

Symposium at the 39th Annual SfN Meeting in Chicago, IL
Scientific Advances in Micro-Electrode Array (MEA) Electrophysiology

Monday, October 19, 6:30 - 9:00 p.m.
McCormick Place Campus, Room S404ABCD

The goal of this symposium is to create awareness among new investigators and established scientists of the diverse applications of micro-electrode array systems. This session will illustrate why this system is ideal for studying network electrophysiology, increasing throughput of conventional microelectrode recordings, and enabling researchers to quickly and easily perform electrophysiological studies regardless of their scientific background. International speakers from industry and academia will present their research enabled by innovative neuroscience research methods with multi-electrode array systems in a variety of preparations. Organized by AutoMate Scientific Inc., Berkeley, CA, USA and Alpha MED Sciences Co., Ltd. Osaka, Japan.

Welcome and Introduction

Enrique G Navarrete, MD

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Electrical Activity in the Ventromedial Nucleus In Vitro Predicts Food Intake and Female Sexual Behaviour

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Introduction: The ventromedial nucleus (VMN) is believed to be the hypothalamic 'satiety center'. Lesioning of the VMN in rats, results in rapid hyperphagia and increased rate of body weight gain whereas stimulation of the VMN results in a decrease of food intake. Lesioning the VMN also decreases female receptivity (lordosis) and electrical stimulation facilitates lordosis. Compounds microinjected into the VMN have also been shown to modulate both food intake and sexual receptivity. The aim of the study was to a) evaluate the role of endogenous glutamate, steroid hormones and diet on neuronal activity within the VMN *ex vivo* and b) understand the pharmacology and underlying mechanism of agents known to modulate food intake and lordosis.

Methods: Brains were removed from male and female Sprague-Dawley rats following cervical dislocation (150-200 g) in compliance with UK Home Office guidelines. Coronal VMN slices (350 μm) were placed onto a 64 microelectrode chip, superfused with aCSF 5 mL min⁻¹ and stimulated (150 μA , 0.1 ms, every 30 s) using MED64 Performer to evoke excitatory postsynaptic potentials (EPSPs). Single-unit activity was recorded at 20 kHz simultaneously from all 64 electrodes for 2 s every 10 s.

Results: EPSPs are abolished by tetrodotoxin (300 nM; $P < 0.005$) and the non-NMDA receptor antagonist DNQX (10 μM , $n = 51$; $P < 0.005$) implying signals are due to nerve-evoked glutamate release. The GABA_A antagonist bicuculline potentiates EPSPs (EC_{50} 1.6 \pm 0.6 μM , $n = 7$) suggesting glutamate release is under endogenous GABA control. Glutamate release was greatest during metestrous and following ovariectomisation. The number of regions within the VMN eliciting single-unit activity was reduced following ovariectomy without changing spike frequency. EPSPs were potentiated from rats fed a high fat diet compared to chow, diet-induced obese or diet-resistant rats whereas single unit activity was attenuated.

Agents increasing lordosis and decreasing food intake via the VMN, decrease nerve-evoked glutamate release (endogenous GABA; noradrenaline EC_{50} 3.8 \pm 1.7 μM , $n = 6$; bombesin EC_{50} 75 \pm 9 nM, $n = 5$; TRH EC_{50} 20 \pm 4 nM, $n = 4$). Conversely, agents decreasing lordosis and increasing food intake via the VMN increase glutamate release (8-OH-DPAT EC_{50} 125 \pm 34 nM, $n = 7$; DAMGO EC_{50} 44.8 \pm 20.9 nM, $n = 9$; NPY EC_{50} < 1 μM , $n = 14$). No effect was observed with GLP, PACAP or melanocortin agonists (1 μM , $n = 5$; $P > 0.05$) nor the adenylate cyclase activator forskolin (100 nM, $n = 5$; $P > 0.05$).

Conclusion: Here we present a novel robust VMN *in vitro* technique that a) suggests glutamate via non-NMDA receptors inhibits lordosis and increases food intake. Activation of G_i-coupled receptors potentiates glutamate release resulting in an increase of food intake and inhibition of female sexual behaviour, whereas activation of G_q-coupled receptors attenuates endogenous glutamate to decrease food intake and facilitate female sexual function. The role of G_s-coupled receptors remains to be determined.

