

2006 Society for Neurosciences, Satellite Symposium
"Recent Advances in Network Electrophysiology Using Multi-Electrode Arrays"
Monday, Oct. 16th. 6:30 – 9 p.m.

OVERVIEW: This symposium will present an overview of the rapidly accumulating knowledge gained from using multi-electrode recordings of neuronal activity in vitro and discuss future directions of research using this technology. Presentations will focus on short and long-term properties of neuronal networks investigated in neuronal cultures and acute and cultured brain slices. The target audience for this event includes all neuroscientists who strive to get reliable, long-term, continuous recordings and two-dimensional, real-time analysis of neuronal activity.

SPEAKERS:

1. Dr. Suguru Kudoh, Neuronics Research Group, research Institute for Cell Engineering, National Institute of Advanced Industrial Science and Technology
"Reorganization of internal connectivity in dissociated neuronal culture by interaction with outer world "
Note: some data/figures are available on his website at: <http://staff.aist.go.jp/s.n.kudoh/>

2. Tim Simeone, Barrow Neurological Institute and St Joseph's Hospital & Medical Center, Phoenix, AZ, USA
"A Planar Multielectrode Array Analysis of Resected Human Hypothalamic Hamartoma Tissue" (*tentative title*)

3. Dr. Suguru Kawato, Dept. of Biophysics and Life Sciences, Graduate School of Arts and Sciences, Univ. of Tokyo at Komaba
"Rapid modulation of hippocampal LTD by estrogen and endocrine disrupters: multielectrode analysis"

4. John Mielke, National Research Council of Canada; Ontario, Canada
"Use of Multi-Electrode Arrays to Study Synaptic Activity in Cultured Hippocampal Slices "

Rapid modulation of hippocampal LTD by estrogen and endocrine disrupters: multielectrode analysis

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Rapid modulation of hippocampal synaptic plasticity by estrogen has long been a hot topic, however, analysis of molecular mechanisms via synaptic estrogen receptors has been seriously difficult. Here, 17 β -estradiol-induced rapid modulation (within 90 min) of long-term depression (LTD) was analyzed using custom-made multi-electrode investigations (Mukai et al., 2006a). Recordings were performed using novel 64 multielectrodes particularly arranged to stimulate the Schaffer collaterals in the stratum radiatum of CA1, the recurrent collateral fibers in the stratum radiatum of CA3, and the medial perforant pathways in the molecular layer of dentate gyrus (DG).

LTD was induced pharmacologically by the transient application (3min) of NMDA to the adult male rat hippocampal slices. Upon application of 30 μ M NMDA, the maximal amplitude of EPSP was transiently decreased to a minimal value and then recovered to reach approximately a plateau level (<100%) within 30-50 min after NMDA application, indicating the LTD-establishment. This LTD was induced by the activation of phosphatase due to a moderate Ca²⁺ influx through NMDA receptors. The plateau EPSP amplitude at 60 min was 80.4% (CA1), 88.8% (CA3) and 95.1% (DG), respectively. A 30 min preperfusion of 10 nM estradiol, for example, significantly enhanced LTD resulting in the EPSP amplitude at 60 min of 59.7% (CA1), 79.1% (CA3) and 92.2% (DG). The dose dependency of enhancement effect by estradiol was heterogeneous in different regions. The LTD enhancement was least significant at 0.1 nM, moderately significant at 1 nM, and most significant at 10 nM for estradiol in both CA1 and CA3. In DG, however, the LTD enhancement was most significant at 1 nM, and smaller enhancement was observed at 0.1 and 10 nM estradiol. Investigations using specific estrogen agonists indicated that the contribution of estrogen receptor ER α but not ER β was essential to these estradiol effects. PPT (ER α agonist) exhibited a significant LTD enhancement in CA1, while DPN (ER β agonist) did induce a suppression of LTD in CA1, suggesting the contribution of ER β opposite to that of ER α to LTD. The coprefusion of 10 nM 17 β -estradiol (used as an antagonist) with 1 nM 17 β -estradiol suppressed the 17 β -estradiol effect on LTD. DES at 1-10 nM enhanced LTD, and DES was more effective at 1 nM than 10 nM. GPR30 (membrane estrogen receptor) agonist, 100 nM ICI, did not affect the NMDA-induced LTD.

