tonio Ball, Simone Cardoso de Oliveira, Martin Nawrot, Tobias Pistohl, Joern Rickett, Stefan Rotter, Andreas Schulze-Bonhage and Eilon Vaadia are gratefully acknowledged. Research funded by BMBF-DIP, Boehringer Ingelheim Fonds, GIF and the Heidelberg Academy of Science.
DEVELOPMENT OF NEUROPROSTHESES FOR THE BLIND: A STATUS REPORT


1University Eye Hospital, Dept. II, Tuebingen, Germany; 2University Eye Hospital, Regensburg, Germany; 3Natural and Medical Science Institute at the University of Tuebingen, Reutlingen, Germany; 4Institute for Micro-Electronics, Stuttgart, Germany; 5Institute for Physical Electronics, Stuttgart, Germany; 6Dept. of Physics, University of Marburg, 7Department of Neurophysiology, University of Bochum, 8Retina Implant AG, Tübingen

Context: There are presently several concepts to restore vision in blind or visually impaired persons by implanting electronic devices into the eye or the visual cortex in order to evoke useful visual sensations. Subretinal microphotodiode arrays (MPDAs) have a number of advantages: degenerated photoreceptors are replaced by MPDAs; the remaining neuronal network of the retina can be utilized for signal processing; positioning and fixation of the MPDAs in the subretinal space is relatively easy; no external camera and external image processing is required; eye movements can be used for localization of objects; semiconductor-based MPDAs are well tolerated by inner retina. A subretinal prosthesis requires functioning optics and a preserved optic nerve. (For a summary see: Zrenner E. SCIENCE 295:1022-1025, 2002).

Objective: After a survey of the various concepts pursued presently would wide our aims are presented to replace degenerated photoreceptors by microphotodiode arrays. Targeted diseases are (1) retinitis pigmentosa, and in a later state possibly also (2) age-related macular degeneration (AMD).

Results: Since 1995 our consortium has produced several prototypes of subretinal silicon “chips”, consisting of hundreds to thousands of microphotodiodes with an active area of 20 µm x 20 µm to 200 µm x 200 µm, equipped with microelectrodes (gold or titanium nitride) in a monopolar or bipolar fashion arranged in arrays, round or square with several millimeters in diameter and 50 µm thickness (Schubert et al, 1999). Lately we also developed a foil-bound active chip powered externally (see below).

In vitro experiments with chicken and RCS rat retinae in a sandwich technique, in which recordings are made by means of multielectrode arrays either from the inner or the outer retina (Zrenner et al, 1999; Stett et al, 2000) revealed:
1) charge injections of about 0,4 nC per electrode are sufficient to excite post-receptoral retinal neurons;
2) electrode distances of 50-150 µm in the outer retina can be resolved in ganglion cell recordings;
3) retinae with completely degenerated photoreceptors (RCS rats, 160 days and older) can still be excited by subretinal electrodes in a proper spatially organized manner;
4) surface coating of MPDAs as e.g. with laminins improves cell adhesion and biocompatibility (Guenther et al, 1999).

In vivo experiments revealed:
1) inner retinal layers are well preserved in the central retina (as shown by comparative histological studies of human and animal forms of degenerative retinal disorders) even in patients with longstanding retinitis pigmentosa (see Zrenner et al., 1997).
2) Two surgical approaches for safe introduction of the devices have been developed: (1) ab interno: via the classical transvitreal acces to the retina, and (2) ab externo: cia a scleral flap near the lombus through the subretinal space (like in a tunnel) to the back of the eye (Sachs et al. 1999; Kobuch et al. 1999)
3) inner retinal layers are well preserved after long term implantation of subretinal MPDAs in pigs (up to 16 months)
4) MPDAs remain fixated at stable subretinal positions and are well functioning up to 16 months as revealed by multifocal electroretinograms in both, rabbit and pigs.
5) MPDAs showed some damage of the silicon oxide surface of the implant. A suited coating has been developed in the meantime.
6) spatially sensitive electrically evoked cortical potentials recorded with mult-ielektrode and optical recording from the visual cortex of rabbit and pig following acute electrical subretinal stimulation via electrode foil strips reveal a spatial resolution of at least 1 degree.
7) In the meantime, an active implant has been developed, consisting of a silicon array of 3 mm x 3 mm in size and 1/10 mm in thickness. This device carries 1,600 pixels (70 x 80 µm), each equipped with light-sensitive diodes and a difference amplifier circuit that calculates for each pixel the difference between the overall luminance and the local luminance. Thereby adaptation of the chip to the environmental light has been achieved. This chip is an active implant, i.e. it requires external energy to be switched through to the stimulating electrode, dependent on the strength of local illumination on each pixel.

Conclusion: Although stimulation characteristics, spatial and temporal properties of subretinal stimulation by microphotodiode arrays and biocompatibility are now well investigated and the possibility of eliciting cortical potentials transmitted via subretinal electrical stimulation devices has shown the feasibility of the subretinal approach, still a number of questions remain to be solved. How well is orientation and movement as well as feature localization maintained at the level of the visual cortex? How can long-term stability of silicon chips, the surface of which is affected after long-time implantation be achieved? Will retinal neurons tolerate a long-term (> 24 months) electrical stimulation without morphological and/or functional alteration? What type of image can be perceived by blind patients through subretinal MPDAs?

Since bonding of the active chip with an "wire-bound" external energy transfer was successfully performed and an encapsulation was developed, that allows biostability for at least 12 months, a study in 8 blind patients suffering from retinitis pigmentosa (RP) is in preparation. For the quantifying assessment of function, a standardized, screen-based test battery was developed: light sensitivity, spatial and temporal discrimination, localization and motion, are important features of that test.