The network dynamics and spontaneous activity in dissociated rat hippocampal culture

Suguru N. Kudoh

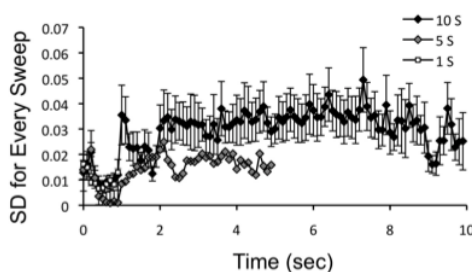
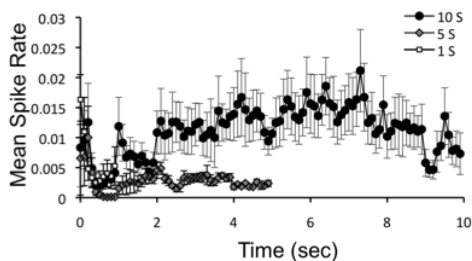
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The dynamics of the neuronal network is determined by the interaction between neurons, and it is highly complicated. A multi electrode array (MEA) dish, the culture dish equipped the bottom with many planar electrodes is very convenient for analyzing the response to the current stimulation, using a small-scale network of the living neurons.

We have analyzed the developmental process of this small-scale semi artificial neuronal network. In this system, the spontaneous action potentials were observed from approximately two weeks *in vitro*. The spatio-temporal pattern of the spontaneous activity became to be complex during culture days, and the hierarchical bursting activity, called “super bursts”, were observed. We found the transient high frequency bursts (HFB) of spontaneous action potentials lasting for one week at an approximately 30 day - 50 days *in vitro* (DIV). The spatio-temporal pattern of the spontaneous action potentials became to be heterogeneous after the transient HFB. The high and low frequency activity of spontaneous action potential were observed through each electrode. The heterogeneity of the rate of spike activity after autonomous expression of HFB activity suggests the synaptic modification was induced by the HFB activity. Thus spontaneous activity is critical for reconstruction of network structure.

Though activity of the neuronal network was self-organized even without external inputs, external electrical inputs were expected to have grate influences on the neuronal activity.

Even a single stimulus transiently changes the whole network activity and repeatedly stimuli may cause long-lasting changes of the network structure. We elucidated the effect of a single stimulus on autonomous electrical activity. The number of spikes decreased transiently and the spike rate gradually recovered within 1-2 s. The response to the same stimulus was approximately reproducible. Therefore, variance of repeatedly evoked spikes at every recording sites in rate reduced for 2 s immediately after the primary evoked activity (figure). In addition, the repetitive stimuli suppress the spontaneously occurring bursting activity in frequency, even though the inter-stimulus- interval was more than 10 sec. These results suggest that the electrical stimulus called up a certain hierarchical state of the network and that state lasted for several seconds. We consider that the spontaneous activity was not only noise but one of the elements of dynamical network state which controlled by electrical stimulus.



Spike recordings from acute slices of the ventral midbrain using a multielectrode array device: a powerful tool for physiological, pharmacological and toxicological studies of the midbrain dopaminergic system

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The ventral midbrain contains neurons providing the main source of dopamine (DA) in the brain. In particular, DA neurons of the Substantia Nigra pars compacta (SNpc) and of the Ventral Tegmental Area (VTA) exert an essential role in motor control as well as motivational and cognitive processes. For this reason, the electrophysiological investigation of these neurons has attracted much interest by the scientific community, for its implication in both neurophysiology of the basal ganglia and neuropathology, in particular Parkinson's disease, schizophrenia and addictive behaviors.

My report will present a new and powerful approach to the study of this neuronal population, consisting on single unit spike recordings from acute slices, using the MED64 multi-electrode array system.

Horizontal midbrain slices (300 μm) are placed in a recording chamber under visual control, in such a way that most of the SNpc area is exposed to the underlying electrode array. The slice is then covered by a lens paper and gently pressed on the electrodes with a nylon mesh glued to a platinum ring, in order to reduce shunting of electrical signals. Particular care is required to maintain a continuous flow of warm (34°C) oxygenated extracellular solution (3-4 ml/min), streaming over the SNpc area, in order to maintain a tonic spontaneous firing rate of the neuronal population.

Using this approach, extracellular spikes have been detected from 30.5 ± 1.6 channels per slice (n=15).

Activity arising from more than a single unit was often observed, therefore, following an appropriate spike sorting procedure, activity from a total of 960 neurons could be obtained (64.0 ± 6.4 cells per slice).

Analysis of the firing of these neurons revealed a large degree of variability with regards to dopamine sensitivity, firing rate and regularity. Moreover, it provided evidence of a significant, though limited, degree of firing synchronicity.

Data will also be shown on the use of this experimental approach for the neuropharmacological investigation of the dopaminergic system. Moreover, preliminary experiments will be shown on recordings obtained from coronal slices of TH-GFP mice. In these slices, it is possible to discriminate electrical activity arising from SNpc and VTA DA neurons, and thus obtain a parallel evaluation of differences in the firing properties or drug sensitivity of these two separate dopaminergic populations.

Correlations between patch and extracellular recording methods using a multi-electrode device (MED64) to test the physiology and pharmacology of transmission in the auditory brainstem

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Our studies are focussed on understanding transmission of information across relay synapses in the auditory brainstem. We have combined whole-cell patch clamp recording and voltage-clamp experiments with immunohistochemical localization of ion channels to study synaptic transmission and voltage-gated ion channel effecting neuronal excitability. The auditory brainstem pathway contains two large synaptic terminals: the Endbulb of Held which is formed by the primary afferent terminal on Bushy cell of the anterior ventral cochlear nucleus; and the Calyx of Held which arises from the projection of the Bushy cell to the contralateral medial nucleus of the trapezoid body (MNTB). This pathway is involved in sound source localization. Although much of our work has been done with patch recording from single neurones, we have been keen to develop extracellular recording techniques from brainstem slices using the Multi-Electrode Device (MED64) as a way to stimulate and record during application of pharmacological agents. The first part of this talk will concern a general description of excitatory synaptic transmission at these large glutamatergic synapses. Second, I will describe the relationship between action potentials and excitatory postsynaptic potentials recorded using whole cell patch clamp compared with the waveforms recorded using the MED. Third, I will demonstrate use of field potential recording with the MED to explore the pharmacology of synaptic transmission in the brainstem and to elucidate changes in the ionic basis of excitability during network behaviour in brain slices from the hippocampus.

Transient Choline-Mediated Depression of Synaptic Transmission in Acutely Prepared Hippocampal Slices

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The hippocampus is a region of the mammalian brain that has been extensively studied due to its role in many forms of memory. Activity within the hippocampus is modulated by broad cholinergic innervation that acts upon a variety of receptors; in particular, the nicotinic acetylcholine receptors (nAChRs), which are ligand-gated ion channels formed from alpha (α 2-10) and beta (β 2-4) subunits. The α 7 homopentamer is one nAChR subtype that has been the focus of considerable interest due to a suspected influence upon both memory-related processes and neurodegeneration. Choline, which is an alcohol moiety present in the synaptic cleft after degradation of acetylcholine, is thought to be a selective agonist of the α 7-nAChR. While previous work has examined the activity of choline at the level of individual neurons, little is known about its effect on synaptic transmission at the population level. As a result, we made use of extracellular recordings to examine how applied choline would affect synaptic activity in acutely prepared hippocampal slices. Choline caused a reversible depression of evoked field excitatory post-synaptic potentials in a concentration-dependant manner (10, 500, and 1000 μ M). To confirm the role of α 7-nAChRs in choline-mediated depression (CMD), two specific antagonists were used (α -bungarotoxin and methyllycaconitine), but neither completely blocked CMD. However, the general nAChR antagonist mecamylamine did block CMD, suggesting participation of another nAChR subtype. In addition to assessing effects on basic transmission, we also examined whether choline might affect synaptic plasticity, which is a well-characterised, population level phenomenon believed to represent a cellular model of memory. Choline was applied after the induction of long-term potentiation (LTP), which is a form of plasticity defined by an induced increase in responsiveness, and, although CMD was observed, potentiation returned upon wash-out. When applied prior to the induction of LTP, choline did not interfere with its ultimate magnitude, but did substantially lengthen the time required for potentiation to develop. From a technical perspective, our study illustrates how multi-electrode arrays can be used to better understand the influence that a compound of interest might have upon synaptic transmission and plasticity. From a physiological perspective, our study provides evidence that choline transiently depresses synaptic activity, and may, therefore, contribute to inhibitory processes in hippocampal function.

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