Not only LTP but also LTD are necessary for complete memory processing. Enhancing effect of LTD by estradiol suggests that estradiol could facilitate erasing wrong memory. In the current study, LTD was chosen to probe the modulation effects of estradiol, because estradiol was much more effective on LTD than LTP. When the enhancement of LTP by estradiol was observed in CA1, an immediate increase by approx. 20% was accompanied upon the onset of estradiol perfusion in the baseline of EPSP slope. The increase has been attendant upon a further approx. 130% increase by high-frequency tetanic stimulation (Foy et al. 1999). However, if we did not have this approx. 20% baseline increase of EPSP slope (before the tetanic stimulation), the enhancement by estradiol was not apparent concerning the pure tetanic stimulation-induced LTP (Mukai et al. 2006b). In other words, the magnitude of tetanic stimulation-induced LTP was nearly the same between in the presence and absence of estradiol. On the other hand, in the current LTD enhancement by estradiol, an immediate increase of EPSP by the onset of estradiol perfusion was not accompanied.

Recently, an issue of 'endocrine disrupters' (low dose environmental chemicals), which are artificial xenoestrogenic substances, has emerged as a social and environmental problem. Typical endocrine disrupters are bisphenol A (BPA), diethylstilbestrol (DES), 4-nonylphenol (NP) and 4-octylphenol (OP) and tributyltin (TBT). While effects of endocrine disrupters on reproductive organs has been intensively investigated (little effect on adult mammals), their effects in the brain are still poorly understood (Kawato, 2004). Here, we demonstrated the rapid effects of 10-100 nM endocrine disrupters on LTD. A 30 min preperfusion of 10 nM BPA significantly enhanced LTD in CA1 and CA3. On the other hand, 10 nM BPA significantly suppressed LTD in DG. NP suppressed LTD in CA1, and enhanced LTD in CA3 and DG. OP suppressed LTD in CA1, and enhanced LTD in CA3, but had no effect in DG. TBT at 10 and 100 nM had no effects on LTD in all CA1, CA3 and DG. The modulation of LTD by BPA and DES was close to that by estradiol (ER α type), however, NP and OP induced ER β type of modulation from that by estradiol. These rapid responses were probably driven via ER α and ER β at synapses of glutamatergic neurons. Localization of ER α at postsynapses as well as nuclei of glutamatergic neurons was demonstrated by immuno-electronmicroscopy with home-made purified antibody RC-19 (Mukai et al., 2006a). Because synaptic endogenous synthesis of estradiol is demonstrated by our group (Hojo et al., 2004), synaptocrine mechanisms (synthesis and action of estradiol at synapses) may play an essential role for rapid synaptic plasticity depending on brain-derived estrogen.

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Use of Multi-Electrode Arrays to Study Synaptic Activity in Cultured Hippocampal Slices

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The hippocampus is an essential component of many pathways leading to memory formation, and is uniquely susceptible to damage. Organotypic hippocampal slice cultures (OHSCs) have become increasingly popular, for they retain an organized cytoarchitecture, express synaptic proteins, and display axospinous synapses. Our group has developed two models wherein slice cultures are paired with multi-electrode arrays (MEAs) to examine hippocampal synaptic activity and dysfunction: inverted MEA-OHSCs, which are cultures maintained on semi-porous membrane pieces and acutely inverted onto an array, and integrated MEA-OHSCs, which are cultures grown directly upon an array. The inverted slice model was designed to provide a high-throughput, endpoint assay of the effect that chronically applied nicotinic receptor agonists have on characteristics of long-term potentiation (LTP). Preliminary work has set the optimal recording conditions, and established that the slices display both early and late-phase LTP induced by multiple applications of high-frequency stimulation. The integrated slice model was intended to provide a means to study chronic changes in defined populations of cells caused by oxygen-glucose deprivation (OGD), an *in vitro* model of ischemia, and β -amyloid ($A\beta$) exposure, a culture model of Alzheimer's disease. To date, we have examined the correlation between OGD severity and the recovery of synaptic activity up to 48 hr later, and the ability of $A\beta$ applied for 24 hr to remove LTP induction in the CA1 subfield. Taken together, our studies have shown that inverted and integrated MEA-OHSCs display synaptic activity and plasticity, and that these platforms allow for the study of changes in hippocampal function beyond the timeframe normally permitted by acutely prepared slices.

