

Using Cryopreserved Brain Neuronal cells For Neurochips

Neuronal cell networks growing on Microelectrode Arrays (MEAs) represent a new tool for assessing neural network dysfunction *in vitro* and screening of pharmacological agents for neuroactive properties. They allow for large scale neural network analysis and at the same time reducing the need for tissue dissection and animal handling.

Researchers from the Neurochip laboratory, Department of Neurology, University of Düsseldorf report¹ that cryopreserved Rat Brain Cortical Cells (QBM Cell Science) are ideally suited for use as a cell-based biosensor on Microelectrode Arrays ("Neurochip"). We provide here excerpts from these studies (with permission)

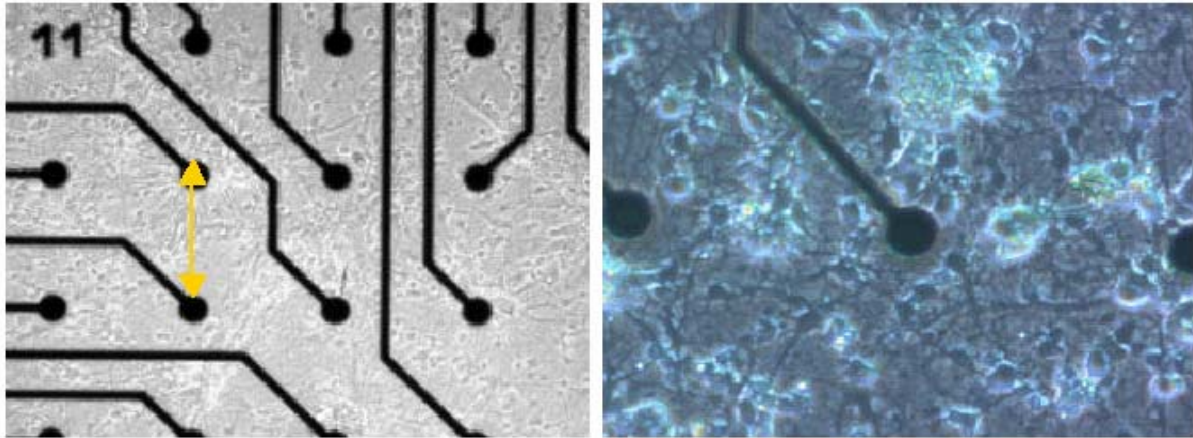


Fig 1. Cryopreserved cortical cells plated on an MEA, 10 days in primary culture. This Neurochip exhibited electrophysiological activity for over 30 days whilst being recorded 3 times/week.

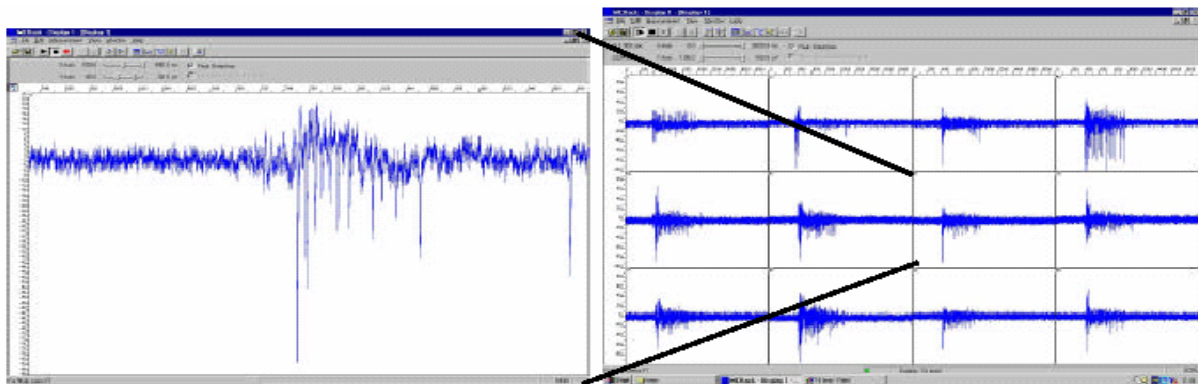


Fig 2. Typical synchronous bursting activity with exposure to the GABA_A antagonist bicuculline (100 μM). The Cryopreserved cortical cells express the most important inhibitory and excitatory ion-channels of CNS neurons with a high sensitivity for typical neuroactive substances.

¹Reports on the applications of Neurochips utilizing Cryopreserved Neuronal cells(QBM Cell Science)
1.SIMEA, Denton, Texas. March 5-8, 2003. Neurophysiological Characterization Of Cryopreserved Rat Cortical Neurons On Microelectrode Arrays. Frauke Otto, Philipp Görtz, Wiebke Fleischer, Mario Siebler
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Neuronal networks growing on microelectrode arrays (MEAs) are promising tools for assessing network dysfunction *in vitro*. Network responses to pharmacological agents have been investigated in cultures of murine spinal cord and cortical neurons as well as on rat cortical and hippocampal neurons so far. Recently, cryopreserved embryonic rat cortical neurons have become commercially available (QBM Cell Science.com). We characterized this cell culture with respect to morphological markers and electrophysiological network properties and further examined their applicability as a cell based biosensor

