The Fourth International Neural Microcircuity Conference

Signal Processing Mechanisms of Cortical Neurons

Program

Kanucha Resort, Okinawa, Japan

June 24th-27th, 2010

Organizing Committee:
Yoshivuki Kubota (Japan) Jackie Schiller (Israel)
Dear participants,

The fourth International Neural Microcircuitry Conference "Signal Processing Mechanisms of Cortical Neurons", will be held in Kanucha Resort, Okinawa, Japan, on June 24th - 27th, 2010.

This meeting is of significant importance for the field of neuronal signal processing. The meeting will present cutting edge data obtained from studies in cortex, hippocampus, and the other area using neurophysiological approaches, molecular manipulation, electron microscopy and theoretical analysis. We are proud to have many distinguished speakers who are in the forefront of their fields from the U.S.A., U.K., Germany, Switzerland, Hungary, Israel, Australia, China and Japan.

We hope you can join us and share this special opportunity.

Organizing Committees
Yoshiyuki Kubota, Ph.D.
Jackie Schiller, Ph.D.
## Program

<table>
<thead>
<tr>
<th>Room</th>
<th>6/24 Thr</th>
<th>Time</th>
<th>Room</th>
<th>6/25 Fri</th>
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<th>6/26 Sat</th>
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<tbody>
<tr>
<td>Karaya</td>
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<td>8:15~8:40</td>
<td>Naoya Takahashi</td>
<td>6:25 Fri</td>
<td>Man Jiang</td>
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<td>Hiroki Kurashige</td>
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<td>Masanori Matsuzaki</td>
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<td>Lucy Palmer</td>
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<td>Tomoki Fukai</td>
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Program

Thursday, June 24th

Reception
14:00 - Hotel Front Building 1st floor

Conference
--- “Karaya Hall” in the Hotel Front Building on 24th and 25th ---
--- “Rain Forest” in the Indoor Pool Building on 25th - 27th ---

15:20 - 15:30 Opening remarks Jackie Schiller

Session 1  Chaired by Jackie Schiller
15:30 - 16:30 IS1 Dan Johnston  (University of Texas, U.S.A.)
    Plasticity of HCN Channels in Soma and Dendrites of Hippocampal CA1 Pyramidal Neurons

16:30 - 17:30 IS2 Yasuo Kawaguchi  (National Institute for Physiological Sciences, Japan)
    Pyramidal and GABAergic Cell Diversity and Connection Selectivity

17:30 - 18:30 IS3 Gábor Tamás  (University of Szeged, Hungary)
    Perspectives for Unitary GABAergic Volume Transmission in Regulating Cortical Microcircuits

18:30 –18:45 commentary by Jackie Schiller

18:45 –19:00 intermission

Banquet
19:00 – 21:00 banquet at “RikaRika” guest lounge in Restaurant Building
Friday, June 25th

Session 2

8:15 – 8:40  ST1  Naoya Takahashi (University of Tokyo, Japan)
Topological Basis for Spike Synchrony in *ex vivo* Neuronal networks

8:40 – 9:40  IS4  Masanori Matsuzaki (University of Tokyo, Japan)
Optical Methods for Revealing the Synaptic Function and Structure in the Local Circuit

9:40 - 10:00  Coffee Break

10:00 – 11:00  IS5  Gina Turrigiano (Brandeis University, U.S.A.)
Visual Deprivation During a Critical Period Selectively Suppresses L5 Pyramidal Neuron Excitability by Modulating the Induction of Intrinsic Plasticity

11:00 – 12:00  IS6  Nelson Spruston (Northwestern University, U.S.A.)
Persistent Firing from the Axon of a Molecularly Identified Population of Inhibitory Interneurons in the Hippocampus

12:00 – 12:25  ST2  Toshihiko Hosoya (RIKEN/Brain Science Institute, Japan)
Mirco-Periodic Functional Organization in Layer V

12:25 – 18:50  Lunch, Free, Dinner

12:40 – 14:00
Organizing committee meeting at Rain Forest Hall
Chaired by Jackie Schiller

Session 3

18:50 – 19:15  ST3  Lucy Palmer (University of Bern, Switzerland)
Dendritic GABA<sub>B</sub> Inhibition Decreases Neuronal Output in *vivo*

19:15 – 20:15  IS7  Jackie Schiller (Technion Medical School, Israel),
Synaptic Integration in Tuft Dendrites of Layer 5 Pyramidal Neurons: an NMDA Spike Story

20:15 - 20:35  Coffee Break

20:35 - 21:35  IS8  Matthew Larkum (University of Bern, Switzerland)
Dendritic Properties of Neocortical Pyramidal Neurons – What have We Learnt from Dendritic Patch Recordings?

21:35 – 22:00  ST4  Masanori Murayama (RIKEN/Brain Science Institute, Japan)
Dendritic Activity in Awake Animals
Saturday, June 25th

Session 4

8:15 – 8:40  ST5  Man Jiang  (Shanghai Institutes for Biological Sciences)
Asynchronous Release of GABAergic Synapses in Neocortex

8:40 – 9:40  IS9  Yousheng Shu  (Shanghai Institutes for Biological Sciences)
Membrane Potential-Dependent Modulation of Recurrent Inhibition in Neocortex

9:40 - 10:00  Coffee Break

Session 5

Chaired by Peter Jonas

8:15 – 8:40  ST5  Man Jiang  (Shanghai Institutes for Biological Sciences)
Asynchronous Release of GABAergic Synapses in Neocortex

8:40 – 9:40  IS9  Yousheng Shu  (Shanghai Institutes for Biological Sciences)
Membrane Potential-Dependent Modulation of Recurrent Inhibition in Neocortex

9:40 - 10:00  Coffee Break

Session 5

Chaired by Matthew Larkum

10:00 – 11:00  IS10  Sacha Nelson  (Brandeis University, U.S.A.)
Physiological Genomics of Cortical Circuits in Health and Disease

Poster Session I

11:00 – 13:00  Chaired by Jackie Schiller and Yoshiyuki Kubota
Commentary by Jackie Schiller, Yoshiyuki Kubota (organizing committee) and the other invited speakers

13:00 – 18:55  Excursion  (Lunch, Free, Dinner)

Session 5

Chaired by Bartlett Mel

18:50 – 19:15  ST6  Allan Gulledge  (Dartmouth Medical School, U.S.A.)
Why do Neurons have Spines?

19:15 – 20:15  IS11  Yoshiyuki Kubota  (National Institute for Physiological Sciences, Japan)
Dendritic Dimensions and Signal Conduction Properties of Cortical Nonpyramidal Cells

20:15 - 20:35  Coffee Break

Chaired by Sacha Nelson

20:35 - 21:35  IS12  Michael Häusser  (University of London, U.K.)
Dendritic Computation

21:35 – 22:00  ST7  Kenji Morita  (University of Tokyo, Japan)
Spatial Noise and Cortical Functions: Potential Functional Roles of the Inhomogeneity across Dendritic Branches of Cortical Pyramidal Cells
Sunday, June 27th

Session 6

8:15 – 8:40  ST8 Hiroki Kurashige (RIKEN/Brain Science Institute, Japan)
Dendritic Plateau Potentials Sustain Noise-Induced Multistability in Neuronal Network Model

8:40 – 9:40  IS13 Bartlett Mel (University of South California, U.S.A.)
“Dark Computation” in the Neocortex: How the Computing Functions of Pyramidal Neuron Dendrites may Depend on 'Invisible' Parameters

9:40 - 10:00  Coffee Break

10:00 – 11:00  IS14 Massimo Scanziani (University of California, San Diego, U.S.A.)
Excitation and Inhibition in Cortical Space

Poster Session II

11:00 – 13:00  Chaired by Jackie Schiller and Yoshiyuki Kubota

Commentary by Jackie Schiller, Yoshiyuki Kubota (organizing committee) and the other invited speakers

13:00 – 18:55  Lunch, Poster, Free, Dinner

Session 7

18:50 – 19:15  ST9 Tomoki Fukai (RIKEN/Brain Science Institute, Japan)
Synaptic Mechanism of Information Transmissions in Low-frequency Asynchronous Firing of Cortical Networks

19:15 – 20:15  IS15 Greg Stuart (Australian National University, Australia)
Somatic and Dendritic Inhibition in Cortical Pyramidal Neurons

20:15 - 20:35  Coffee Break

20:35 - 21:35  IS16 Peter Jonas (University of Freiburg, Germany)
Mechanisms Underlying the Fast Synaptic Output of GABAergic Interneurons

21:35 – 21:45  Concluding Remarks

Yoshiyuki Kubota
Poster session

P1 Masatoshi Takita (AIST, Japan)
Bidirectional Feed-Forward Control in the Local Collateral Circuit of the Hippocampal-Prefrontal Pathway Involved in Working Memory

P2 Fuyuki Karube (National Institute for Physiological Sciences, Japan)
Axonal Bouton Distribution of Layer IV and VI Spiny Neurons over Functional Maps in the Cat Primary Visual Cortex

P3 Mieko Morishima (National Institute for Physiological Sciences, Japan)
Projection Type-Specific Temporal Synaptic Characteristics in Frontal Cortex

P4 Takeshi Otsuka (National Institute for Physiological Sciences, Japan)
Cell Diversity and Connections between Callosal Projection Neurons in the Frontal Cortex

P5 Naoki Shigematsu (National Institute for Physiological Sciences, Japan)
Distribution Patterns of Cortico-Cortical and Thalamo-Cortical Inputs to Parvalbumin-Expressing GABAergic Neurons in Rat Frontal Cortex

P6 Yoshifumi Ueta (National Institute for Physiological Sciences, Japan)
The Relationship between Corticocortical and Corticospinal Projections in Rat Secondary Motor Cortex

P7 Mika Ushimaru (National Institute for Physiological Sciences, Japan)
Firing Patterns of Neocortical and Thalamic Neurons in the Slow Wave

P8 Shun Tsuruno (RIKEN/Brain Science Institute, Japan)
Simple Experimental System For Mapping Inputs To Neocortical Layer 5 Subcerebral Projection Neurons

P9 Genki Minamisawa (University of Tokyo, Japan)
Visual Stimulation-Induced Resetting of Membrane Voltage Responses of V1 Neurons in Awake Mice

P10 Daisuke Ishikawa (University of Tokyo, Japan)
Fluorescent Pipettes for Optically Targeted Patch-Clamp Recordings
P11 Toshiaki Omori (University of Tokyo, Japan)
   Statistical Estimation of Non-Uniform Dendritic Membrane Properties

P12 Kaori Ikeda (Australian National University, Australia)
   Properties of Visually Evoked Responses in Binocular Primary Visual Cortex

P13 Michele G Cavazzini (Australian National University, Australia)
   Action Potential Backpropagation Cortical Interneurons

P14 Mazahir T Hasan (Max Planck Institute for Medical Research, Germany)
   Optical Recording of Neuronal Activity with Genetic Ca²⁺ Sensors in Anesthetized and Freely Moving Mice
Abstract

Oral
Plasticity of HCN Channels in Soma and Dendrites of Hippocampal CA1 Pyramidal Neurons

Daniel Johnston¹, Rishikesh Narayanan¹, Darrin Brager¹, Sachin Vaidya¹, Nikolai Dembrow², Kevin Dougherty², Randy Chitwood², Richard Gray¹, Alan Lewis², and Dane Chetkovich²

¹Center for Learning and Memory, University of Texas at Austin, Austin, TX 78712 USA
²Department of Neurology, Northwestern University, Chicago, IL 60611 USA

The dendrites of hippocampal pyramidal neurons express numerous types of voltage-gated ion channels. The properties and/or distributions of these channels in the dendrites are very non-uniform and highly regulated. We have investigated long-term changes in voltage-gated channels in hippocampal CA1 pyramidal neurons following the induction of long-term potentiation (LTP) and long-term depression (LTD). We have found that there are activity-dependent, and bi-directional, changes in the intrinsic excitability of these neurons with LTP and LTD. The changes in ion channels occur in parallel to those at the synapse and affect both the local and overall excitability of the neuron. The change in local excitability can increase the probability that a given synaptic input fires the cell while a change in the overall excitability of the neuron may act to stabilize its firing rate following experience dependent plasticity.