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A Planar Multielectrode Array Analysis of Resected Human Hypothalamic Hamartoma Tissue

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Rationale: The hypothalamic hamartoma (HH) represents a rare but important model of subcortical epileptogenesis. Clinical studies, based primarily on intracranial electrode recordings have established that the HH itself is epileptogenic, but the mechanisms are unknown. In the present study, we examined the network properties of surgically-resected human HH tissue using a planar multielectrode array recording system.

Methods: HH tissue was obtained with patient consent and upon resection was immediately submerged in ACSF bubbled with 95% O₂/5% CO₂. Tissue slices (400 μm) were placed in a microelectrode dish (Alpha Med Systems, Osaka, Japan) and perfused with warmed (35°C) oxygenated ACSF. The electrodes were arranged in an 8x8 grid with 150 μm separation. Slices were arbitrarily placed over the entire electrode grid.

Results: Paired-pulse stimulation (50 ms interval) to random points within the tissue elicited small negative field potentials and stimulation-evoked single unit firing within specific regions of the tissue. A 5-20% paired-pulse depression of the field potential was evident in most areas, and was converted to a similar magnitude of paired-pulse facilitation by picrotoxin (100 μM) suggesting the presence of both excitatory and inhibitory synaptic components. Furthermore, the stimulation-evoked single unit firing increased (~4-fold) with the second pulse consistent with paired-pulse facilitation of an excitatory component. Picrotoxin substantially increased evoked unit firing suggesting that endogenous GABAergic neuron activity is present normally, and inhibition or shunting of an excitatory component is prominent. In addition, we recorded spontaneous single unit firing of individual cells from multiple electrodes with frequencies ranging from 1-14 Hz and spontaneous negative slow wave activity with amplitudes ranging from 5-300 mV. Interestingly, the frequency of spontaneous slow wave activity decreased with application of picrotoxin and increased with muscimol (30 μM).

Conclusions: Our observations indicate that HH tissue is composed of neurons that are strongly modulated by GABA_A receptor-mediated mechanisms. This is consistent with earlier observations demonstrating positive immunoreactivity of HH tissue to glutamic acid decarboxylase (GAD65/67). A novel finding in this study is that populations of neurons also give rise to spontaneous slow wave discharges. It is of interest to determine if the spontaneous activity will become synchronous across the tissue and develop into epileptiform activity in response to provocation. Future studies will characterize the spontaneous activity and the functional architecture of HH tissue.

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Reorganization of internal connectivity in dissociated neuronal culture by interaction with outer world.

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The dissociated neurons autonomously re-organized their functional neuronal networks on multielectrode array dish, go along with elongating neurites and establishing synaptic connections. Functional assemblies of neurons must be basic constitutions of higher brain function, and the spatio-temporal patterns of spontaneous activity in the cultured networks might be a reflection of functional neuron assemblies.

To visualize the functional connections between neurons, we have analyzed spontaneous activity of synaptically induced action potentials, using "connection map" devised for this purpose. We found the almost matured neuronal networks were not random networks but unneutral, self-organized networks with a few hub-like neurons possessing many inputs from other neurons. In addition, the functional connections were dynamically modified by induction of synaptic potentiation and the process may be required for reorganization of the functional group of neurons.

We also set up the system in which the living neuronal network interacts to feedback stimulation system. Bursting-like stimulation pattern was generated by the interaction of neuronal network and the real-time feedback system. In this system, the living neuronal network can control the duration and frequency of this bursting stimulation according to its internal state. Consequently, even after the 24hr feedback stimulation, drastic change of activity pattern occurred. The result suggests that neurons in dissociated culture autonomously re-organized their functional neuronal networks interacted with their environment. Now we perform precise analysis of the effects of electrical feed-back inputs on developmental changes in spatio-temporal pattern of spontaneous action potentials.

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