for detection of neuroactive substances in human cerebrospinal fluid. Cryopreserved cortical cells were plated on MEAs with a square grid of 60 electrodes (30 μm diameter, 200 μm spacing; Multi Channel Systems, Germany) at a density of $2 \times 10^5/\text{cm}^2$. For each substance examined, the IC_{50} was determined from a dose-response curve based on the change of spontaneous spike rate. Staining of neuron-specific markers (NF, MAP-2, synaptophysin), astrocytes (GFAP) and DNA (DAPI) was performed on coverslips for immunocytochemical characterization. **Results:** **1.** Double-labeling experiments with GFAP and NF revealed a neuron/astrocyte-ratio of approximately 50/50. Neurons further expressed MAP-2 and synaptophysin. **2.** Cryopreserved neurons formed a neurite network. Spontaneous spike activity and periodic burst patterns could be recorded repetitively from 11 to 84 days in culture similar to acutely prepared neurons. **3.** Dose response curves revealed the following electrophysiological properties: TTX, GABA and APV inhibited the spontaneous spike activity by prolonging the interburst intervals in a dose-dependent manner with an IC_{50} of 4. 1 nM, 1 μM and 18 μM , respectively. NMDA and glutamate responses were biphasic, inhibitory at higher concentrations (IC_{50} =1.8 μM and 10 μM), but excitatory below their IC_{50} . **4.** Application of cerebrospinal fluid (CSF) from patients with benign intracranial hypertension led to a two-fold increase in network activity compared to artificial CSF. So, human CSF seems to contain network activating substances.

Cryopreserved cortical cells on MEA developed electrophysiologically active neuronal networks with stable spike and burst activity. The IC_{50} values demonstrated a higher sensitivity to neuroactive substances than published results of devices using murine spinal cord neurons (Gross et al., *Biosensors & Bioelectronics* 1995,10:553-567). We conclude that cryopreserved neurons on MEA are promising tools for detection of clinically relevant neuroactive substances in the CSF of patients.

2.German Neuroscience Assoc. June 12-15, 2003. Goettingen, Germany. *Establishing Dissociated Neuronal Cultures as Biosensors for Neuroactive Substances in Acute Encephalopathy.* Philipp Görtz, Frauke Otto, Wiebke Fleischer, Annika Hoinkes, Mario Siebler Dept of Neurology, Heinrich-Heine-University Düsseldorf, Germany

Acute encephalopathy is a common symptom of the central nervous system (CNS) caused by different disorders like metabolic diseases, hepatic failure or inflammatory processes. The associated neurological dysfunction is reversible which indicates that not structural but functional properties are disturbed. Abnormalities in patients electroencephalogram reflect the interference of pathological agents with neuronal network function in vivo. Human brain tissue itself is virtually inaccessible for examination, leaving the cerebrospinal fluid (CSF) for diagnosis in patients with encephalopathy. Classical diagnostic parameters, however, often show no abnormalities, although patients are affected dramatically. Thus, we examined dissociated neuronal cultures on microelectrode arrays (MEAs) as an in vitro model of CNS neurophysiological network activity to screen for mediators inducing encephalopathy.

For recordings we used microelectrode arrays (MEAs) with a square grid of 60 electrodes (30 μm diameter, 200 μm spacing) (Multi-Channel-Systems Reutlingen, Germany) and a head stage heating system. We developed a semi-automatic system for application of substances and determined the IC_{50} from dose-response curves fitted to spontaneous spike rate (SSR) data. Primary dissociated embryonic cortical neurons from Wistar rats (DC), embryonic rat brain cortex Cells (CC) (Sprague-Dawley rat, QBM CellScience.com) and hNT neurons derived from a human teratocarcinoma cell line were established on the MEAs and compared concerning their maintenance and electrophysiological properties.

Spontaneous activity could be first detected after day 5 in vitro (DIV) in DC, after 11 DIV in CC and after 14 DIV in hNT neurons. CC and hNT neurons could be measured for more than 70 days. The fraction of spontaneous active cultures was highest for CC (75 %), followed by hNT (30 %) and DC (25 %). All cultures could be measured for hours repetitively on consecutive days. hNT neurons usually developed irregular spontaneously spiking, whereas stable synchronized burst activity could be recorded in DC and CC with a similar burst rate (0.1-1 Hz). The assessment of monosubstances revealed a high sensitivity of the DC and CC for glutamate (IC_{50} =10 μM), NMDA (IC_{50} =1.8 μM) and APV (IC_{50} =18 μM). The IC_{50} of TTX (1.1 nM) is in accordance with the concentration range known to be effective at the sodium channel. We could record spontaneous neuronal network activity of CC and hNT but not of DC under incubation with pure CSF and serum. The CSF even showed excitatory effects in CC and hNT increasing the SSR compared to artificial CSF (HEPES or bicarbonate buffered) or growth medium.

We examined substances which are suspected to induce encephalopathy in patients with metabolic disorders of the homocysteine pathway. We could demonstrate for the first time that not homocysteine

(IC₅₀= 401 μM) itself is the relevant agent of the acute neuronal network dysfunction but its oxidized forms homocysteic acid (IC₅₀= 1.3 μM) and homocysteinesulfinic acid (IC₅₀ = 1.9 μM). In conclusion, the MEA with different neuronal cell types allows to screen for neuroactive substances with a high sensitivity. Thus, the "Neurochip" provides a promising tool for the identification and the monitoring of clinical relevant substances in encephalopathy.