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Literature
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Early safety and pharmacological profiling of lead compounds: be faster and better with Multi-Electrode Arrays

Bruno Buisson

Department of Pharmacology, TROPHOS, Marseille, France

Trophos is a French Biotech that develops new drugs for neurodegenerative diseases (Motoneuron Diseases, Alzheimer's Disease, Huntington's Disease). These new chemical entities are expected to slow-down or stop neurodegeneration. *In vitro* cell-based screening has identified effective molecules among our 40,000 compound chemical library. Early drug selection is based on standard ADMET criteria such as plasmatic and metabolic stability as well as transit across the intestinal and blood brain barrier. With the aim to select and to develop better compounds, Trophos has introduced early tests to investigate potential adverse effects of lead compounds on principle neuro- and physiological properties, namely action potential propagation and synaptic transmission. To be as close as possible to the physiological situation, the molecules are evaluated by using brain slices recorded on Multi-Electrode Arrays. The hippocampal slice is used to evaluate possible interactions at glutamate or GABA synapses whereas spontaneous action potentials of Purkinje neurons are recorded in cerebellar slices. The two models have been validated with conventional pharmacological tools for voltage and ligand-gated channels and the effect of known commercial drugs will be illustrated.
Neuronal Networks on Passive Microelectrode Arrays: Emerging Histiotypic Nature of Pharmacological Responses

Guenter W. Gross

Department of Biological Sciences and Center for Network Neuroscience, University of North Texas, Denton TX 76205

In the past decade, substantial progress has been made with substrate-integrated microelectrode arrays and the growth of viable neuronal networks on such arrays. Signal-to-noise ratios of 20:1 are not uncommon, adhesion is strong enough to survive 50g tangential acceleration, and long-term, stable, multisite recording is limited in time only by appropriate life-support techniques. However, such progress has not been accompanied by the acceptance of dissociated (primary) neuronal cultures as reliable systems for pharmacological and toxicological studies.

Given that quasi monolayer cultures allow long-term (weeks to months) investigations, optical access to major components of the network circuitry, multisite recording of spatio-temporal action potential patterns, and the monitoring of action potential waveshapes for assessment of channel pharmacology, it is essential that such systems be experimentally validated as representative of the parent tissue. Experimental validation must involve not only general trends of activity changes classified as excitatory, inhibitory, disinhibitory (epileptiform), reversible and irreversible, but should also include concentration-response curves and EC\textsuperscript{50} values. Comparisons to in vivo data must also be approached systematically. However, such comparisons are not trivial as behavioral changes and even life support failures in animals are usually not presented with electrophysiological data. For example, respiratory insufficiency occurs before total cessation of electrical activity in the respiratory centers and is generally not defined on an electrophysiological basis.

Appropriate dissociation and culture maintenance techniques can generate spontaneously active networks with remarkable similarity to parent tissue responses in vivo. In our laboratory, histiotypic responses have been quantified with compounds such as ethanol, anandamide and other n-acyl ethanolamines, tetrodo toxin, trimethylolpropane phosphate (TMPP), trimethyl-tin chloride, fluoxetine, the L-type calcium channel blockers verapamil and diltiazem, and the antimalarial drug chloroquine. This presentation will summarize the histiotypic nature of pharmacological and toxicological network responses and emphasize recent data on botulinum toxin and quinine.
The Retinasensor – a MEA-based tool to study drug effects on retinal function

Elke Guenther, Thoralf Herrmann, Alfred Stett

NMI – Natural and Medical Sciences Institute, Reutlingen, Germany

**Purpose:** The retina is a peripheral, easily accessible part of the central nervous system. Incidence of light results in a complex signalling within the retinal neurons that is reflected in the electroretinogram (ERG). Retinal function can be affected by acute injuries, intoxications or retinal diseases, resulting in visual impairment or blindness. Under these conditions, the shape and amplitude of an ERG is altered, making it of clinical value as a diagnostic tool.

A retinasensor, based on multisite recording of local ERGs *in vitro* has been developed to easily and effectively assess effects of pharmacological compounds on retinal activity making it a valuable tool for secondary screening and toxicity testing.

**Methods:** Retinal segments with the pigment epithelium attached, dissected from explanted chicken or rat retinas, were placed ganglion cell side down on the translucent surface of a microelectrode array (MEA). Experiments were started after a 30 min. recovery period under stationary perfusion with oxygenated and temperature controlled. Under these conditions, measurements could be performed for up to 4 hours. Full-field stimulation with light impulses of defined strength was carried out with a halogen lamp and electro-magnetic shutter. The light was projected through the objectives of an inverted microscope homogeneously onto the retinal sample. Intensity and wavelength was controlled by colour and neutral filter. The full-field light intensity on the retina level without filter (white light) was 100 mW/cm² (500 kLux), 20 mW/cm² (blue light) and 40 mW/cm² (red light), respectively.

**Results:** Light stimulation resulted in the parallel recording of 60 local electroretinograms (*microERGs*) at a filter setting of 0.5 Hz-2.8 kHz. Offline filtering with a highpass filter (200 Hz) allowed extraction of ganglion cell action potentials. Bath application of the sodium channel blocker TTX resulted in a complete loss of ganglion cell activity. Application of AP4 (10 µM), a compound known to block signal transmission from photoreceptors to ON bipolar cells resulted in a loss of the b-wave. After washing b-wave amplitude was restored to control levels before drug application. In addition, different other drugs, such as chloroquine, ouabain and sildenafil, known to affect ERGs in humans were tested.

**Conclusions:** The retinasensor is a fast and effective test system to screen compound effects on retinal activity. It can be used to assess effects of pharmacological compounds and putative therapeutics, drug side effects as well as consequences of degeneration-related processes on retinal signalling.
Potential Applications of Neuronal Networks on MEAs

Mario Siebler, Philipp Görtz, Frauke Otto, Wiebke Fleischer

Neurochip Laboratory, Department of Neurology, University of Düsseldorf

Introduction: Neuronal networks of different cell types on microelectrode arrays (“neurochips”) allow screening of pharmacological substances and provide a perspective to elucidate complex neurophysiological interactions. Neurochips may serve as models for special CNS diseases. Here, we present data how to use the neurochip as a clinical tool for the investigation of neurological diseases.

Methods: Cryopreserved primary dissociated neurons from rat and mice cortex and from rat striatum (“CryoCells”, QBM Cell Science, Ottawa, ON, Canada) as well as human NT2 neurons were seeded on micro electrode arrays. Electrophysiological recordings were analyzed by a special software to determine neuronal network parameters such as the spike and burst rate or the correlation between channels. Classical neuroactive substances like TTX as well as substances identified in metabolic diseases with neurological impairment were applied onto the neurochip. Cerebrospinal fluid (CSF) that was tested on the neurochip was acquired from patients with normal pressure hydrocephalus, multiple sclerosis and head injury by diagnostic or therapeutic ventricular lumbar puncture.

Results: Imbalances of the homocysteine metabolism are related to several diseases that affect the central nervous system – for example homocystinuria, stroke, and Alzheimer’s disease. Homocysteine is an intermediate of the methionine catabolism and a key substance in the cysteine pathway. We investigated the acute effects of homocysteine and its oxidized forms homocysteic acid (HCA) and homocysteinesulfinic acid (HCSA) on neuronal network function, determining the dose response curve for D,L-homocysteine with an IC₅₀ = 401 µM. Interestingly, inhibitory effects of L-HCA (IC₅₀ = 1.3 µM) and L-HCSA (IC₅₀ = 1.9 µM) set in at significantly lower concentrations.