One type of bi-directional change in excitability associated with synaptic plasticity appears to involve the hyperpolarization activated "h" channel, which is comprised of HCN1 and HCN2 subunits in CA1 neurons. There are also bi-directional changes in h channels at different time points following a sustained seizure. These changes in h channels (or Iₜ) can decrease and increase, respectively, the overall excitability of CA1 neurons and represent a form of homeostatic plasticity. In the present work we explored some of the mechanisms involved in the regulation of Iₜ in these neurons. We found that the depletion of intracellular calcium stores, or what has been called "ER stress", produced an increase in Iₜ. This increase in Iₜ required IP3 receptors, store operated calcium channels, and protein kinase A activity. We propose this as a
homeostatic mechanism that protects neurons after depletion of calcium stores triggered through altered network activity during pathological conditions. In other work stemming from the changes in $I_h$ associated with epilepsy, we explored the role of an important auxiliary subunit (TRIP8b) that regulates the trafficking and surface expression of h channels. A mouse in which the gene for TRIP8b was deleted shows profound deficits in $I_h$ in both soma and dendrites of these neurons. H channels appear to be very “plastic” and highly regulated in CA1 neurons.

Supported by NIH Grant MH048432.
Pyramidal and GABAergic Cell Diversity and Connection Selectivity

Yasuo Kawaguchi

National Institute for Physiological Sciences, Okazaki, Japan;
The Graduate University for Advanced Studies (SOKENDAI), Okazaki, Japan;
JST, CREST, Japan

The neocortex is composed of numerous types of excitatory principal neurons and inhibitory interneurons. Pyramidal cell excitability is regulated by various GABAergic cell types. Despite their importance in regulating excitatory networks, little is known about how inhibitory interneurons are incorporated into excitatory intra- and interlaminar subnetworks.

We quantitatively identified GABAergic nonpyramidal cell subtypes on the basis of their physiological characteristics, differential molecular expression, and axonal and dendritic arborization. Excitatory pyramidal cell subtypes were classified based on their extracortical projections, physiology, and dendritic arborization. GABAergic cells exhibited subtype-specific preferences for forming synaptic connections onto specific target-cell surface domains. Similarly, pyramidal neurons exhibited selective connectivity, with several internal excitatory pathways segregated according to the extracortical targets of their efferent projection neurons. Layer 5 fast spiking (FS) and pyramidal cells made reciprocally connected modules. These modules did not preferentially receive common inputs from layer 2/3. On the other hand, layer 5 non-FS/pyramidal cell pairs were preferentially targeted for common input from layer 2/3 cells. These suggest that Layer 5 inhibitory interneurons form distinct intra- and interlaminar subnetworks with pyramidal cells, depending on inhibitory cell types.
Perspectives for Unitary GABAergic Volume Transmission in Regulating Cortical Microcircuits

Szabolcs Oláh¹, Márton Rózsa¹, Gergely Komlósi¹, Eszter Boldog¹, Rita Báldi¹, Pál Barzó² and Gábor Tamás¹

¹Research Group for Cortical Microcircuits of the Hungarian Academy of Sciences, Department of Physiology, Anatomy and Neuroscience, University of Szeged, Közép fasor 52, Szeged, H-6726, Hungary
²University of Szeged, Department of Neurosurgery, Semmelweis u. 6. Szeged, H-6725, Hungary

Gamma-aminobutyric acid (GABA) is predominantly released by local interneurons in the cerebral cortex to particular subcellular domains of the target cells. This suggests that compartmentalized, synapse specific action of GABA is required in cortical networks for phasic inhibition. However, GABA released at the synaptic cleft diffuses to receptors outside the postsynaptic density and thus tonically activates extrasynaptic GABAA and GABAB receptors, which include subtypes of both receptor families especially sensitive to low concentrations of GABA. The synaptic and extrasynaptic action of GABA is in line with idea that neurons of the brain use synaptic (or wiring) transmission and nonsynaptic (or volume) transmission for communication. However, reuptake mechanisms restrict the spatial extent of extrasynaptic GABAergic effects and it was proposed that concerted action of several presynaptic interneurons or sustained firing of individual cells or increased release site density is required to reach ambient GABA levels sufficient to activate extrasynaptic receptors.

We found that individual neurogliaform cells release GABA sufficient for volume transmission within the axonal cloud and thus neurogliaform cells do not require synapses to produce inhibitory responses in the overwhelming majority of nearby neurons. Neurogliaform cells suppress connections between other neurons acting on presynaptic terminals which do not receive synapses at all in the cerebral cortex and, moreover, reach extrasynaptic, δ subunit containing GABAA (GABAAδ) receptors responsible for tonic inhibition. We reveal that GABAAδ receptors are localized to
neurogliaform cells preferentially among cortical interneurons. Neurosteroids and ethanol, which are modulators of GABA\textsubscript{δ} receptors, alter unitary GABAergic effects between neurogliaform cells. Transmitters released by unitary volume transmission might reach non-neuronal elements of the cortex. Our preliminary experiments monitoring Ca\textsuperscript{2+} signals in the network during multiple patch clamp recordings show unprecedented effects for GABAergic neurons: spikes of individual neurogliaform cells trigger simple Ca\textsuperscript{2+} responses simultaneously in several astrocytes within the axonal field of a neurogliaform cell followed by longer lasting Ca\textsuperscript{2+} oscillations around blood vessels. In contrast to the specifically placed synapses formed by other interneurons, the output of neurosteroid and ethanol sensitive neurogliaform cells represents the ultimate form of spatial unspecificity in GABAergic systems leading to long lasting network hyperpolarization combined with widespread suppression of communication in the local circuit. We propose that the spatial unspecificity of neurotransmitter action leads to unprecedented functional capabilities for a single neuron simultaneously acting on neuronal, glial and vascular components of the surrounding area.
Topological Basis for Spike Synchrony in \textit{ex vivo} Neuronal Networks

Naoya Takahashi\textsuperscript{1}, Takuya Sasaki\textsuperscript{1}, Norio Matsuki\textsuperscript{1}, and Yuji Ikegaya\textsuperscript{1,2}

\textsuperscript{1} Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan; \textsuperscript{2} Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, Kawaguchi, Japan

Spike synchronization underlies information processing and storage in the brain. But how can neurons synchronize in noisy networks? By exploiting a high-speed multineuron imaging technique and a large-scale synapse mapping method, we directly compared spontaneous activity patterns and anatomical connectivity in hippocampal CA3 networks \textit{ex vivo}. As compared to non-synaptic pairs, synaptically coupled neurons shared more common presynaptic neurons, received more correlated excitatory synaptic inputs, and emitted more synchronized spikes. Importantly, common presynaptic parents \textit{per se} synchronized strongly more than unshared upstream neurons. Dynamic-clamp simulations revealed that common presynaptic inputs alone could not account for the realistic degree of synchronization unless the presynaptic spikes were synchronized. On a macroscopic scale, network activity exhibited a power-law scaling of synchronization, which engaged varying sets of highly synchronized and densely inter-wired neuron groups. Thus, locally coherent neuronal activity converges on specific cell assemblies, thereby yielding complex ensemble dynamics. The segmental synchronization may serve as information modules that flow in associative and parallel network channels.
Optical Methods for Revealing the Synaptic Function and Structure in the Local Circuit

Masanori Matsuzaki$^{1,2}$, Graham C.R. Ellis-Davies$^3$, Srinivas Kantevari$^3$, Yuya Kanemoto$^1$, Tatsuya Hayama$^1$, and Haruo Kasai$^1$

$^1$Graduate School of Medicine, University of Tokyo, Tokyo, Japan;
$^2$PRESTO and CREST, Japan Science and Technology Agency, Saitama, Japan;
$^3$Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, USA

Two-photon (2P) excitation is a method that has revolutionized many areas of biological science as it enables three-dimensionally defined excitation of chromophores in biological tissue. We have developed 2P uncaging methods to reveal the microarchitecture of synaptic connections at a level of single synapses. Recently, we synthesized two novel caged-GABA compounds. 2P excitation of the first caged GABA produced rapid activation of GABAergic currents in neurons in brain slices with an axial resolution of approximately 2 µm and enabled high-resolution functional mapping of GABA-A receptors. The other caged GABA, when combined with an appropriate caged glutamate, allowed bimodal control of neuronal membrane potential with subcellular resolution using optically independent 2P uncaging of each neurotransmitter. We used two-color, 2P uncaging to fire and block action potentials from rat hippocampal CA 1 neurons in brain slices with 720-nm and 830-nm light, respectively. In addition, we have combined 2P uncaging of glutamate over a broad area with 2P calcium imaging in a narrow region. The former was used for systematic activation of layer 2/3 pyramidal cells in the rat motor cortex, while the latter was used to detect the dendritic spines of layer 5 pyramidal cells that were innervated by some of the photoactivated cells. This technique allowed identification of various sizes of innervating spine located <140 µm laterally from the postsynaptic soma. Our new methods will be powerful tools for clarifying the microarchitecture of synaptic connections, including the positional and structural characteristics of excitatory and inhibitory synapses.
References:
Visual Deprivation During a Critical Period Selectively Suppresses L5 Pyramidal Neuron Excitability by Modulating the Induction of Intrinsic Plasticity

Kiran Nataraj, Sandrine LeFort, Marc Nahmani, and Gina Turrigiano

Dept of biology MS 08, Brandeis University, Waltham, MA 02454

Sensory cortex is highly sensitive to disruption by sensory deprivation during an early critical period (CP). In visual cortex monocular deprivation (MD) reduces the ability of the deprived eye to activate visual cortex, but the underlying cellular plasticity mechanisms are incompletely understood. Here we show that MD selectively reduces the intrinsic excitability of layer 5 (L5) pyramidal neurons, a major output pathway of cortex. Deprived neurons were less excitable, and when driven to fire at high frequencies had enhanced long-term potentiation of intrinsic excitability (LTP-IE). These excitability changes occurred through endocytosis-dependent changes in a persistent $K_v$ current, were tightly correlated with the classical visual system CP, and (like the functional effects of MD) could be rapidly reversed when vision was restored. These data suggest that LTP-IE plays an important role in the activity-dependent refinement of visual cortical circuits, by regulating the excitability of a major output pathway of cortex.
Inhibitory interneurons are famously diverse, consisting of many subtypes with different morphologies, molecular identities and neurophysiological fingerprints. One study estimated the number of subtypes at sixteen in the CA1 region of the hippocampus alone [1]. In order to study a molecularly identified population of hippocampal interneurons, we obtained a BAC transgenic mouse expressing EGFP under the control of the gene for the 5-HT 5B receptor (Htr5b) [2]. A population of EGFP-positive interneurons with diverse anatomical properties but unique physiological properties was identified in the CA1 region of these mice. Labeled interneurons were present in all CA1 subfields, but we focused our study on those with cell bodies near the border of stratum radiatum and stratum lacunosum-moleculare.