No sufficient explanation is given why some patients with head injuries lose consciousness. When CSF of patients with head injuries was applied onto the neurochip we observed a dramatic decrease of network activity. First analysis provides an indication of an insufficient NMDA receptor input mediated by the CSF – an effect that could not be eliminated by proteolysis of the CSF.

The pathophysiological mechanism of multiple sclerosis is not well understood and only recently axonal damage has been recognized in early phases of the disease. Neurochips may be suited to monitor the disease process via CSF of multiple sclerosis patients. CSF of patients on neurochips changed the network activity compared to CSF from healthy subjects. On the other hand, QYNAD, a pentapeptide that was found in multiple sclerosis and thought to be a key substance in neuronal dysfunction, had no significant impact on neurochip activity.

Patients with CNS diseases like Parkinson’s disease and stroke may benefit from neuronal grafting therapies. HNT neurons were already used in pioneering grafting studies. HNT neurons placed on MEAs developed spontaneous activity with different qualities compared to cortical cells: they displayed a more uncorrelated firing and were less sensitive to neuroactive substances. Thus, hNT neurons may be not ideal to replace network lesions.

Conclusion: The MEA technique in combination with cultured neuronal cells has strong potential to answer clinical relevant questions.
Substrate-integrated multi-electrode and sensor arrays: Applications in neuropathology, safety pharmacology and drug testing

Dieter G. Weiss, Simone Stüwe, Alexandra Gramowski, Liane Mehnert, Werner Baumann, Dietmar Schiffmann, Sandra Witt

Institute of Cell Biology and Biosystems Technology, Departments of Animal Physiology and of Biophysics, University of Rostock, Rostock, Germany.

We report on current applications of multi-electrode arrays and neurosensor chips to various fields in biology and medicine. Electrically active neuronal networks from embryonic mouse spinal cord or brain are cultured on glass/ITO- or silicon-based multi-electrode arrays with stable cell-electrode coupling for several months. This allows the monitoring of the onset of electrical activity, of bursting activity stabilization and of the development of histiotypic native and drug-modified electrical activity patterns. The glass neurochip sensor system (CNNS, Denton TX) was extensively used over the last years to monitor states of toxic or metabolic impairment of neurons accompanied by characteristic electrical activity changes. Network activity is classified and characterized at the level of spike and burst patterns using 38 different activity-describing variables to quantify the effects of neuro-active drugs on network electrical activity states.

In order to evaluate if changes in the network activity pattern can be used to monitor and different stages of impairments of consciousness in humans, preparations of blood plasma from healthy subjects and coma patients with hepatic encephalopathy (HE) were added to neuronal networks in order to study their influences on the electrical activity patterns. HE results from the accumulation of toxic substances in the body, reflected by high levels in blood plasma. Following addition of ultrafiltrates and extracts changes in network activity were classified and characterized at the level of spikes and bursts using a set of activity-describing variables. Besides the hepatic encephalopathy study, other applications included studies on the effects on the electrical activity of neurotoxins, ammonia and other putative encephalopathy-causing compounds, neurosteroids, benzodiazepines, anaesthetics and anticonvulsive drugs as well as studies on detecting neuronal side-effects of lead substances.

A new standard CMOS silicon chip with unique features has recently been introduced. Besides the recording electrodes for action potentials, oxygen sensors, temperature diodes and ion sensitive field effect transistors (ISFET) were integrated to measure oxygen consumption, temperature and pH changes of the cultures at the silicon chip.

Based on our results and experience we conclude that multi-electrode systems provide a platform suitable for pharmaceutical drug development, for high-content drug screening, and for safety pharmacology as well as for diagnostics of human diseases. The future developments of the Rostock Biosystems Technology Consortium aim at improvements in the silicon neurosensor chip system, methods for the characterization of the neuronal cell biology during long-term recording, the application of advanced information science tools for precisely describing the different activity patterns characteristic for drug treatment states, and making the accumulated results on standard drugs accessible in a data base.

Supported by the DFG Innovationskolleg “Komplexe und Zelluläre Sensorsysteme”, Grant INK 27, the Landesforschungsschwerpunkt and the European Community (EFRE).
Utilization of the Multi Electrode Array system for studying electrophysiological remodeling by pathophysiological stresses in cultured neonatal rat ventricular myocytes.

Ofer Binah

Rappaport Institute, P.O.Box 9697. Haifa 31096, Israel

Since the study of electrophysiological remodeling by cardiac perturbations pathologies in vivo is a formidable task, rendered even more complex by the three-dimensional geometry of the ventricles, we have developed and utilized an experimentally sound, yet more rigorously controlled model of the myocardium. My presentation will focus on our studies in which we have employed the Multi Electrode Array (MEA) data acquisition system to record extracellular electrograms from cultured neonatal rat ventricular myocytes (NRVM) under a variety of experimental conditions. This superb experimental system enabled us to apply versatile stimulation capabilities and long-term noninvasive recordings of myocytes electrophysiological activity, under a variety of physiological and pathophysiological conditions. The major advantages of our system are: (1) The ability to perform long-term studies under carefully-controlled experimental conditions. (2) Each culture serves as its own control. (3) Pharmacological agents can be added and washed out without disturbing the microenvironment of the culture. (4) This system may enable in the future, genetic manipulations while continuously monitoring the culture's electrophysiological properties.

My presentation will include representative findings from the following topics: (1) The relationship between beating rate, impulse conduction and the waveform of extracellular electrograms; (2) Electrophysiological remodeling by altered activation patterns; (3) Gap junctional and electrophysiological remodeling by hypoxia; (4) Electrophysiological remodeling by activation of the Fas receptor; (5) Electrophysiological maturation of human embryonic stem cells.
Higher Throughput and Lower Costs in MEA Technology

Meyer, T; Möller, A; Boven K-H; Lobitz N.

1 Multi Channel Systems MCS GmbH & NMI Reutlingen
2 Natural and Medical Sciences Institute, Reutlingen Germany

Purpose:
Microelectrode Arrays (MEA) are a standard tool in advanced electrophysiology since many years. However, they never made the step to a standard tool in pharmaceutical industry for screening purposes. One of the main reasons for this are the high costs of the MEA chips. In many cases the added information provided by the multitude of electrodes is not even required. This encouraged us to search for layouts and manufacturing procedures enabling us to produce MEAs and MEA derived plates at substantially lower costs. The second aim was to improve throughput by applying standard 96 well formats.

Methods:
ecoMEAs
By applying printed circuit board (PCB) manufacturing processes with specialized material allowing the culture of cells on it, it was possible to reduce the production costs of MEAs substantially. Standard materials for PCBs are not suitable for biological applications due to high concentrations of Bromine. With our customized material it was possible to culture cardiac myocytes and neurons on the MEAs.

96Well MEAs
As mentioned above, for many screening purposes it is not required to retrieve data from 60 or more electrodes. Using the multitude of electrodes to obtain multiple independent experiments is the goal here. By applying the same techniques as above, we could record signals from cardiac myocytes from a 96 well plate. A special amplifier setup for these layouts was developed as well and is marketed for specialized applications in safety pharmacology as “QT-Screen”.

Results:
Various forms of ecoMEAs have been tested successfully with cardiac myocytes, cortical neurons (Univ. Düsseldorf) and acute brain and heart preparations. Recordings from 96 well plates with one electrode per well have been performed successfully as well. A photo of a well of a 96 well plate with a gold electrode in the center and a octagonal shaped reference electrode is shown om the right.

Conclusions:
For many applications, were high throughput and costs per data point matter, the new developments will broaden the application field of MEAs and generally of extracellular recording techniques. However, limitations exist and whenever very high spatial resolution is required, the novel MEAs will not replace standard MEAs.
Determination of electrical properties of ES cell derived cardiomyocytes using MEAs

M. Reppel, MD¹; M. Halbach¹; U. Egert, MD²; BK. Fleischmann, MD¹; J. Hescheler, MD¹

¹ Institute of Neurophysiology, University Cologne;
² Institute for Biology III, Albert-Ludwigs-University Freiburg, Germany.

Pluripotent embryonic stem cells (ES cells) provide a new tool to study cardiomyogenesis under in-vitro conditions. ES cells kept in permanent culture are differentiated within aggregates („embryoid bodies“, EBs) in which among other cell types cardiomyocytes appear 3 - 4 days after plating. These form spontaneously beating clusters mostly consisting of expanded regions of cardiac cells connected with narrow tissue strands. To record the electrical activity of these contracting areas we used Multi Electrode Arrays (MEAs) consisting of 60 substrate-integrated electrodes. We investigated the influence of Na⁺-, K⁺- and Ca²⁺-channel blockers on the electrical signal generation and propagation as well as on the shape of field potentials (FPs). We also used ES cell derived cardiac myocytes as a multicellular in vitro model for cardiac development. Long-term recordings with MEAs enabled the examination of electrophysiological properties during the ongoing differentiation process. During time in culture the beating aggregate of cardiac myocytes differentiating from ES cells increased in size (7-fold). This change was accompanied by an increase of the beating frequency from 1 to 5 Hz and a decrease of the FP duration. Furthermore a shortening of the FP upstroke velocity could be observed concomitant with a functional segregation of slow upstroke velocities in the area of the pacemaker. Our data indicate a functional differentiation and segregation of the cells into pacemaker and myocard-like regions. This in vitro development of a three-dimensional heart like structure closely follows the development known from mouse embryonic heart. The preparation thus forms an ideal model to monitor the development of electrical activity in embryonic cardiac myocytes for wild type and genetically modified ES cells, thereby taking into account the functional differentiation of the tissue. Our data suggest that EBs plated on MEAs provide a suitable tool for pre-screening of cardioactive substances.
High Throughput Toxin Evaluation
K. Varghese, A. Natarajan, A. Jamshidi, P. Molnar, J.J.

Hickman Department of Bioengineering, Clemson University, Clemson, SC, USA

Purpose:
The objective of this study is to develop a high throughput cell based biosensor using Metal Microelectrode Arrays (MEAs) and Field Effect Transistors (FETs) and compare and contrast the results obtained with the two different devices. The sensor thus developed should be able to detect acute and chronic effects of environmental toxins and pharmacological substances. The cell based biosensor consists of a monolayer of cultured embryonic chick cardiac myocytes on MEAs and FETs.

Methods:
Day 6 chicken embryos were dissected to obtain the hearts. The hearts were then trypsinized with 0.05% Trypsin, centrifuged at 400 rpm and plated at a density of 400,000 cells/ml on MEAs and FETs coated with a combination of Polyornithine and Laminin. Leibovitz’s L-15 media supplemented with 10% Fetal Bovine Serum was used as the culture media and the cells were incubated in a non-CO₂ environment. The feasibility of using serum-free media to grow these cells is also being studied. The cells were observed over a period of 6 days and recordings were done when the monolayer was at its healthiest – indicated by the strength and frequency of cellular contractions viewed using a microscope. The recording set up from Multichannel Systems consisted of an MEA chamber and amplifier connected to a computer and a stimulus generator. The cell biopotentials were analyzed using the MC Rack software. The cells were tested for the acute effects of cadmium chloride and mercuric chloride, known environmental toxins. With each toxin, the time and concentration required to totally stop the heartbeats was noted. The change in frequency and amplitude of the potentials was studied until this point was reached.

Results:
Mercuric chloride toxicity:
Spike activity of the cardiac myocytes was observed to significantly decrease when 100µM of mercuric chloride was added. The toxin was fatal at a concentration of 300 µM.
Cadmium chloride toxicity:
Preliminary results with cadmium chloride show that 100 µM of cadmium chloride kills the cells within 7 minutes.

Ongoing work:
• Along with testing other toxins, the above mentioned experiments are being replicated with serum-free media conditions.

Conclusions:
Cadmium chloride, a Ca²⁺ channel blocker and a relatively common environmental toxin, is acutely toxic at 100µM. Though mercuric chloride causes a noticeable decrease in the spike activity at 100µM, the cells don’t completely stop beating till the concentration is increased to 300 µM.
GABA<sub>A</sub> Receptor-Specific Substances Induce Characteristic Changes of the Electrical Activity of Neuronal Networks on Microelectrode Arrays

L. Mehnert<sup>1</sup>, U. Mehnert<sup>2</sup>, D. G. Weiss<sup>1</sup>, D. Schiffmann<sup>1</sup>

<sup>1</sup>Institut für Zellbiologie und Biosystemtechnik, Universität Rostock, Germany;
<sup>2</sup>Mediclin Müritz-Klinikum Waren, 17192 Waren, Germany

For pharmacological applications of receptor-specific drugs and anesthetics it is necessary to achieve a locally defined binding to the receptor, to enhance effects and to minimize adverse effects and interactions. As an experimental system we have used primary dissociated nerve cells from mouse frontal cortex grown on microelectrode arrays which form functionally active neuronal networks. The influence of neuroactive drugs on their electrophysiological behaviour can be monitored by analyzing the recorded activity.