In whole-cell recording from hippocampal slices, labeled interneurons exhibited a remarkable pattern of persistent firing following the delivery of multiple depolarizing current steps in the soma. Persistent firing lasted an average of about one minute, but continued for several minutes in many cells (max. 13 minutes). The number of action potentials required to evoke persistent firing was variable, and depended on the duration of each current injection and the time between current injections. Higher frequencies of firing were generally more effective, and repeated firing was more effective than epochs of firing interrupted by seconds-long periods with no stimulation. Firing patterns recorded from hippocampal interneurons in vivo evoked persistent firing very effectively.

During persistent firing, the action potentials arose rapidly from the holding potential, even when the somatic recording was hyperpolarized below the usual resting potential of about -66 mV. The persistent action potentials resembled antidromic spikes in this regard. Hyperpolarization of the soma sometimes resulted in the appearance of spikelets, which were also observed during somatic hyperpolarization and antidromic stimulation of the
axon. Computational modeling suggested that the spikelets observed under these conditions are likely to be axonal action potentials that fail to actively propagate to the soma. Furthermore, the model indicated that variability in spikelet amplitude between cells is likely due to failure of antidromic action potentials at different electrotonic distances from the soma.

Persistent firing could be evoked by repetitive antidromic stimulation of the axon, even when the soma was hyperpolarized to prevent full-sized action potentials from propagating to the soma. In paired recordings from EGFP-positive interneurons, current injection into one neuron sometimes (3/19 pairs) evoked persistent firing in the second neuron, despite the absence of direct electrotonic coupling of subthreshold membrane potential.

Together, these observations suggest that action potential firing leads to a slow and cumulative process that triggers spontaneous persistent firing in the axon of some inhibitory interneurons in the hippocampus. The resulting action potentials appear to be generated in the axon, far enough from the soma that their initiation is resistant to hyperpolarization of the soma. Our results suggest that a network of inhibitory interneurons, coupled via as of yet unidentified axo-axonal interactions, can fire persistently in their axons, independent of any depolarization of the soma or dendrites.

References:
The neocortex has a large variety of neuronal types. If a small functional unit composed of specific neuronal types is repeated in the circuit, analysis of single repeats would contribute to the understanding of the whole neocortical circuits. We found that the arrangement of subcerebral projection neurons (SCPNs), a major type of pyramidal neurons in layer V, is not random, but has a significant periodicity in the tangential direction. The wavelength of the periodicity was typically 30-35 µm and much narrower than the widths of cortical columns (200-500 µm). The periodic organization was found in multiple cortical areas including the somatosensory and the visual cortices. We examined functional significance of the repeated structure by investigating whether neurons within single repeats have related functions. For this purpose we analyzed the repeated structure in the binocular visual area, where sensitivity to single eye stimulation is varied among neurons. Analysis of their activities by measuring cFos expression revealed that sensitivity to single eye stimulation is similar among neurons within the same repeats, but not similar among adjacent repeats, indicating that neurons in single repeats have related functions. Our results therefore suggest that layer V has micro-periodic functional organization in which a small functional unit composed of SCPNs is periodically repeated.
Dendritic GABA\textsubscript{B} Inhibition Decreases Neuronal Output in vivo

Lucy M. Palmer, Masanori Murayama, Sean C. Murphy and Matthew E. Larkum

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The dendrites of layer 5 pyramidal neurons are strongly inhibited by cortical interneurons. Dendritic inhibition has two different time courses; short (up to 150 ms) GABA\textsubscript{A} mediated and long (up to 500 ms) GABA\textsubscript{B} mediated inhibition. Although inhibition has drastic effects on local dendritic activity, the effect on the output of the neuron is unknown. We investigated dendritic inhibition in layer 5 (L5) pyramidal neurons in the hindlimb somatosensory cortex in vivo using both population and single-cell calcium imaging and patch-clamp electrophysiology. Electrical contralateral hindlimb stimulation generates a two-phase EPSP in the dendrite that can lead to an increase in action potential firing in the soma (spontaneous 0.9±0.3 Hz; evoked 4.8±1.0 Hz; n=13). This response is modulated by interhemispheric input as paired stimulation of the ipsilateral hindpaw 400ms before contralateral hindpaw stimulation causes a significant decrease in both the population fluorescence response recorded in the apical dendrite (by 36.3±0.8%) and in the evoked firing rate recorded in the soma (control 4.8±1.0 Hz; paired, 3.1±0.7 Hz; n = 13). Due to the temporal scale of inhibition and the fact that the decrease in L5 pyramidal neuron firing was replicated by the local application of GABA\textsubscript{B} antagonist Baclofen (50 µM) in layers 2/3 during contralateral hindlimb stimulation (control, 1.0±0.4Hz; baclofen, 0.3±0.1Hz; n=7), this interhemispheric modulation of L5 action potential firing is largely mediated by dendritic GABA\textsubscript{B} inhibition. Furthermore, when postsynaptic GABA\textsubscript{B} receptors are absent interhemispheric input did not inhibit action potential firing (control 7.6±1.8 Hz; paired 8.7±1.9 Hz, n=7) and the application of Baclofen to the upper layers also did not alter firing (control 9.0±2.3 Hz; baclofen, 12.0±4.5 Hz, n=5; GABA\textsubscript{B}1b KO). In contrast, L5 pyramidal neurons which were lacking presynaptic GABA\textsubscript{B} receptors still resulted in a significant decrease in evoked firing during interhemispheric input (control, 12.2±4.5 Hz, paired, 8.5±5.0 Hz, n=3; GABA\textsubscript{B}1a KO). These results not only illustrate GABA\textsubscript{B} mediated dendritic inhibition in vivo but also imply a causal relationship of dendritic
activity on action potential generation in cortical L5 pyramidal neurons.
Synaptic Integration in Tuft Dendrites of Layer 5 Pyramidal Neurons: an NMDA Spike Story

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Tuft dendrites are the main recipients of long range feedback connections thus are thought to be important for top down processing of information in the cortical column. In this talk I will present data from our direct recordings using scanning Dodt contrast and two photon imaging in the fine tuft dendrites all the way up to layer 1. The integrative properties of tuft dendrites including the size and attenuation of basic synaptic events, active and passive characteristics will be described including calcium, sodium and NMDA spikes. Using our experimental and modeling data a model for the role of NMDA spikes and their possible importance will be described.
Dendritic Properties of Neocortical Pyramidal Neurons – What Have We Learnt from Dendritic Patch Recordings?

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The advent of targeted, direct and multiple dendritic patch recordings from neurons in brain slices signaled the beginning of a new phase for the understanding of signal processing in neurons. Pyramidal neurons remain the most studied for dendritic recordings partly because of their central importance in the cortical circuit and the fascinating diversity of their dendritic properties but also because of the sheer size of their apical shaft dendrites. As time progresses, smaller and smaller dendrites become accessible to direct dendritic recordings making it possible to record from smaller structures. I will present data from recent projects showing the dendritic properties of L2/3 and L6 neurons as well as from the basal and tuft dendrites of L5 pyramidal neurons. Each of these studies involved multiple patch recordings from the somata and dendrites of neocortical pyramidal neurons in the somatosensory cortex of rats. From this work it has become clear that all pyramidal neurons of the neocortex have active dendrites including mechanisms for the generation of sodium, calcium and NMDA spikes. NMDA spikes appear to be confined to the thinnest dendrites in the basal and tuft dendritic trees. Furthermore, specific mechanisms for the block of local spike electrogenesis are also found in all layers of the cortex.

With the newest available data it is finally possible to put together an overview of the signaling properties of the whole dendritic tree in pyramidal neurons from every layer in which they are found. This knowledge also has to be integrated into the larger picture of the cortical circuit in which each pyramidal neuron receives specific inputs with their basal tuft dendrites in different layers. Lastly, both the anatomical and physiological information needs to be put into a framework for understanding the functional role of pyramidal neurons throughout the cortex.
Dendritic Activity In Awake Animals

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Very little is known about dendritic activity in living animals and even less about sensory processing and its relationship to behavior. Here, we measured dendritic calcium activity in layer 5 (L5) pyramidal neuron in the sensorimotor cortex of awake and anesthetized rats following sensory stimulation by using a fiberoptic imaging method (Murayama et al., *J Neurophysiol.*, 2007; Murayama & Larkum, *Nat. Protoc.*, 2009). Dendrites of L5 pyramidal cells in somatosensory area were bolus-loaded with Ca\(^{2+}\)-sensitive dye. Hind limb stimulation evoked bi-phasic (fast and slow) dendritic responses. The strength of sensory stimulation was encoded in the fast dendritic calcium response of a local population of L5 pyramidal cells in a graded manner. Using various forms of pharmacological interventions we could show that the slope of the stimulus–response function was under the control of a particular subset of inhibitory neurons (Martinotti cell) activated by synaptic inputs predominantly in L5. Recordings from single apical tuft dendrites *in vitro* showed that activity in L5 pyramidal neurons disynaptically coupled via interneurons directly blocks the initiation of dendritic calcium spikes in neighbouring pyramidal neurons. A model of the results revealed that the microcircuit is organized so that local populations of apical dendrites can adaptively encode bottom-up sensory stimuli linearly across their full dynamic range. (Murayama et al., *Nature*, 2009).