For functional studies of the benzodiazepine, neurosteroid and barbiturate binding site of the GABA<sub>A</sub> receptor, diazepam, flumazenil, pregnanolone and thiopental were tested. The tranquilizer diazepam is an agonist of the benzodiazepine binding site, while flumazenil is the corresponding antagonist. Application of diazepam (7 to 175µM) induced a fast, significant, dose-dependent response, with maximum decrease of network activity at 87.5 µM. Medium change restored the original activity, indicating reversibility of the effect. The antagonist flumazenil also reversed the effect of diazepam. Due to a lower receptor affinity, a higher concentration (165.5 µM) was needed, but little differences in the reversed activity pattern were observed. The anticonvulsant pregnanolone acts as an agonist at the steroid binding site of the GABA<sub>A</sub> receptor. Application of pregnanolone (200nM to 2µM) decreased the neuronal activity in a concentration-dependent manner. The effect was reversible. Thiopental, a barbiturate, also caused a concentration-dependent, reversible reduction in network activity. In this case an increasing dissolution of the typical rhythmic activity pattern was observed.

Further, the interactions of different binding sites at the same receptor were analysed in a preliminary study. The diazepam/flumazenil experiments were repeated under the influence of the GABA<sub>A</sub> receptor antagonist bicuculline which did not change the observed responses.

Altogether, our results show that substance-specific effects on the GABA<sub>A</sub> receptor can be precisely analyzed using neuronal networks cultured on microelectrode arrays.
PSPICE Modelling of Cell-Electrode Coupling

Erik Schreiber¹, Werner Baumann¹, Guido Krause¹, Angela Podssun¹, Susanne Homma¹, René Schrott¹, Ralf Ehret², Ingo Freund³ and Mirko Lehmann³

¹Biophysics, Institute of Cell Biology and Biosystems Technology, Department of Biological Sciences, University of Rostock, Rostock, Germany, ²Bionas GmbH., Rostock, Germany, ³Micronas GmbH., Freiburg, Germany

Different Cell Monitoring Systems (CMS) for on-line monitoring of cellular reactions are under development by our group. They are based on microchips in order to use all the advantages of standard CMOS techniques such as high quality production as well as the possibility to integrate sensor electronics like filters and preamplifiers on chip. As one result a commercial device for characterisation and monitoring of cell physiological as well as culture parameters (e.g. acidification, temperature, adhesion) is now available [www.bionas.de]. For future applications this specialised chip has now been combined with a multi-electrode array to measure electrical signals of neuronal networks cultured on neurosensor microchip at the same time as the physiological conditions of the culture.

To understand the influence of our electrode properties on signal transmission different simulation experiments have been carried out with the PSPICE (Simulation Program with Integrated Circuit Emphasis) tool. Modelling the whole system by equivalent circuit diagrams the influence of different parameters in coupling impedance, sealing and leakage have been investigated. The results are compared with measurements using patch clamp micro-electrodes in combination with our silicon chip recording electrodes and with conventional electrodes on glass chips. The improvement due to electrode coating by porous metal surface will be evaluated.

Our new silicon chip is a standard microelectronic device allowing combined measurements of electrical activity and parameters for metabolic characterisation of cell cultures. This promises a deeper understanding in the relationship of electrical and metabolic cell physiology.

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FPGA and DSP-Based Online Spike Detection and Classification

René Schrott\textsuperscript{1}, Andreas Keuer\textsuperscript{2}, Jan Taube\textsuperscript{1}, Danilo Schmück\textsuperscript{1}, Helmut Beikirch\textsuperscript{1}, Werner Baumann\textsuperscript{3}, Erik Schreiber\textsuperscript{3},

\textsuperscript{1}Faculty of Computer Science and Electrical Engineering, University of Rostock, Rostock, Germany, \textsuperscript{2}Bionas GmbH, Rostock, Germany, \textsuperscript{3}Department of Biological Sciences, Biophysics, University of Rostock, Rostock, Germany,

Currently a cell monitoring system (CMS\textsuperscript{®}) for online and non-invasive measurements of the metabolic and electrical activity of a neural network is developed by an interdisciplinary consortium at the University of Rostock [1]. For this purpose a multi electrode array on a silicon substrate is used to perform measurements of the cellular reactions of cultured neurons. To be able to perform the highly parallel data pre-processing the threshold computation for the action potential detection is implemented on a field programmable gate array (FPGA) due to its flexibility and speed. Subsequently the spike classification is carried out on a digital signal processor (DSP).

The reduced signal-to-noise ratio (SNR) which is caused by the non-invasive measurement and additional disturbances requires a conditioning of the recorded signal before the actual spike detection can be performed. This can be achieved by multi resolution analysis (MRA) described in [2]. In comparison to an optional finite impulse response (FIR) filter the calculational effort of the signal processing is reduced due to the effectiveness of this “in place” algorithm. This transform divides the signal into several frequency components when applied repeatedly. Effective noise suppression and an adaptive detection threshold are obtained. Even under varying signal conditions this threshold can be applied to the measured data without interaction. The approximation of the transform coefficients allows the efficient implementation of this method on an FPGA filter bank structure [3].

The “point-to-point” signal analysis and the correlation were chosen in order to meet the requirements of the online classification and the desired implementation of these algorithms on a compact hardware platform. The exemplary implementation of the action potential detection and threshold computation for eight input channels could be realised successfully on a Xilinx VirtexE FPGA. Cross-correlation as a means of classification could be implemented on a DSP evaluation board as well. Benchmark tests and parallel simulations are necessary to optimise existing classification methods and to look for new approaches. This method will permit successful integration of the data processing part into the cell monitoring system.

References
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Extracellular multi-site recordings from patterned neuronal network

M.Sokolov, A.Beattie, R.Tang, D. Mccloy, C.Wilkinson, A.Curtis

Centre for Cell Engineering, University of Glasgow, Glasgow, UK

Purpose:
-To obtain stable, long-lasting, multi-site extracellular recordings of neuronal activity.
-To develop method of creating functional neuronal network of desired configuration.
-To analyse if neuronal activity could be controlled by network design.

Methods: Modified standard microfabrication methods were used to develop different types of MEA for recordings of neuronal activity. CNS neurons were cultured on flat surfaces or surfaces caring specific pattern, using either topography or chemical (or in combination) cues to guide neuronal network development. Electrophysiological recordings were performed using MEA or glass micropipette electrodes.

Results: Our work consists from several parts. As first step – a flexible top-down MEA (platinum-gold on polyimide substrate) was fabricated. Immediately after platinization the impedance of electrodes was in range of 20-40 kOms (at 1 kHz), but dramatically increased (up to 100-150 MOhms) if we tried to bend polyimide. To check flexible MEA, we made recordings from cultured cardiomyocites and cortical/hippocampal cultures, to see if we could obtain good signal-to-noise ratio recordings for further single cell activity analysis. Although we could obtain fine signal from cardiomyocites, recordings of single neuron activity was not reliable.

To control network formation, we grew cultures on different substrates: glass with micro stamped proteins, polycaprolactone (PCL) and silicone polymers with nanostructures on it. In general cells show tendency to follow designed pattern. To characterise this cultures (cell type, synapse formation etc.) immunocytochemistry will be done.

Conclusions: Flexible MEA is suitable for electrophysiological recordings. Further adjusting and improving of recording conditions is necessary, as well as further optimisation of methods to control patterned network development.
The drug discovery business challenges the development of novel electrophysiological instrumentation for probing ion channel activity and its modulation on the molecular, cellular and tissue level. The driving force is the need for automated systems to increase the throughput of the compound screening process by orders of magnitude, and for assays that help to reduce the risk of compound failure at late stages of pre-clinical and clinical trials due to adverse cardiovascular and CNS side effects.

The key enabling technologies for innovations in electrophysiology are microsystem technique and microelectronics. These techniques have enabled the development of microchip-based tools like automated patch clamp devices and planar electrode systems like MEAs and FET arrays.

While there are outstanding prospects for the successful implementation of automated patch clamp systems into the industrial screening process, MEA applications have not yet become established and widespread assays for industrial compound testing. Since its introduction 30 years ago, the MEA technology and the related cell and tissue culture methods have been permanently improved. However, so far their domain are academic and applied research where numerous applications give proof of the reliability of the technique.

The talk will give an overview on the state-of-the-art in microchip-based electrophysiology. Against the background of the needs and trends in the drug discovery process, opportunities for extracellular electrophysiological methods like MEAs and FETs for ion channel screening and safety pharmacology are discussed.

Additionally, a short view is given to the neurotechnology market and its opportunities for MEA applications in the field of neuroprosthesis like retinal implants.
Effects of acute valproate exposure on the electrical activity of primary frontal cortex networks grown on microelectrode arrays

Simone Stüwe, Alexandra Gramowski and Dieter G. Weiss

Institute of Cell Biology and Biosystems Technology, University of Rostock, 18051 Rostock, Germany

Valproate, one of the major anti-epileptic drugs, is effectively used in the treatment of bipolar disorders, neuropathic pain and migraine prophylaxis. Its wide therapeutic spectrum is reflected by several different mechanisms of action, i.e. effects on amino acidergic neurotransmission, brain metabolism and direct effects on excitable membranes.

We tested the influence of valproate on the electrical activity patterns in primary cell cultures of murine frontal cortex. Therefore mature networks (n=16) grown on 64-microelectrode arrays were acutely exposed (30 min stable phase per step) to increasing concentrations of valproate (100 nM – 20 mM, 10 steps). Simultaneous extracellular recordings of network activity revealed a concentration-dependent decrease of activity beginning at 10 µM (p=0.05) with cessation of activity between 10 and 20 mM. The network patterns remained stable over a broad concentration range, activity synchronization and regularity were finally lost at 5 mM valproate. At 10 mM valproate 26 of the 30 evaluated activity features had changed significantly compared to native activity with an EC50 of 2.2 mM for spike and burst rate. The effects were reversible by medium changes.

Blockage of the GABA-A receptor by 40 µM bicuculline prior to valproate exposure shifted the EC50 to 4.2 mM (n=5). This confirms reports that the clinical efficacy of valproate is partially related to its ability to enhance central GABAergic neurotransmission.

Thus, neuronal networks grown on microelectrode arrays provide a powerful tool to elucidate pharmacological mechanisms.

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Recording from living neural network with transparent microelectrode arrays

R. Tang, K. Mathieson, M. Sokolov, A. Beattie, C. Wilkinson, A. Curtis

Department of Electrical & Electronic Engineering, University of Glasgow, Glasgow, UK

**Purpose:** Long term recording from and stimulating spinal cord neurons or hippocampal neurons, which were guided grown to form a network (Jude pattern).

**Methods:** Transparent indium tin oxide (ITO) microelectrode arrays (MEA) and the novel PDMS nerve cell container.

The microelectrode arrays (MEA) is made in the transparent conductor indium tin oxide (ITO) by CH4/H2 reactive ion etching on a glass substrate. This ITO layer is coated with a layer of silicon nitride. At the positions where electrodes and contact pads are, vias through this protective and insulating layer are made by SF6 RIE. Finally electrodes are coated with platinum black. The whole MEA and its substrate are transparent and so allow observation from underneath, which is convenient for cell observation and device alignment.

These Nerve cell culture is incubated on a PDMS structure, which is embossed on glass slice substrate. The embossing mold is made by SF6 ICP dry etch on silicon wafer. The design of the PDMS structure is considered of having a proper amount of oxygen diffusion through and giving a reasonable distance from cells to electrodes.

The growth of nerve cells are guided by both surface topography and chemical means. Our former research has shown different responses of nerve cells to various topographic features.

The nerve cell container and MEA are aligned and assembled together carefully to ensure each electrode gets in contact with each neuron.

**Results and Conclusion:** The SF6 ICP dry etch recipe for fabrication of silicon mold proved to be a success. Adhesion problem and differential thermal expansion problem in embossing are solved with using new material. Fabrication of recording devices has been completed.
We report on the fabrication of out-of-plane electrodes designed for intracellular biopotential measurements on living cells. The electrodes are based on silicon microneedles with a height of less than 5 µm and a diameter below 1 µm, realized using deep reactive ion etching. The silicon microneedles were uniformly covered by 525 nm silicon oxide (SiO₂) using chemical vapor deposition (CVD). Then 250 nm of titanium tungsten (TiW) is sputtered and patterned by lift-off, and a 525-nm-thick CVD SiO₂ passivation is deposited. Finally the passivation is opened at the electrode tip in order to provide a sub-µm contact for local electroporation and potential measurements. Arrays of 8×8 electrodes were realized.