In the awake state, there was a prominent slow, delayed response whose integral was ~14 fold larger than in the anesthetized state. These changes were confined to layer 5 pyramidal dendrites and were not reflected in the activity of layer 2/3 neurons in general. Inactivating a higher cortical, supplementary motor area (SMA) with tetrodotoxin suppressed dendritic activity as did transecting the region between the two cortical areas. Conversely, stimulation of the supplementary motor area resulted in increases in dendritic activity. Anterogradely labeling of L5 neurons with a neural tracer in the SMA showed corticocortical projections to primary sensory area. These
physiological and anatomical findings demonstrate the SMA drives the large dendritic activity in sensory area. We conclude that during the awake state, top-down connections control the duration of dendritic activity which is functionally related to behavior (Murayama & Larkum, *PNAS*, 2009).
Asynchronous Release of GABAergic Synapses in Neocortex

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The neocortex is composed of many types of neurons, including numerous pyramidal cells (PC) and various types of interneurons. Different kinds of interneurons have different intrinsic properties and play distinct roles in the central nervous system. In this study, through dual or triple whole-cell recording in acutely isolated slices from rat prefrontal cortex, we demonstrated that GABAergic transmission from the inhibitory fast-spiking (FS) interneuron was highly asynchronous in response to high-frequency stimulation. The amount of asynchronous release was dependent on the frequency and the number of action potentials in FS interneurons. These features were quite universal in GABAergic synapses made by FS interneurons, including FS-PC synapses, FS-FS synapses, and FS autapses. Since we obtained similar results using perforated patch recording technique, the asynchronous release may take place under physiological condition. Further experiments revealed that the asynchronous release was dependent on the calcium dynamics after high-frequency firing because bath application of EGTA-AM could block the asynchronous release. Consistently, we also observed asynchronous release in FS synapses in human cortical slices obtained from patients with epileptic seizures. Together, these results suggested that the asynchronous release was a common feature for FS synapses and might contribute to the maintenance of excitation-inhibition balance and cortical information processing.
Membrane Potential-Dependent Modulation of Recurrent Inhibition in Neocortex

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A dynamic balance of excitation and inhibition is crucial for network stability and cortical processing, but it is unclear how this balance is achieved at different membrane potential ($V_m$) states, e.g., during persistent activity or slow $V_m$ oscillation. It has been shown that cortical excitatory neurons communicate not only through the generation of all-or-none action potentials (digital mode) but also through a presynaptic $V_m$-dependent modulation of transmitter release (analog mode)$^{1,2}$. Considering that interneurons within cortical microcircuits are driven by excitatory neurons, leading to recurrent inhibition$^{3,4}$, we hypothesized that the amount of recurrent inhibition might be subjected to modulation in a manner that depends on the state of depolarization of the excitatory neuron. Whole-cell recording from paired layer-5 pyramidal cells (PCs) in rat somatosensory cortical slices revealed that the amplitude of disynaptic IPSPs mediated by low-threshold spiking and fast spiking interneurons were increased in a manner directly proportional to the level of depolarization of the presynaptic PC. Further PC-interneuron pair recording showed that this modulation was attributed to PC depolarization-induced increase of EPSP amplitudes (analog communication) that elevated interneuronal firing and inhibition of neighboring PCs. Thus, an immediate $V_m$ state-dependent modulation of recurrent inhibition dynamically maintains excitation-inhibition balance of local circuits and thereby contributes to cortical information processing.

References:


Physiological Genomics of Cortical Circuits in Health and Disease

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Understanding the structure and function of neural circuits requires proper identification of the neural cell types that comprise them. Although in some structures, like the vertebrate retina, there is broad agreement on the component cell types, in other brain structures, like the cerebral cortex such agreement is lacking. We have combined anatomy, electrophysiology and gene expression studies to try to understand the taxonomy of cell types within the mouse neocortex and other related structures. Using this approach we have tried to understand how specific cellular phenotypes develop, how they are specialized across cortical regions, and how they are altered in the developmental disorder Rett Syndrome. I will also describe ongoing efforts to develop strains of mice and reagents capable of providing genetic access to identified neuronal cell types.
Why do Neurons have Spines?

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Most excitatory cortical neurons receive glutamatergic excitatory input onto specialized neuronal processes called dendritic spines. Dendritic spines are complex structures, and malformation of spines is characteristic of many disease states associated with decreased cognition. What advantage do spines provide spiny neurons? And why are spines exclusively associated with excitatory glutamatergic synaptic transmission? In the absence of spines, the amplitude and kinetics of excitatory postsynaptic potentials (EPSPs) at the site of synaptic input are highly variable and dependent on the local dendritic geometry. Here we demonstrate a fundamental biophysical attribute of spines that limits location-dependent variability in EPSP properties at the site of synaptic input. In simplified and reconstructed neuronal morphologies, simulated excitatory synaptic conductances onto spines generate local (within spine-head) EPSPs with limited location-dependent variability in amplitude, peak latency, and half-width. The same synaptic conductances onto dendritic shafts generate local (within shaft) EPSPs whose amplitude and shape are highly location-dependent. The impact of spines on local EPSP properties is independent of synaptic conductance, but is negatively correlated with spine neck resistance. We propose that one function of spines is to standardize the amplitude and kinetics of local EPSPs, making them less dependent on synapse location within the dendritic tree. A consequence of spine-dependent EPSP standardization is that local voltage-dependent processes will be more uniformly activated at all synaptic locations. Indeed, we found that spines limited location-dependent variability in NMDA receptor activation and standardized AMPA/NMDA ratios. Because NMDA receptors and other voltage-sensitive process underly many forms of synaptic plasticity, the ability of spines to standardize local EPSP properties may allow neurons to utilize similar post-synaptic molecular mechanisms at all synaptic locations. These advantages
may explain why spines occur almost exclusively at glutamatergic synapses showing dynamic, Hebbian-like plasticity.
Dendritic Dimensions and Signal Conduction Properties of Cortical Nonpyramidal Cells

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Neurons receive thousands of synaptic inputs onto their dendrites and soma, and spatially and temporally integrate these inputs to produce appropriate output in the form of action potentials generated in axons. The morphology of dendrites can influence the integration of synaptic input, as well as effect the pattern of action potentials generated by suprathreshold stimuli. Using three-dimensional reconstructions from light and electron microscopic observations, we quantified the morphologies of the dendritic trees of four cortical interneuron subtypes present in the rat frontal cortex: Martinotti cells, fast spiking basket cells, double bouquet neurons, and large-basket neurons. Our ultrastructural data reveal four conserved principles governing the dendritic dimensions of these neurons. First, the cross-sectional area at any given point within a dendrite is proportional to the summed length of distally located dendrites beyond it, including all subsequent dendritic branches. Second, the total cross-sectional area is conserved at dendritic bifurcation points. Third, dendritic cross-sections are typically irregular ellipsoids rather than circles. Finally, in all neurons we found branch diameters consistent with Rall's conductance matching assumption for dendritic bifurcations. These conserved features may facilitate even distribution of cellular components, as well as
somatic depolarization, into all compartments of the dendritic tree, and may also limit the
effects of dendritic topology on action potential generation.
Dendritic Computation

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The computational power of dendrites has long been predicted using modelling approaches, but actual experimental examples of how dendrites solve computational problems are rare. We have combined patch-clamp recordings with two photon imaging and glutamate uncaging to demonstrate that cortical pyramidal neurons can discriminate spatiotemporal sequences of synaptic inputs along single dendrites. This provides a dendritic mechanism for pyramidal neurons to compute direction and velocity, and shows how dendrites can be used to decode spatiotemporal patterns of input.
Spatial Noise and Cortical Functions: Potential Functional Roles of the Inhomogeneity across Dendritic Branches of Cortical Pyramidal Cells

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The shape of the neuronal input-output relationship (I-O curve), namely, the dependence of the neuronal firing rate on the strength of the input has significant impacts on the dynamics of the neural circuit. In the cortex of behaving animals, input to a pyramidal cell is thought to be accompanied with temporal irregularity, called temporal noise, and in vitro studies have shown that the temporal noise can smoothen the initial part of the I-O curve (Shu Y et al., 2003, J Neurosci). It has been suggested that such a smoothening may play important roles in several cognitive functions executed by cortical circuits. Specifically, the smoothening of the I-O curve may underlie the contrast-invariant orientation tuning of the neurons in the visual cortex (Anderson J et al., 2000, Science). It may also implement a multiplicative operation (Hansel D & van Vreeswijk C, 2002, J Neurosci; Murphy BK & Miller KD, 2003, J Neurosci), which is hypothesized to be used in sensori-motor coordinate transformation as well as in top-down attentional control (Ardid S et al., 2007, J Neurosci).

Similar to the existence of the temporal noise, input to cortical neuron in vivo is considered to be also accompanied with spatial irregularity, namely, inhomogeneity of the local input across dendritic branches. Moreover, it was shown that the property of the branch itself can be significantly variable (Losonczy A et al., 2008, Nature). Here I propose that such a spatial inhomogeneity, referred to as spatial noise, can potentially play comparable or complementary roles to the temporal noise in the cortical functions. In particular, I show by computational modeling that the spatial noise can endow a recurrent neural circuit with an ability to respond to an external input in an intensity-insensitive manner. Moreover, I also show that well-organized input-inhomogeneity across branches shaped through the dendritic clustered plasticity rule can effectively operate as spatial "noise". This might be homologous to a notion (Buzsáki G, 2006, "Rhythms of the Brain") that oscillations, well-organized temporal
fluctuations, can effectively work as noise so as to enhance the detectability of weak signal, thereby suggesting that the long-lasting argument on the neural coding, i.e., whether irregularity represents signal or noise (Mainen ZF & Sejnowski TJ, 1995, Science; Shadlen MN & Newsome WT, 1998, J Neurosci), can also be applied to the spatial domain.

References:
Dendritic Plateau Potentials Sustain Noise-induced Multistability in Neuronal Network Model

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Dendritic plateau potential is a form of dendritic spikes commonly found in dendrites of pyramidal neurons [1, 2]. The potential is characterized by nonlinearity, locality and long duration. In the present study, we investigate the effect of dendritic plateau potentials on the network dynamics of a neuronal circuit. We propose a neuronal field model that takes an effect of dendritic plateau potential into account. Like in typical cortical models [3], we observe spatially localized activity (“bump”) in our model, which can be interpreted as an attending point if the model represents the visual cortex, or interpreted as a memory trace if it represents a brain area storing memory.

Our simulations with constant background inputs demonstrate the localized network activity spontaneously moving and running away, irrespective of the presence of the dendritic plateau potential. The runaway activity can be regarded “exploration” in the visual space or “wondering thought” depending on the context. Our simulations with noisy background inputs demonstrates the localized network activity that stands still when the duration of dendritic plateau potential is set to be long enough, which can be interpreted as “fixation” in the visual space or “retrieved memory” depending on the context (figure 1). No stable activity is formed when the duration of dendritic potential is too short.

Interestingly, the runaway activity under constant input with a long-lasting dendritic plateau can be converted into standing cluster by a noise increase in the input following temporal inhibition to somata, which is related to the long duration of dendritic plateau potential. In contrast, the simple increase of noise could not work. These observations suggest the existence of hysteresis effect and it was confirmed with additional analysis (figure 2).

The effect observed here goes beyond the previously proposed mechanism [4], where the localized activity was immobilized as a result of the degeneration of two forms of
activity running in the opposite direction. Our effect represents noise-induced multistability, where the localized activity can be either as the stationary activity or the runaway activity. Our results provide the insight into the mechanism for activity control in the cortical circuits caused by dendritic nonlinearity.

References:
“Dark Computation” in the Neocortex: How the Computing Functions of Pyramidal Neuron Dendrites may depend on 'Invisible' Parameters

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Both experimental data and modeling studies over the past 25 years support the view that dendrites contribute substantially to the computing functions of CNS neurons. A guiding concept in the realm of dendritic computation has been that of a computational "subunit" which combines two ideas proposed by Christof Koch in the 1980's, that (1) dendrites tend to compartmentalize voltage signals within separate dendritic subregions, thus defining groups of synapses that interact with each other "as a unit", and (2) the idea that nonlinear membrane mechanisms can effect logical, algebraic, and/or temporal computations that occur in parallel with computations ongoing in other subunits. In the past few years, we have begun to explore within-subunit effects in the thin dendrites of pyramidal neurons both in computer models and brain slice experiments in collaboration with Jackie Schiller's lab, and have found that differences in the absolute and relative locations of excitatory and inhibitory synapses within a dendrite, and in the relative amounts of AMPA vs. NMDA conductance expressed by different groups of synapses, can lead to profoundly different subunit computations. In this talk, the biophysical basis of a variety of within-subunit computations will be discussed, including those that may underlie nonlinear contextual and attentional modulation effects, divisive vs. subtractive normalization, and temporal order sensitivity.
Excitation and Inhibition in Cortical Space

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In the cerebral cortex the flow of information is continuously processed by ensembles of neurons arranged along two major functional cortical subdivisions namely layers and columns. Layers and columns span the tangential and radial dimension of the cortical sheet and are interconnected via axonal projections that ensure the coordination of neuronal activity along these two orthogonal dimensions. While this pattern of connectivity sets anatomical constraints on signal propagation across layer and columns, it alone does not determine the flow of neuronal activity through cortical circuits. Indeed, the relationship between excitation and inhibition in cortical space is just as crucial in directing the flow of information. We address the logic by which the specific spatial relationship between excitation and inhibition impacts the routing of information across layers and columns. We find a remarkably precise spatial overlap of the two opposing conductances across these two cortical dimensions. Despite this overlap, however, layer specific differences in the ratio between excitation inhibition channel information flow by generating lateral suppression and feed-forward facilitation. Thus, finely tuned spatial match between excitation and inhibition precisely orchestrates signal propagation across two main cortical subdivision, layers and columns.
Synaptic Mechanism of Information Transmissions in Low-frequency Asynchronous Firing of Cortical Networks

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In vivo cortical neurons typically exhibit irregular asynchronous firing at relatively low rates, and reverberating synaptic input is considered to generate such persistent firing. However, the mechanism to retain the stable low-frequency persistent firing remains elusive. In this presentation, we demonstrate in a computational model that a neuronal network can stably generate low-frequency asynchronous firing when the weights of recurrent excitatory synapses are distributed according to a distribution with a long tail, typically a log normal of distribution\(^1\). In this weight distribution, only a few synapses can be strong on a single neuron, while the remaining synapses should be very weak. Interestingly, a few very strong synapses may generate an EPSP that is large enough to induce firing of a postsynaptic cell and transmit information about both rate and timing of the presynaptic spike trains mediated by these synapses. Thus, our model also accounts for precisely-timed spike sequences reported previously in some experiments. We show that the information transmission is maximized when weak synapses retain the average subthreshold membrane potential about 10 mV below the firing threshold. Furthermore, we propose a class of multiplicative spike-timing-dependent plasticity rules\(^2\) that can generate a log normal distribution of synaptic weights.

References:
IS–15

Somatic and Dendritic Inhibition in Cortical Pyramidal Neurons

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I will discuss how somatic and dendritic inhibition can impact on information processing in cortical layer 5 pyramidal neurons. The primary inhibitory neurotransmitter in the brain is GABA, which acts at ionotropic GABA-A and metabotropic GABA-B receptors. GABA-A receptors directly activate a chloride conductance, whereas GABA-B receptors either activate a potassium conductance or down-regulate voltage-activated calcium channels. In the first half of my talk I will focus on the interaction of action potentials (APs) with somatic and dendritic GABA-A mediated inhibition. This work indicates that somatic GABA-A inhibitory postsynaptic potentials (IPSPs) can be substantially reduced by appropriately timed APs due to “shunting” via the large conductance changes that occur in the axon during AP generation. In contrast, dendritic IPSPs could be boosted by APs due to an increase in driving force for inhibitory current flow during the backpropagating AP. In the second half of my talk I will focus on somatic and dendritic GABA-B mediated inhibition. Somatic GABA-B receptor activation led to a hyperpolarization of the resting membrane potential and a decrease in input resistance via a barium-sensitive potassium conductance. In contrast, dendritic GABA-B receptor activation had no effect on dendritic membrane properties, but could block dendritic calcium electrogenesis evoked by high-frequency action potential trains. This effect was mediated by a down-regulation of dendritic calcium channels. Together, these findings indicate that GABA-A and GABA-B receptor-mediated inhibition acts to modulate excitability of layer 5 pyramidal neurons via different, location-dependent mechanisms.
Mechanisms Underlying the Fast Synaptic Output of GABAergic Interneurons

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Fast-spiking, parvalbumin-expressing GABAergic interneurons play a key role in the function of neuronal networks. They mediate fast feedforward and feedback inhibition and are involved in the generation of oscillatory activity in the brain. For all of these functions, the rapid transmitter release at the outputs synapses of these interneurons is critically important. However, the mechanisms underlying rapid transmission are insufficiently understood. We addressed these questions at the basket cell – granule cell synapse in the dentate gyrus.

To examine whether tight coupling between Ca^{2+} channels and exocytotic Ca^{2+} sensors contributes to rapid transmission, we mapped the distance by introducing exogenous Ca^{2+} chelators, combining paired recording and patch pipette perfusion. GABA release at basket cell-granule cell synapses was sensitive to millimolar concentrations of the fast Ca^{2+} chelator BAPTA, but insensitive to the slow Ca^{2+} chelator EGTA. Quantitative modeling indicates that Ca^{2+} source and Ca^{2+} sensor are tightly coupled at this synapse, with distances in the range of 10 – 20 nm.

The tight coupling between Ca^{2+} channels and Ca^{2+} sensors places constraints on the number of Ca^{2+} channels involved in synaptic transmission. To determine the number of open Ca^{2+} channels necessary for transmitter release at basket cell output synapses, we combined presynaptic Ca^{2+} imaging and paired recording. Our results suggest that the opening of three or fewer Ca^{2+} channels triggers transmitter release. Furthermore, a small number of Ca^{2+} channels can evoke release with high temporal precision, despite stochastic Ca^{2+} channel opening.

In conclusion, several factors contribute to rapid exocytosis at the output synapses of fast-spiking, parvalbumin-expressing GABAergic interneurons. These factors contribute to fast feedforward and feedback inhibition in hippocampal microcircuits.
References:
Abstract
Poster
Bidirectional Feed-Forward Control in the Local Collateral Circuit of the Hippocampal-Prefrontal Pathway Involved in Working Memory

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Anatomical evidence suggests that intermediate hippocampal afferents collaterally innervate excitatory pyramidal neurons and inhibitory interneurons, forming a triangular local microcircuit in the medial prefrontal cortex in rats\textsuperscript{1}. The intermediate hippocampal route specifically processes working memory on the order of seconds as assessed by delayed alternating task\textsuperscript{2} and the ventral hippocampal route does on the order of minutes\textsuperscript{3}. To compare these routes, we examined paired-pulse responses across varied stimulus intensities (0.04, 0.08, 0.16, 0.24, and 0.32 mA) and interstimulus intervals (ISIs; 25, 50, and 100 ms) and tested the effect of long-term potentiation (LTP) under urethane anesthesia\textsuperscript{4}. The intermediate route showed paired-pulse changes as facilitation-like feed-forward activation at lower intensities and depression-like feed-forward inhibition at higher intensities. The bidirectional feed-forward control, which GABA receptor system underlies\textsuperscript{5}, was observed at each ISI and paired-pulse responses were higher at longer ISIs. LTP efficiently shifted the single pulse strength-response relationship and increased paired-pulse changes at 25 ms ISI. In the ventral route, the paired-pulse response varied with ISI rather than intensity, and increased at 50- and 100-ms ISI after LTP without shifting the single pulse strength-response relationship. We will discuss structural function of the local collateral circuit in the hippocampal-prefrontal pathway following additional analysis of relationship between spontaneous activities in hippocampus and prefrontal cortex \textit{in vivo}. 

65
References:
Axonal Bouton Distribution of Layer IV and VI Spiny Neurons over Functional Maps in the Cat Primary Visual Cortex.

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We investigated bouton distribution of layer IV and VI spiny neurons over orientation preference and ocular dominance maps using in vivo optical imaging, unit recordings, and single neuron reconstructions in the cat visual cortex, area 18. Since thalamocortical axons terminate within layer IV and VI, these neurons must take important roles for visual signal integration as the first stage of cortical circuitry. Our aim is to reveal relationship between horizontal connections of these neurons and functional architecture. On average, axons of layer IV neurons extended ~1.5 mm in horizontal radius (14 star pyramidal and 9 spiny stellate cells), suggesting contribution to long range connections. In the vicinity of the soma (<400 µm), boutons appeared chiefly at iso-orientations. However, at more distal regions, 7 cells preferentially projected to non-iso-orientation domains. Boutons of each cell were partitioned into distinct clusters (range: 1-15) based on the mean-shift algorithm. Preferred orientation was varied among individual clusters, as 57 clusters preferred iso-orientations and 43 clusters preferred cross-orientations. Each cluster showed sharp "tuning" in orientation preference. Unlike layer III/V pyramidal cells preferring chiefly iso-orientations, layer IV cells engaged with broad orientations because each bouton cluster from the same cell can show different orientation preference.

For layer VI cells, 23 spiny cells were reconstructed, which could be divided into three morphological types based on apical dendrite morphology. Each type also differentiated axonal distribution for both horizontal and vertical dimensions, and one type showed wider horizontal extent than others. In general, boutons of layer VI cells more often occupied iso-orientation domains than layer IV cells, especially in the wide extent axon type, although iso-orientation preference was weaker than layer III/V cells. We also examined bouton distribution over ocular dominance map. Layer VI cells tended to
innervate the domains representing the same ocular dominance more frequently than other layer cells.

In summary, (1) layer IV and VI cells innervate non-iso orientation domains more often than superficial layer cells, especially, layer IV cells showed strong cross-orientation preference; (2) layer VI cells seem to relate information flow for ocular dominance. These results suggest that two thalamic recipient layers differentially contribute visual signal integration in the primary visual cortex.
Projection Type-Specific Temporal Synaptic Characteristics in Frontal Cortex

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3 JST, CREST, Okazaki, Japan

The neocortical local circuit is now considered to consist of multiple subnetworks formed by selective connection dependent on the pre- and postsynaptic neuron type specified by morphological, physiological and extracortical projection properties. However, the synaptic transmission and spatial relationship of connected neuron pairs remain to be investigated between the subnetworks.

Layer 5 of the frontal cortex has two major types of pyramidal cells different in extracortical targets: one sending axons to the pons (corticopontine cells) and the other innervate both sides of the striatum and not projecting to the brainstem (crossed corticostriatal cells). To reveal the synaptic transmission dependency on extracortical projection, we compared the synaptic property and dendritic arborization between connected corticopontine and connected crossed corticostriatal pairs, identified by retrograde labeling from the pons or the contralateral striatum in slice preparation. To investigate the dendritic property, we reconstructed the dendrites of connected pairs by Neurolucida.

We found that connection probability was similar, but the mean EPSC amplitude of corticopontine pairs was larger than crossed corticostriatal pairs. The short term plasticity of corticopontine pairs was more facilitatory than that of crossed corticostriatal pairs. The nearest distance between apical dendrites of connected neurons were shorter in corticopontine pairs than crossed corticostriatal ones.

These findings suggest that temporal synaptic characteristics and apical dendrite configurations are different between excitatory subnetworks formed on the extracortical projection difference.
Cell Diversity and Connections between Callosal Projection Neurons in the Frontal Cortex

Takeshi Otsuka and Yasuo Kawaguchi

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2 The Graduate University for Advanced Studies (SOKENDAI), Okazaki, Japan
3 JST, CREST, Japan

Recent advances have established that multiple subnetworks of synaptically coupled excitatory neurons provide distinct pathways for information flow through the cortical circuit. This excitatory subnetworks is now assumed to consist of functionally segregated channels corresponding to projection systems to subcortical areas. The cortex is, however, composed of two hemispheres. Disfunction of information transfer between cortical hemispheres by callosal transaction causes dramatic sensation and perceptual deficits, indicating the importance of information transfer and integration between cortical hemispheres. To understand how the cortex process the information to transfer to the other hemisphere, we investigated physiological and morphological properties of callosal projection neurons and local connections between them. Callosal projection neurons were identified by the injection of retrograde fluorescent tracer to the contralateral frontal cortex, and found in both supragranular and infragranular layers. We then obtained whole-cell recordings from labeled cells in the slice preparations. Our results suggest that callosal projection neurons are heterogeneous in morphological and physiological properties, and form subnetworks between them.

References:
Distribution Patterns of Cortico-Cortical and Thalamo-Cortical Inputs to Parvalbumin-Expressing GABAergic Neurons in Rat Frontal Cortex

Naoki Shigematsu\textsuperscript{1,3}, Yoshiyuki Kubota\textsuperscript{1,2,3} and Yasuo Kawaguchi\textsuperscript{1,2,3}

\textsuperscript{1} National Institute for Physiological Sciences, Okazaki, Japan
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\textsuperscript{3} JST, CREST, Japan

Parvalbumin-expressing neurons (PV neurons) account for large populations of neocortical GABAergic cells. Interaction between PV neurons and their excitatory inputs generates neocortical fast rhythms, probably important for the local circuit integration. However patterns of these excitatory inputs have been rarely studied. PV neurons receive excitations from two major sources, pyramidal and thalamo-cortical cells. To reveal the synaptic interaction differences of two input types, we compared these input densities between the somatic/dendritic domains or between the sublaminae of rat frontal cortex. Cortico-cortical terminals were visualized by immunohistochemical staining of vesicular glutamate transporter type 1 (VGluT1), and thalamo-cortical ones by that of type 2 (VGluT2). It has been established that these molecules localize in each excitatory axon terminal (bouton) separately. PV neurons were visualized by its immunolabeling, or Lucifer yellow injection into \textit{vicia villosa} agglutinin-positive cell, most likely PV neurons, in the fixed slice preparation. The bouton appositions were identified by the reconstructions of confocal laser scanning images. PV dendrites and somata had much more appositions to the terminal boutons positive for VGluT1 than to those for VGluT2 in individual layers. VGluT2 appositions to the somata were found mostly in layer 4, but much fewer to the layer 4 dendrites. These results suggest that PV neurons receive excitatory inputs from cortical pyramidal cells to both dendrites and somata abundantly in every layer, whereas the direct thalamo-cortical interaction with the PV population occurs exclusively at their somata of layer 4.
The Relationship between Corticocortical and Corticospinal Projections in Rat Secondary Motor Cortex

Yoshifumi Ueta¹,² and Yasuo Kawaguchi¹,²,³

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² The Graduate University for Advanced Studies (SOKENDAI), Okazaki, Japan;
³ JST, CREST, Japan

The rodent motor cortical area consists of two parts: caudal primary (M1) and rostral secondary motor cortex (M2). M1 and M2 are mutually connected and both project to the spinal cord. Since M2 is considered to be a higher area than M1, it would control spinal cord motor system through the corticospinal direct pathway and corticocortical projection to M1. We know little if anything about the relation of corticospinal and corticocortical pyramidal neurons and their local synaptic wiring. This knowledge would shed light on the cortical motor control system as well as organization rules of frontal regions.

We defined rat motor areas by intracortical microstimulation: limb or whisker movements were induced at much lower stimulus intensity in M1 than M2. The physiological distinctions of the two areas coincided well with the different immunoreactive patterns for neurofilament heavy subunit. We investigated corticospinal and corticocortical projection patterns using fluorescent retrograde tracers. First, we observed that corticospinal projection patterns were different between M1 and M2. Second, corticocortical projections from M2 to M1 were formed according to the M1 body movement map. Third, in M2, the projection neurons to M1 located in layer 2 to upper layer 5, whereas corticospinal neurons in deeper part of layer 5.

These results suggest the functional differences between corticospinal projection from M1 and M2, cortical output separation to the spinal cord and adjacent cortical areas, and some degree of preservation of M1 body movement map in higher areas of rats.
Firing Patterns of Neocortical and Thalamic Neurons in the Slow Wave

Mika Ushimaru\textsuperscript{1,2,3}, Maria Victoria Puig\textsuperscript{1} and Yasuo Kawaguchi\textsuperscript{1,2,3}

\textsuperscript{1} National Institute for Physiological Sciences, Okazaki, Japan;
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The electroencephalogram (EEG) during deep sleep exhibits slow wave (SW) synchronized oscillations which desynchronize when the animals are awake. Up-state depolarizations during SW are assumed to be generated within cortex and to propagate to thalamus, whereas spindle waves of 7 to 14 Hz are generated in thalamus and propagate to cortex. To understand the functional role of SW and spindle oscillations, and the mechanism of desynchronization (DS) from SW, we investigated the firing phase of cortical and thalamic neurons within these oscillations. In anesthetized rats, we identified SW and DS state using local field potential signal recorded from cortical layer 5. Simultaneously, we performed single unit recordings and identified corticothalamic (CTh) and crossed-corticostriatal (CCS) cells in layer 5 of frontal cortex by antidromic excitation. In thalamus, we identified neurons in three subregions using juxtacellular labeling and calbindin immunoreactivity: neurons in the ventro-lateral (VL) nucleus receiving excitation from the cerebellum, the ventro-anterior/medial nucleus (VA/VM) nucleus receiving inhibition from the basal ganglia, and the thalamic reticular (Rt) nucleus. We found that firing time within Up-states and firing frequency change from SW to DS differed among neuron subtypes. In addition, cortical and thalamic cells had distinct spindle firing phases. These results suggest that firing pattern dependency on the local oscillations as well as the global brain states is divergent not only between cortex and thalamus but also among neuron subtypes in individual regions.
Simple Experimental System For Mapping Inputs To Neocortical Layer 5 Subcerebral Projection Neurons

Shun Tsuruno¹, Hisato Maruoka¹, Rumi Kurokawa¹, Toshihiko Hosoya¹

¹RIKEN BSI, Wako, Japan

Subcerebral projection neurons (SCPNs) in layer 5 (L5) are the major output neurons of the neocortex. They receive their major interlayer excitatory inputs from layer 2/3 (L2/3) neurons. The connection between these two groups of neurons is important to understand the functional mechanism of neocortex and has been extensively studied. However, it has been technically difficult and time-consuming to assess their connectivity in large scale; it usually requires patch clamping a large number of pairs of neurons in the two layers. In addition, identification of SCPNs requires either assessment of their electrophysiological properties or retrograde tracing from subcerebral areas.

Here, we propose a less technical and less time-consuming method utilizing ChR2-assisted circuit mapping and transgenic mice with readily identifiable SCPNs. GFP was expressed in the transgenic mice under the control of the promoter of a SCPN marker. We confirmed that GFP was exclusively expressed in SCPNs in L5 with two methods: retrograde tracing from pons and simultaneously staining GFP with a SCPN marker. ChR2 was exclusively expressed in L2/3 excitatory neurons by in utero electroporation. Then we mapped the excitatory inputs to L5 SCPNs from L2/3 in acute brain slices by scanning the laser in L2/3 while recording EPSCs from GFP (+) neurons. The inputs from an area of 350 µm x 750 µm of L2/3 to a L5 SCPN could be mapped in less than 5 min. The spatial resolution of the map was improved by minimizing simultaneous spike induction in multiple L2/3 neurons. This was achieved by minimizing the laser power, reducing the density of ChR2 (+) neurons and excluding ChR2 from axons. This simple experimental system enables high-resolution mapping of functional inputs to L5 SCPNs with minimal cost.
Visual Stimulation-Induced Resetting of Membrane Voltage Responses of V1 Neurons in Awake Mice

Genki Minamisawa¹, Norio Matsuki¹ and Yuji Ikegaya¹,²

¹Grad School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan
²Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, Saitama, Japan

Most studies that addressed neuronal representation in primary sensory cortex have been carried out with anesthetized animals and have focused on supra-threshold responses. Here we investigated the sub-threshold dynamics of L2/3 neurons in primary visual cortex in awake, head-restricted mice (P18-22, male). Membrane voltage responses to full-field flashes (0.1 ms, 30 klx) were monitored in the whole-cell current-clamp configuration. Each response consisted of two components of depolarization, i.e., a fast transient component (latencies of 50-200 ms) and a slow sustained component. The latter was often prolonged to a couple of seconds, seemingly reflecting persistent activity in the neocortex. We compared the responses of the identical neurons between awake and urethane-anesthesia states and found that the response properties, such as the latency and amplitude of the response, were changed after induction of anesthesia. Thus, natural responses must be monitored in awake animals in order to determine the 'true' dynamics of the sensory cortex. We next applied paired flash stimuli with various inter-stimulus intervals (ISIs). Irrespective of ISI, the second stimulus in each paired flash triggered visual response that resembled the response elicited by a single stimulus. This indicates that the ongoing visual response to the first flash was reset by the subsequent second flash and restart from the beginning as would be the case if no preceding input was given.
Fluorescent Pipettes for Optically Targeted Patch-Clamp Recordings

Daisuke Ishikawa¹, Naoya Takahashi¹, Takuya Sasaki¹, Atsushi Usami¹, Norio Matsuki¹, and Yuji Ikegaya¹,²

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² PRESTO, Japan Science and Technology Agency, kawaguchi, Japan

Targeted patch-clamp recordings are a promising technique that can directly address the physiological properties of a specific neuron embedded in a neuronal network. Typically, neurons are visualized through fluorescent dyes or fluorescent proteins with fluorescence microscopy. After switching to transmitted light microscopy, neurons of interest are re-identified and visually approached in situ with patch-clamp pipettes. Here we introduce a simpler method for neuron targeting. With fluorophore-coated pipettes, fluorescently labeled neurons and the pipette tips are simultaneously imaged at the same fluorescence wavelength in the same microscope field, so that the neurons and even their neurites are targeted without suffering from chromatic aberration or mechanical complication in optics. We did not find that the coated fluorophores affected the electric properties of pipettes or neurons. The novel technique will be widely available for pipette micromanipulation under online visual control.
Statistical Estimation of Non-Uniform Dendritic Membrane Properties

Toshiaki Omori\textsuperscript{1,2}, Toru Aonishi\textsuperscript{3,2}, and Masato Okada\textsuperscript{1,2}

\textsuperscript{1}Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Japan
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\textsuperscript{3}Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology, Yokohama, Japan

Recent experimental findings such as dendritic spikes and backpropagating action potentials suggest that dendrite contributes more to neural computation than previously thought. Thus, elucidating dendritic computation is an important issue in both theoretical and experimental neurosciences [Häusser et al., 2000; Johnston et al., 2000; Häusser and Mel, 2003; Polsky et al., 2004; Larkum and Nevian, 2008; Spruston, 2008; Stuart et al., 2008]. A recent combined theoretical and experimental study suggested that membrane resistance is spatially segregated in the dendrite of hippocampal CA1 pyramidal neurons and that this segregated distribution may correspond to the segregation of synaptic inputs conveying sensory and memory information from the entorhinal cortex and the CA3, respectively, to the CA1 [Omori et al., 2006, 2009]. This suggests that the non-uniform membrane properties over the dendrite play a critical role in information processing closely related to local neural circuits. However, the non-uniform distribution of membrane properties over the dendrite, such as those of ion channel densities and the membrane resistance, remains unclear since it is difficult to directly measure membrane properties over dendrite; the spatiotemporal resolution in the recordings from dendrites is limited and data observable by imaging are noisy.

To address the difficulty in measuring this hidden but important feature of dendrite, we propose a statistical estimation algorithm based on data assimilation method [Omori et al., 2008]. We estimate the non-uniform distribution of membrane properties over dendrite from partially observable noisy data by using a distributed constant type Kalman filter and an expectation maximization algorithm. To evaluate the effectiveness of this method for imaging data, we investigate the dependency of the estimation
accuracy on the spatiotemporal resolution in the imaging experiments. We found using
simulated data that the non-uniform membrane properties over the dendrite can be
estimated even from partially observable noisy data recorded from dendrites.

This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas
“The study on the neural dynamics for understanding communication in terms of
complex hetero systems (Research Area No. 4103)” [No. 22120506 (T.O.)],
Grant-in-Aids for Scientific Research (C) [Nos. 20509001 (T.O.) and 20500201 (T.A.)],
a Grant-in-Aid for Scientific Research (A) [No. 20240020 (M.O.)], and a Grant-in-Aid
for Exploratory Research [No. 22650041 (M.O.)] of the Ministry of Education, Culture,
Sports, Science, and Technology, Japan.
Properties of Visually Evoked Responses in Binocular Primary Visual Cortex

Ikeda, K., Longordo, F. and Stuart, G.J.

Department of Neuroscience, The John Curtin School of Medical Research
Australian National University, Canberra ACT 0200, Australia

Sensory information received by the eyes is processed in the brain to form a single and coherent view of the outside world. To achieve this, input from the overlapping visual fields of the two eyes must be combined. This processing is first thought to occur at the level of the primary visual cortex. It is known that in rodents, as in higher mammals, visual information from the ipsilateral and contralateral eyes terminates in defined and separate areas in the thalamus before converging in the binocular region of primary visual cortex. However, how inputs from the two eyes are integrated at the single cell level is unclear. We investigated this issue by performing in vivo patch-clamp recordings from layer V pyramidal neurons in the binocular region of the visual cortex of adult C57BL/6J mice. First, we assessed differences between neuronal responses evoked by contralateral and ipsilateral eye stimulation by performing selective full-field retinal illumination using light emitting diodes. Analysis of subthreshold responses revealed that stimulation of the contralateral eye evokes a large excitatory postsynaptic potential (EPSP) followed by a smaller inhibitory postsynaptic potential (IPSP). A smaller and delayed EPSP was observed during stimulation of the ipsilateral eye. Notably, when both eyes were simultaneously stimulated, the amplitude of the evoked response was similar to that observed during activation of the contralateral eye alone. Comparison of EPSPs evoked by stimulation of both eyes with the expected linear sum of the responses generated following activation of each eye on its own indicates that the inputs were integrated sub-linearly. Next, we characterized how neurons in binocular visual cortex respond when the two eyes are activated by visual stimuli presented selectively in the binocular visual space. For this purpose visual stimuli were displayed on a monitor subtending – 60° X 60° of the visual field. Preliminary data show that the characteristics of neuronal responses are qualitatively different under these conditions. In particular,
they change as a function of the area of stimulated visual space. Increasing the stimulus area resulted in faster EPSP onset, larger EPSP amplitude and decreased EPSP half-width. Moreover, a smaller and delayed IPSP was evoked in response to stimulation of the entire binocular visual space. Future experiments will address the contribution of the contralateral and ipsilateral eye to these responses. This research will help to identify the underlying cellular mechanisms involved in the integration of binocular visual information in cortex.
Inhibitory interneurons play a critical role in the control of cortical excitation and network synchrony. This is achieved through reciprocal synaptic coupling, as well as dendritic gap junctions and dendritic transmitter release. These latter two processes require robust propagation of action potentials (APs) into the dendritic tree. Here we investigate the efficiency of AP backpropagation in cortical interneurons using voltage-sensitive dyes (VSD). This technique allows the direct recording of transmembrane potential simultaneously at multiple locations, which is difficult to achieve with conventional electrophysiological methods. We focus on cortical layer 2/3 bitufted interneurons, identified by their morphology, firing pattern in response to somatic current injection and somatic AP waveform. After identification, interneurons were filled with VSD (JPW1114) via a somatic recording pipette and fluorescent signals generated in response to APs were recorded at multiple dendritic regions up to 230 µm from the soma. The amplitude of AP signals at each dendritic location was calibrated by comparing the fluorescent response to hyperpolarizing steady-state voltage changes (generated by somatic current injection) with that predicted from morphologically realistic models. On average, dendritic AP signals attenuated to approximately 50% of the somatic AP amplitude at a distance of 100 µm from the soma (n=6). These data demonstrate that APs invade the dendrites of cortical layer 2/3 bitufted interneurons in a decremental manner, suggesting that their impact will be greatest at proximal dendritic locations. Further investigations will examine the role of dendritic voltage-gated channels in regulating AP backpropagation in these neurons.
Optical Recording of Neuronal Activity with Genetic Ca2+ Sensors in Anesthetized and Freely Moving Mice

Henry Lütcke¹, Masanori Murayama²,³, Thomas Hahn⁴,⁵, David J. Margolis¹, Simone Astori⁴,⁶, Stephan Meyer zum Alten Borgloh⁴, Werner Göbel¹, Ying Yang⁴, Wannan Tang⁴, Sebastian Kögler⁷, Rolf Sprengel⁴, Takeharu Nagai⁸,⁹, Atsushi Miyawaki⁸, Matthew E. Larkum², Fritjof Helmchen¹ and Mazahir T. Hasan⁴

¹ Brain Research Institute, University of Zurich, Zurich, Switzerland;  
² Department of Physiology, University of Bern, Bern, Switzerland;  
³ Behavioral Neurophysiology Laboratory, Brain Science Institute, RIKEN, Saitama, Japan;  
⁴ Max Planck Institute for Medical Research, Heidelberg, Germany;  
⁵ Central Institute for Mental Health, Mannheim, Germany;  
⁶ Department of Cell Biology and Morphology, University of Lausanne, Switzerland;  
⁷ University of Göttingen Medical School, Göttingen, Germany;  
⁸ Laboratory for Cell Function Dynamics, Brain Science Institute, RIKEN, Saitama, Japan;  
⁹ Research Institute for Electronic Science, Hokkaido University, Hokkaido, Japan

Long-term, population imaging of neuronal activity with single-cell, single action-potential (AP) resolution has a tremendous potential for understanding how information is encoded in neuronal circuits and modified by experience. To enable direct correlation between cellular signals and animal behavior, a major challenge in systems biology is to perform studies in freely-moving mammals. This goal requires the ability to identify neural correlates of various aspects of animal behavior, from sensory processing to decision-making and motor output. Recent advances in the design and delivery of genetically encoded calcium indicator proteins (FCIPs) demonstrated the feasibility of optical detection of single APs (Wallace et al., Nat Methods 5:797-804, 2008) and even in freely moving mice (Lütcke et al., Front. Neural Circuits 4:9, 2010). Here we show results on the functional characterization of YC3.60 for recording neuronal activity both in vitro and in vivo. With recombinant adeno-associated virus (rAAV) equipped with the human synapsin promotor, we can achieve long-term neuron-specific YC3.60 expression. Three weeks after injection of rAAV-hSYN-YC3.60 into the barrel cortex of young adult mice, we could observe...
wide-spread (ca. 1 mm) and dense expression around injection site with labeling of neuronal somata, dendrites and background neuropil clearly visible. Simultaneous two-photon calcium imaging and cell-attached recording in barrel cortex of urethane-anaesthetised mice allows the optical detection of single APs in vivo and sensory evoked responses (with airpuffs, 1 s at 5 Hz). Stimulation elicited APs and corresponding calcium transients with fast kinetics both in soma and neuropil. Implantation of a cranial window over YC3.60 expressing cortex allows two-photon calcium imaging of identified neuronal populations over months. To test whether YC3.60 can also be used for recording neuronal activity in freely moving animals, we used a single optical fiber (Murayama and Larkum 2009, Nat. Protocols 4:1551-1559) to both excite the CFP (in YC3.60) with a blue light-emitting-diode and collect the emitted YFP light (in YC3.60). The fiber was placed on top a dura (or a thinned skull) of isoflurane-anesthesized mice. These mice showed Ca2+ transients with variable response amplitude in different trials. To evoke sensory responses, we delivered five successive air-puffs every 5s and observed single trial Ca2+ responses. We could also detect complex activity dynamics of variable amplitude when mice were either passively sitting or actively exploring.

References:
Information

1) Outline
   June 24th (Thr) ~ 27th (Sun)
   Conference room: Karaya Hall (24th, 25th, Kanucha map ①)
                      Rain Forest Hall (25th~27th, Kanucha map ⑪)
   Reception starts at 14:00 pm on 24th
   Place: 1F lobby at Kanucha Hotel Front Building (24th, 25th, Kanucha map ①)
          Foyer at Rain Forest Hall (25th~27th, Kanucha map ⑪)
   Registration fee: 13,000 yen (on-site), Poster registration fee: 5,000 yen

2) Banquet
   Place: “RikaRika” guest lounge in Restaurant Building (Kanucha map ⑬)
   Date & Time: at 19:00 - 21:00 on June 24th, Fee: 7,000 yen (on-site)

3) Dining
   There are 8 restaurants and 2 bars in Kanucha. (see Kanucha map)
   Breakfast (7:00 – 10:00), Lunch (11:30 – 14:30), Dinner (18:00 – 22:00)
   Tea Time (14:30 – 18:00), Poolside Bar (10:00 – 18:00), Bar (19:00 – 24:00)

4) Local restaurant
   There are local small restaurants in nearby villages; Abu village, Mihara village and
   Teama village. They are very reasonable price restaurant for the natives and serve
   local food, such as Okinawa noodle and dishes. They are located about 1 km away
   from Kanucha Resort. Take taxi or rent-a-car to the restaurant.

5) Excursions
   Please enjoy free hours in the afternoon on 25th – 27th. There are many choices:
   water sports, activities, culture, outdoors, pools, beach, entertainment, relaxation and
   wellness in Kanucha. Apply at Leisure desk in Hotel Front Building or at Beach
   counter in beach area by yourself. You should find English speaking person at the
   Leisure desk.
   On 28th, we go to Makishi public market in Naha city to enjoy sightseeing and
   Okinawa local meal for lunch. You are more than welcome to join us. Please take
   Kanucha shuttle No.2 departing at 8:50 on 28th. We meet you at check in counter
   area in Naha airport around noon. Let’s take Yui monorail to go to Makishi station
   for sightseeing the market. We should be back Naha airport at about 14:00 p.m.

6) Internet
   Wired LAN is available in your room and conference rooms. The Ethernet code is
   available at hotel front desk.

7) Related conference
   The fifth international neural microcircuitry conference, JST session “Microcircuitry
   of Cortex" will be held in Miraikan in Tokyo immediately following this conference,
   June 29th and 30th, 2010.
**Oral Presentation Instruction**

Please bring your own note PC and check the connection with the projector in advance during the coffee break or before the session. Some one should wait for you to help checking the connectivity between your note PC and the projector during the coffee break or at least 15 - 30 minutes before the session.

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**Poster Presentation Instruction**

Poster size: 120 cm (v) × 180 cm (h)  

Poster display board for one poster

1) Poster boards are located at the rear side of “Rain Forest” Hall where the conference is held on 25th – 27th.

2) Please display your poster on the poster display board (right photo) where you found your poster number. Two display boards are for one poster area.

3) Please display your poster after 16 pm on 25th and remove it before 14 pm on 27th.

4) The poster sessions are scheduled at 11:00 am – 13:00 pm on 26th and 27th.

ポスターは、レインフォレストの後方にあるご自分の発表番号のポスターボードに、25日の16時以降にお貼り下さい。また、27日の午後２時までには撤去をお願いします。ポスター発表時間は、26日27日の11時—13時です。その間は、ポスターの説明をお願いします。
Kanucha Resort Shuttle Bus

fee per persons: 1,880 JPY
Capacity: 40 persons
Reservations or information: (098) 861-5489 (HOT Okinawa)

* Upon exiting the baggage claim area at Naha Airport, please report to the Naha Bus information counter located near the center of the arrival hall.

From Naha Airport

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* Shuttle no. 12 operates on weekends and holidays only.

< Depending on departure location, please report to one of the following >

Naha Airport: Naha Bus information counter, Naha Airport Domestic Terminal Arrivals Hall 1F
Kanucha Golf Range: Range parking area
Hotel: Front Lobby or Leisure Desk (located in the lobby)

1. When we do not get confirmation at the meeting time / a set place, we consider it to be refusal to pick up passenger and leave it on schedule.
2. Please allow time for unforeseen circumstances such as heavy traffic.
3. Shuttle buses are booked in advance; please confirm your reservation at the relevant check-in point.
4. Both northbound and southbound shuttle buses stop for a 5-minute toilet break.
5. Please make sure you will arrive at the airport in plenty of make your flight.
6. Northbound shuttles stop only at Asahibashi Bus Terminal.
7. Shuttles 8, 10, 12, and 14 stop at Kanucha golf Range.
ご案内

1）国際研究集会概要
日程：2010年6月24日（木）～27日（日）
場所：沖縄県名護市カヌチャリゾート
　カラヤホール（24, 25日、map ①）レインフォレスト（25～27日、map ⑫）
受付：24日午後2時～
受付場所：フロント棟1階フロント横（24, 25日、map ①）
　レインフォレストホール前 ホワイエ（25～27日、map ⑫）
参加費：13,000円（事前申込12,000円、琉球割引有）ポスター登録費：5,000円

2）懇親会
開催場所：リッカリッカ（レストラン棟、マップ⑬）
開催時間：24日19:00-21:00
会費：7000円（当日申込可）

3）食事（地図参照）
カヌチャリゾート内には8つのレストランと2つのバーがあります。
朝食（7:00～10:00）、昼食（11:30～14:30）、夕食（18:00～22:00）
喫茶（14:30～18:00）、プールサイドバー（10:00～18:00）、バー（19:00～24:00）

4）近隣の食堂
1kmほど離れた安倍地区、三原地区や沖縄（てぃーま）地区には、郷土料理や沖縄そばの食堂があります。千円以下で食事ができる地元の方相手の小さな食堂です。
あぶ海産物料理（0980-55-8705、名護市字安倍504）、やんぱる（0980-55-9404、名護市字南130-2）
さらばんじ（0980-55-8684、名護市字南367-5）、やんぱる産業（0980-55-8250、名護市字安倍504）
いしろ（0980-55-8081、名護市字安倍505）、サザンナード（0980-55-8732、名護市字三原387）
はなはな（0980-55-8406、名護市字安倍119-1）、朱呂屋（0980-55-9302、名護市字三原214-3）
やんぱる料理の店あぶ（0980-55-8705、名護市字安倍504）

5）エクスカーション
25-27日の午後は自由時間です。リゾートライフをお楽しみください。各種のアクティビティがあります。フロント棟のレジャーデスク、ビーチのビーチカウンターにて各自で申込をお願いします。
また、28日に、エクスカーションとして那覇市内の牧志公設市場に行きます。朝8時50分発の、カヌチャシャトル2便に乗車いただき、空港で手荷物のチェックインを済ませた後、12時にチェックインカウンター付近にご集合下さい。ユイレールで牧志駅まで行き、市場を訪問の後、2階の食堂で昼食をとり、午後2時頃には空港に到着し解散する予定です。事前に申込む必要はありません。

6）インターネット
ご宿泊のお部屋と、会議場は有線LANが完備されています。LANコードはフロントで貸出しサービスがあります。ご利用下さい。

7）日本科学未来館の国際研究集会
6月29日～30日に、東京のお台場の日本科学未来館にて、第5回神経局所回路国際会議JST sessionを開催します。ご参加をお待ちしております。

89
### カヌチャリゾートシャトルパス

#### 内容
- 船上：40名
- 予約・お問い合わせ：098-861-5489 (株)ホット沖縄
- 空港に到着したら、那覇空港到着ロビー1Fの那覇バス空港案内所にて受付手続きが必要となります。

#### 那覇空港発（下り便）

<table>
<thead>
<tr>
<th>便名</th>
<th>那覇空港</th>
<th>カヌチャリゾート</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>10:00</td>
<td>11:50</td>
</tr>
<tr>
<td>5</td>
<td>11:30</td>
<td>13:10</td>
</tr>
<tr>
<td>7</td>
<td>13:30</td>
<td>15:10</td>
</tr>
<tr>
<td>9</td>
<td>15:00</td>
<td>16:40</td>
</tr>
<tr>
<td>11</td>
<td>17:00</td>
<td>18:40</td>
</tr>
<tr>
<td>13</td>
<td>18:30</td>
<td>20:10</td>
</tr>
</tbody>
</table>

#### カヌチャリゾート発（上り便）

<table>
<thead>
<tr>
<th>便名</th>
<th>カヌチャリゾート</th>
<th>那覇空港（バス停）</th>
<th>那覇空港</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8:50</td>
<td>10:30</td>
<td>10:40</td>
</tr>
<tr>
<td>4</td>
<td>11:00</td>
<td>12:40</td>
<td>12:50</td>
</tr>
<tr>
<td>6</td>
<td>12:30</td>
<td>14:10</td>
<td>14:20</td>
</tr>
<tr>
<td>8</td>
<td>14:00</td>
<td>15:40</td>
<td>15:50</td>
</tr>
<tr>
<td>10</td>
<td>16:00</td>
<td>17:40</td>
<td>17:50</td>
</tr>
<tr>
<td>12</td>
<td>19:00</td>
<td>20:30</td>
<td>20:40</td>
</tr>
</tbody>
</table>

#### 集合場所：那覇近郊－カヌチャ行き  ※出発の10分前までに集合
- 那覇空港発：那覇バス空港案内所（空港到着ロビー1階）集合
- 那覇近郊発：カヌチャリゾートプラザ駐車場に集合
- ホテル発：フロントロビーもしくはレジャーデスクに集合

#### 那覇空港到着ロビーの1F

- ※10分前に到着ロビー内「那覇バスカウンター」へご集合お願いします。
- 那覇空港到着ロビー内にいることに注意してください。
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*1 同社製品TM-1000
*2 一般的な顕微鏡倍率比

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インターネットでも事前確認しております。以下URLへアクセスしてください。
URL http://www.hitachi-hitec.com/tm/

94
細胞培養観察装置

BioStation CT

多量のサンプルを安定した環境で培養しながら自動観察！

- 安定環境のインキュベータ内で、培養サンプルを自動で顕微鏡撮影します。
- いつでもどこでも、同じクオリティで大量サンプルの多点・複数倍率のタイムラプスデータを簡単に取得できます。
- 培養容器全体を高明像で撮影するフルスキャン機能により、樹立効率の低いIPS・ES細胞も確実にイメージします。
- 各種フラスコ、ディッシュ、ウェルプレートに対応できます。

生細胞タイムラプスイメージング装置

Bio Station IMq

培養から高感度タイムラプス画像取得・解析まで、これ一台で簡単に！

- 培養、画像取得、解析の3つの機能を一体化、顕微鏡やカメラの操作に慣れていない方でも簡単に観察・撮影が可能です。
- 長時間観察で大きな問題となるフォーカスドリフトを、温度変化・振動の両面からも解消しました。
- タイムラプス撮影しながら培地交換可能なオプションユニットと、コントロール実験可能な4分割ディッシュをご用意。
ハヤシはモノづくりの全工程をお手伝いします。

【試薬】 分析化学用、食品分析用、有機合成用、生物化学用、高分子合成用、ケミカル合成用、医薬品合成用、電子材料合成用、電気化学合成用、電子材料合成用、化学分析用、電子材料合成用

【設備】 機械、化学、機械、化学、機械、化学、機械、化学、機械、化学、機械、化学、機械、化学、機械、化学、機械、化学、機械、化学、機械、化学、機械、化学、機械

【環境対策】 エネルギー利用効率化、環境対策、持続可能な経営

協賛企業：

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