For the analysis of living cells well-established measurement methods, such as the patch clamp technique, are used to determine, e.g., intracellular potentials. A drawback of this method is that it is a serial process and requires skilled staff. Systems with planar CMOS-integrated electrode arrays have been reported enabling a parallel measurement of extracellular action potentials. This study focuses on the development of three-dimensional (3D) electrodes that can be integrated in a post-CMOS process on IC-chips. This could, after the local opening of the cell membrane, enable the absolute intracellular potential to be measured. In comparison to other on-chip patch-clamp methods adherent cells can be used and the preparation of non-adherent cells is not necessary. At the same time, this approach benefits from the massive parallel data processing capability of IC chips.

Figure 1(a) shows a transmission electron microscopy (TEM) image of the cross-section of a ca. 4.2-µm-high silicon microneedle covered by the SiO₂/TiW/SiO₂ layer sandwich. The image clearly shows the successful removal of the final SiO₂ layer at the electrode tip. The total diameter of the electrode is 3 µm with an exposed contact of about 1 µm². The electrode shape and dimensions were found to be uniform across the entire array. After the fabrication of the electrode arrays neural cells were cultured on top of the passivated topography. Figure 1(b) illustrates that the neural cells were successfully cultured on individual electrodes.

Fig. 1: (a) TEM image of the cross-section of a silicon microneedle covered with 525 nm SiO₂, 250 nm TiW, 525 nm SiO₂ (total diameter 3 µm, height 4.2 µm); top passivation opened at the electrode tip; (b) SEM image of a neural cell cultured on a structured electrode chip.
Neural networks cultured on multielectrode arrays are characterised by synchronous firing periods (bursts) among neurons (for review see Corner et al. 2002). We have recently shown that in dissociated rat neocortical networks, profiles of spontaneously occurring network bursts change with the development of the culture (Van Pelt et al., 2004 a, b). Among others, the width of averaged bursts showed significant changes during development and went through a broadening and shortening phase in about 4 weeks. To investigate the influence of electrical stimulation on the profile of spontaneous network bursts, we measured spontaneous activity before and after stimulation and analysed the network bursts as previously reported for experiments without stimulation (Van Pelt et al., 2004 a, b).

Neurons from E18 Wistar rats were dissociated and plated on multielectrode arrays (60 electrodes with diameters of either 10, 20 or 30 µm). Culture density was approximately 100,000 cells/plate. Measurements of extracellular spikes were performed on cultures of 2–4 weeks in vitro. The following stimulation protocol was applied sequentially at 4–6 sites: a train of 20–40 biphasic voltage pulses of either ±2V or ±0.3V was delivered. Stimulation frequency varied and was between 0.1 Hz and 0.5 Hz.

We found, that electrical stimulation not only changes the temporal profile of the averaged spontaneous bursts, but can also silence or recruit sites which contribute to these network bursts. We conclude that stimulation can change the routing of spontaneous activity through the network.

References:


Application of multielectrode recording to the retina of a genetically engineered Basson-knockout mouse

Lars van Ahrens, Malte T. Ahlers, Martin Greschner, Josef Ammermüller

Neurobiology, Carl von Ossietzky University Oldenburg, 26111 Oldenburg, Germany

The mouse has become very important in life sciences especially due to the genetical access. In this context a setup for multichannel extracellular recording with a 100 electrode array has been assembled in order to investigate the response characteristics of mouse retinal ganglion cells.

A mutant mouse deficient of the functional Bassoon protein (Bsn -/-) has been examined and the data were analysed with respect to the wild type (Bsn +/+). Bsn is a large protein (420 kDa) and in the retina it is localized at the presynaptic active zone of the photoreceptor ribbon synapse. Bassoon appears to be concentrated close to the ribbon base and is supposed to be involved in the formation and the function of photoreceptor ribbon synapses [1].

Extracellular recordings from ganglion cells were performed in the isolated mouse retina. The preparation was placed on a piece of agarose gel and subsequently mounted ganglion cell side up on a glass slide. During a recording session the retina was continuously superfused with bicarbonate-buffered Ames medium. A passepartout made of filter membrane bared the retina from floating. Recordings were made at 35°C. Constant temperature was guaranteed by a heated chamber underneath the retina plus additional temperature control of the superfusion medium. Full field light stimulation was applied to the retina’s photoreceptor layer from using a LED. Stimuli were established according to previously performed ERG studies with the Bsn -/- mouse.

Retinal ganglion cells of BSN deficient mice older than one year showed a similarly complex response pattern as it was seen in the wild type. Possibly the deficiency caused by the lack of Bsn is compensated by synaptic plasticity. The latency of the response to the stimulus offset (light off) was significantly higher among the majority of cells in the -/- mice. The latency of the response to the stimulus onset (light on) also peaked a little later. This is consistent with the latency of the a-wave originating from the ERG of the mutant mouse.

Extracellular Recording of Individual Mammalian Neurons with Low Noise Field Effect Transistors

Moritz Voelker, Peter Fromherz

Max-Planck-Institut für Biochemie, Abteilung Membran und Neurophysik, Martinsried, Germany

Abstract:

Noninvasive recording of electrical activity of individual nerve cells in culture is a prerequisite for the study of designed neuronal networks and neuron-based pharmacological sensors. We employ open field effect transistors to record the extracellular signals beneath the cells. While invertebrate neurons yield large signals, the smaller rat neurons could be recorded previously only by signal averaging. Here we report on extracellular recording of individual neurons from rat hippocampus as well as of dense cultures. By using buried channel field effect transistors built with a low noise process, we detect extracellular signals from individual neurons with an amplitude of about 100 µV and from dense cultures with signals up to 4 mV, considerably more than with planar metal electrodes. The extracellular voltages with individual neurons and dense cell cultures are discussed in terms of capacitive and ionic currents in the planar core-coat conductor of cell-silicon junctions.
Patterned Neuronal Networks to Study Information Processing in vitro on Microelectrode Arrays

Bruce C. Wheeler,1), Yoonkey Nam1), David Khatami1), Rudi Scharnweber3), Gregory Brewer4)

1) Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA, 2) Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA, 3) Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL, USA, 4) Department of Medical Microbiology and Immunology, Southern Illinois University School of Medicine, Springfield, IL, USA

We are designing and building neuronal circuits in vitro by controlling the pattern of the growth of neurons. By combining this scheme with surface microelectrode arrays, we are beginning to study information processing in these defined neural circuits. This approach is made possible by advances in photo-lithography, surface chemistry, cell culture techniques, and biosensors.

Our work uses, principally, a cell adhesive biomolecule (poly-D-lysine, PDL), which is patterned on microelectrode array by photolithography or micro-stamping [1, 2]. Alternatively, laminin and laminin/polylysine mixtures are used. The cell repulsive area is defined by polyethylene glycol [3], or, as is usually successful, the uncoated polyimide that constitutes the insulation on some of our electrode arrays. Hippocampal neurons (BrainBitsLLC.com) from 18-day gestation Sprague-Dawley rat embryos were dissected mechanically and cultured at 37°C, 5% CO2, 9% O2, in serum free B27/- Neurobasal medium (Invitrogen, Gaithersburg, MD) with 0.5 mM glutamine and 25 μM glutamate.

We have amply demonstrated that we can create micropatterns of neurons that are alive and functional on top of the microelectrode array [1, 2, 4]. In particular the lithography permits a high degree of localization to individual electrodes, prompting investigations underway as to the nature of the neuron/ electrode coupling and the frequency of finding electrodes judged active by the presence of extracellularly recordable action potentials. In current work we have examples of activity propagating along the geometrically defined network--both at speeds appropriate to axonal conduction as well as to multi-neuron / multi-synapse networks; enhancement of recording by localization to electrodes; elementary plasticity; and sequential activation of different pathways in a network. The goal of the work is to make such recordings reproducible and robust so as to be useful in scientific investigations regarding neuronal information processing.


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Modulation of Neuronal Network Activity by SDF-1 Chemokines

Tom Wiegand, Jessica Opatz, Patrick Küry, Hans Werner Müller

Molecular Neurobiology Laboratory, Department of Neurology
University of Düsseldorf, Düsseldorf, Germany

Purpose:
Stromal cell-derived factor-1 (SDF-1, CXCL12) is the only known ligand of CXC chemokine receptor 4 (CXCR4). CXCR4 is a member of the G-protein-coupled receptor family and is able to modulate the activity of neurons through regulation of \([Ca^{2+}]_i\). We focus on the functional difference between the SDF-1α and the SDF-1γ isoforms. While both isoforms bind to the CXCR4 receptor, they differ in a 30 AA carboxy-terminal peptide extension present in SDF-1γ.

Methods:
To neuronal networks consisting of dissociated primary cultures of neocortical neurons from embryonic rat grown on Multielectrode Arrays the chemokine isoforms SDF-1α and SDF-1γ were applied at different concentrations. The electrophysiological activity of the network was monitored before and after application of the chemokines. Activity was monitored and registered as spike wave form with Multichannel Systems MC_Rack™ and analysed as events/time. To trigger the recording of spikes, an individual threshold level was given for every experiment. Application of substances was performed without interruption of the recording procedure.

Results:
While SDF-1α stimulates electrophysiological network activity, SDF-1γ inhibits the neuronal network activity following a certain time lag. The effects are reversible and can be observed over a broad range of chemokine concentrations. At a concentration of 3µM SDF-1γ the network activity is completely abolished.

Conclusions:
We could clearly demonstrate that the two isoforms of SDF-1 differentially affect the electrophysiological properties of a neuronal network. This finding suggests that the 30 AA carboxy-terminal extension modulates the interaction of the chemokine with its receptor CXCR4 or that additional interactions with yet unknown neuronal binding sites could be involved.
Extracellular recording of the activation kinetics of EAG potassium channels

Günter Wrobel¹, S. Ingebrandt¹, U.B. Kaupp², A. Offenhäusser¹

¹Institute of Thin Films and Interfaces (ISG2), ²Institute of Biological Information Processing (IBI1), Forschungszentrum Jülich GmbH, Jülich, Germany

Purpose:
The extracellular recording of cellular activity provides for a powerful, non-invasive technique that holds great promise for biomedical studies. It has been shown that non-metallized field-effect transistors (FET) can register the electrical activity of cells attached to the gate region. Changes in the extracellular potential were registered as changes in the drain-source current of the FET. Although the proof-of-principle has been achieved more than 10 years ago, however, the mechanisms underlying the cell-transistor coupling are not completely understood. The basic unsterstanding of the extracellular recorded signal shapes is important for the application of cell-transistor hybrids.

Methods:
In this study we examine the electrical coupling between FETs and HEK293 cells expressing a voltage-gated potassium channel type of the subfamily of ether-á-gogo (EAG) channels. The activation kinetics of the EAG channels is largely determined by the holding potential in voltage-clamp mode and by extracellular divalent cations. The EAG channels can adopt slow and fast gating modes depending on the holding potential from which the channels become activated. The resulting currents obtain a different but characteristic sigmoidal rising shape in dependence of the holding potential. This decelerated activation is particular pronounced in the presence of extracellular divalent cations that promote the transition of the channel from fast to slow mode of activation. Therefore this system provides an ideal test, whether the extracellular voltage signal recorded by the FET tracks the membrane current recorded with the patch-clamp technique faithfully.

Results and Conclusions:
We examined the electrical coupling between the EAG expressing HEK cells and p-channel as well as n-channel FETs and observed significant differences in signal shape and amplitude for the extracellular recordings. These differences recorded by the two transistor types cannot be explained by an enhanced K⁺ conductance in the cell-transistor junction. We therefore suggest that electrodiffusion of accumulated K⁺ ions in the cleft between the attached cellular membrane and the transistor surface is affecting the recorded FET-signals.
Input impedance effects on extracellular signals in metal microelectrode configuration

Y. Zhang, G. Wrobel, N. Wolters, S. Ingebrandt, H.-J. Krause and A. Offenhäusser
Forschungszentrum Jülich, Institut für Schichten und Grenzflächen (ISG-2), Jülich, Germany

Abstract:
In previous studies in our group we have developed an 64-channel extracellular recording system for planar metal-microelectrode arrays. It uses a specially designed electronic circuit incorporating low-noise junction field-effect transistor (JFET). The ability of this system for extracellular recordings from cardiac myocyte cultures with planar, unmodified gold electrodes in micrometer size has already been shown in previous works [1, 2]. In recent studies we are aiming at a system, where the metal electrode is directly connected to the input of an operational preamplifier (OP). Generally the electronic contact of a single cell with an extracellular sensor is described by an equivalent electrical circuit, the point-contact model. In this electrical equivalent circuit the metal electrode has a large resistance to an extended electrolyte, which is set to electric ground potential. We varied the input impedance of the preamplifier OP by using shunt resistors and capacitors. It was found that not only the amplitudes of the extracellular recordings but also the transfer function is changing, compared to signals without any shunt element at the OP input. The effects were more pronounced with decreasing shunt resistance. Our results strongly suggest that in order to readout a correct extracellular signal, one should use a preamplifier with a very high input resistance (> 50M Ohms) and reduce any parasitic capacitances of the electrode array in future designs.

References: