Instituto Juan March
de Estudios e Investigaciones

111 CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Co-sponsored by
COLUMBIA UNIVERSITY

Workshop on

Dendrites

Organized by

R. Yuste and S. A. Siegelbaum

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The lectures summarized in this publication were presented by their authors at a workshop held on the 5th through the 7th of June, 2000, at Columbia University, New York (USA).

Depósito legal: M-31.675/2000
Impresión: Ediciones Peninsular. Tomelloso, 27. 28026 Madrid.

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Introduction

R. Yuste and S. A. Siegelbaum

The role of dendrites in neuronal signal processing has been a topic of research for more than a century, since Cajal's original postulate that dendrites constitute the input side of the neuron. Cajal concluded that dendrites were passive structures. However, studies initiated by Rafael Lorente de Nó, and extended by Eric Kandel and Alden Spencer in the hippocampus and by Rodolfo Llinás in the cerebellum, later found that dendrites could produce action potentials. The workshop on Dendrites, sponsored by the Instituto Juan March and held at Columbia University in New York, on June 5-7, 2000, confirmed that dendrites are, indeed, very active participants in neuronal signaling and plasticity, as well as an extremely exciting and productive area of research.

Recent studies have greatly expanded our view of the functional properties of mammalian dendrites through novel experimental approaches such as two-photon microscopy and dendritic patch recording. These techniques have provided direct evidence for the presence of several types of voltage-gated conductances in dendrites and for the chemical compartmentalization of local biochemical signals in dendritic spines. Although the logic of dendritic processing is still unclear, a host of new findings indicate that mammalian dendrites are richer functionally than previously thought, and suggest that individual neurons are capable of sophisticated information processing.

The meeting was organized into presentations that focused on five main topics: 1. Electrical excitability; 2. Signaling through intracellular calcium; 3. Receptor localization and postsynaptic biochemical signaling; 4. Local protein synthesis and transport; 5. Developmental processes. In addition the meeting contained two special lectures. Rodolfo Llinas lectured on the importance of dendritic active conductances in the context of neuronal circuitry, and in particular of oscillating electrical activity in re-entrant loops in ensembles of neurons. Eric Kandel spoke on the importance of local protein synthesis in tagging active synapses for synapse-specific long-term plasticity at the Aplysia sensory to motor neuron connection.

During the meeting, several recurrent themes appeared, as evidenced by the abstracts in this booklet. First, the generation of dendritic action potentials and their ability to propagate to the neuronal cell body is a complex process that can be influenced by the

specific timing and pattern of synaptic activity. Modulation of dendritic voltage-gated channels by second messenger cascades can further regulate this electrical excitability. Second, there is a dynamic cycling of transmitter receptors in the dendritic postsynaptic membrane. This cycling can be controlled by synaptic activity, giving rise to long-term plasticity. Third, the dendritic spines themselves are plastic and undergo actin-based motility and shape changes. This can lead to formation of dendritic filopodia, totally new spines or a change in the structure of preexisting spines, which in turn can influence spine calcium dynamics.

Studies in the future are needed to determine the physiological function of dendritic excitability, spine calcium dynamics and spine motility.

R. Yuste and S. A. Siegelbaum

Session 1: Active properties

Chair: Daniel Johnston

Perspectives on the active properties of dendrites

G. M. Shepherd

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The concept that neuronal dendrites support active transmission and processing of neural information goes back to the origins of modern cellular neuroscience. Cajal used the olfactory mitral cell and the cerebellar Purkinje cell as the two clearest examples showing that dendrites are directly involved in transmitting neural activity, presumably similar to the impulse in peripheral axons.

When single cell recordings began in the CNS in the 1950s, a working hypothesis was therefore that action potentials would be found to be generated in the dendrites. However, experiments in the motoneuron and in the crustacean stretch receptor showed that the site is located in the axonal initial segment. This became the classical model of action potential generation in nerve cells. However, it was further found that action potentials could invade motoneuron dendrites antidromically, and that synaptic excitation could generate action potentials that spread orthodromically in the apical dendrites of hippocampal pyramidal cells. Also, evidence for fast prepotentials generated at active sites in dendrites was found. Thus, many of the critical issues regarding the presence and significance of active properties of dendrites were raised by the earliest single neuron recording studies.

Rigorous analysis of dendritic properties began with the studies of Rall in the late 1950s, leading to the introduction of compartmental modeling methods for this purpose in the 1960s. The early studies of olfactory dendrites included both active and passive dendritic properties. The application of this approach to other neurons emphasized mainly passive properties, giving rise to the modern view that active properties were classically underappreciated. But the modelers could only model what the physiologists recorded, and, with the exception of several pioneering reports in the cerebellum, hippocampus, and neocortex, active properties were the exception rather than the rule in the studies of most neurons until the 1990s.

There is now increasing evidence that active properties are present throughout the dendritic membranes of most neurons, with their densities varying to different degrees, as will be documented at this workshop. This has expanded the possibilities for dendritic functions, but has also raised severe problems in obtaining experimental evidence for specific functions involved in information processing, and in generating models that are adequately constrained to give insight into those specific functions.

Taking our cue from Cajal, we can use neurons in the olfactory pathway to demonstrate unequivocally several specific dendritic functions.

These include:

Distal dendrites receive and process specific afferent input;

Dendrites couple specific afferent input in distal dendrites to the soma and initial axonal segment;

The sites of action potential initiation can shift between initial axonal segment and distal dendrites dependent on the intensity of distal excitatory synaptic input and of soma and basal dendritic inhbitory synaptic input;
Dendrites have synaptic outputs;
Dendritic spines function as compartments with local synaptic inputs and outputs;
Neurons can have synaptic outputs in the absence of propagating action potentials;
Active spine interactions support simple logic operations;
Spines serve as metabolic compartments.

These functions will be illustrated with examples from olfactory neurons, and the presence or possibility of similar properties and functions in other central neurons will be discussed.

Several issues regarding dendritic function that are currently under investigation in the olfactory system and in other systems will be addressed. These include how active channels get distributed in dendritic trees, evidence for Ca dependent transmitter release through NMDA channels, and what are the contributions of active and synaptic dendritic properties to memory mechanisms.

A final question is how important are dendritic properties to the functions of neural networks? Most neural networks are based exclusively on different weights of axonal connections, with no representation of dendritic properties, an approach that Golgi and other reticularists would have found delightful. One of the most critical challenges for neuroscience is identifying essential dendritic information processing properties, and specifying the extent to which they must be represented in order for a neural network to qualify as representing how the brain actually carries out its functions.

Role of active dendrites in long-term synaptic plasticity

Daniel Johnston

The dendrites of hippocampal pyramidal neurons express a wide variety of voltage-gated ion channels, including several types of sodium, calcium, and potassium channels. One of the surprising findings in this regard, is the remarkably large gradient in the density of transient, or A-type, potassium channels recently found in apical dendrites of CA1 pyramidal neurons. There is an increasing gradient of these channels, which varies some five-fold from the soma to at least 3/4 of the end of the dendritic tree. (A similar gradient also exists for hyperpolarization-activated channels.) We have been investigating the modulation of the dendritic A-type channels and whether the channels play a role in the induction and/or expression of long-term potentiation (LTP) of Schaffer collateral synapses on these neurons. The molecular identity of the dendritic potassium channels is not known, but the Shal-type Kv4.2 subunit is the most likely candidate. Data will be presented to indicate that the modulation of dendritic potassium channels by PKA and PKC is mediated by a common downstream signalling pathway, extracellular signal-regulated kinase, ERK. Furthermore, data will be presented to suggest that dendritic potassium channels can control the induction of LTP with certain stimulus paradigms and that a decrease in potassium channel activity in dendrites occurs with the expression of LTP.

Three forms of dendritic excitability in hippocampal CA1 dendrites

Nelson Spruston, Nace Golding, Hae-yoon Jung, Timothy Mickus

Northwestern University

The integration of synaptic inputs in dendrites is central to the function of most neurons. It is now clear that this process is shaped, in large part, by voltage-gated channels present in dendrites. Activation and deactivation of these voltage- and/or calcium-dependent channels by synaptic potentials alters dramatically the voltage changes that occur in the dendrites, soma, and axon. To determine how voltage-gated channels in dendrites contribute to the process of synaptic integration, we have used simultaneous dendritic and somatic patch-clamp recording from CA1 pyramidal neurons. All recordings were performed *in vitro*, in hippocampal slices prepared from 6-10 week-old Wistar rats. Slices were maintained in artificial cerebrospinal fluid at 34±2°C.

In response to synaptic activation with stimulating electrodes in *stratum radiatum* or *stratum lacunosum moleculare*, we find that the postsynaptic action potential occurs first in the soma (Spruston et al. 1995), suggesting that action potential initiation normally occurs near the soma. Experiments performed by Colbert and Johnston suggest that this low-threshold action potential initiation zone is in the first node of Ranvier of the axon (Colbert et al. 1996). A relatively low dendritic sodium channel density and high dendritic potassium channel density (compared to the axon) are likely to be responsible for the higher action potential threshold in the dendrites (Colbert et al. 1996; Hoffman et al. 1997). Following their initiation in the axon, action potentials actively backpropagate into CA1 dendrites in an activity-dependent manner (Spruston et al. 1995; Jung et al. 1997; Colbert et al. 1997; Mickus et al. 1999).

With stronger synaptic stimulation, we find that dendritically recorded spikes sometimes precedes somatic action potentials. We also find, however, that these dendritic spikes often occur in the absence of a somatic action potential (Golding & Spruston 1998). Taken together, these findings suggest that regenerative spikes can be initiated in dendrites, but that these events fail to reliably invade the soma and axon. Similar events can be triggered by large, brief current injections via the dendritic electrode. These events are blocked by TTX, suggesting that this form of dendritic spike is mediated largely by voltage-gated sodium channels (Golding & Spruston 1998).

Calcium channels can also contribute to dendritic excitability, in the form of dendritically generated calcium spikes. These events can be triggered by strong synaptic stimulation or dendritic current injection (Schwartzkroin & Slawsky 1977; Golding et al. 1999). Their larger amplitude and longer duration distinguishes these events from dendritic sodium spikes. Calcium spikes are not evoked by somatic current injection, and calcium spikes evoked by dendritic current injection are blocked by dendritic, but not somatic, application of the broad-spectrum calcium-channel blocker nickel (5 mM in the pressure application pipette) (Golding et al. 1999). We find that dendritic calcium spikes are shaped by at least two distinct subtypes of voltage-gated potassium channels (Golding et al. 1999). A slowly inactivating, voltage-gated potassium channel (D type) raises the threshold for calcium spikes; blocking these channels with 100 μM 4-AP or 0.5 μM dendrotoxin lowers the calcium

spike threshold dramatically and causes calcium spikes in response to somatic current injection. A voltage- and calcium-activated potassium channel (BK type) influences the duration of calcium spikes; blocking these channels with 250 μM TEA or 70 nM charybdotoxin dramatically prolongs calcium spikes.

Thus, we have identified three distinct forms of dendritic excitability in CA1 pyramidal neurons: backpropagating action potentials, dendritically initiated sodium spikes, and dendritically generated calcium spikes. Each of these events have also been observed *in vivo* (Kamondi et al. 1998) and we speculate that each is likely to be uniquely involved in the process of synaptic integration and plasticity.

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Synaptic weight is independent of synapse location in CA1 pyramidal neurons

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Like many other neurons in the CNS, hippocampal CA1 pyramidal neurons receive tens of thousands of excitatory synaptic contacts over several hundred microns of their apical dendritic arborizations. The widely distributed nature of this synaptic input has led to the controversial idea that distal synapses are less effective than proximal synapses in firing a neuron. We report here, however, that the amplitude of unitary excitatory synaptic input at the soma of CA1 pyramidal neurons does not depend on the dendritic location of the synapse. Using dual whole-cell recordings from the soma and apical dendrites of these cells we measured the amplitude of presumably unitary or single terminal EPSPs simultaneously at both the dendritic input site and the soma. It was found that the amplitude of the somatic EPSP remained ~0.2 mV for synaptic input ranging from 50 to 325 µm from the soma while the local dendritic EPSP amplitude increased nearly four fold for the same distance (0.25 to 0.8 mV). To determine the level of location-dependence to be expected from a uniform synaptic input, EPSC-shaped currents were injected into the dendrites and the local dendritic and propagated somatic voltage transients ($EPSP_i$) were measured. There was a large location-dependence to the $EPSP_i$, with somatic $EPSP_i$ amplitude decreasing to less than half as the input location was moved distal (0.5 to 0.19 mV from 50 to 325 µm). The amplitude of the $EPSP_i$ at the dendritic injection site appeared to increase slightly (<50%) but not nearly as much as the synaptically-evoked EPSP amplitude increased with distance. To further examine the conductance of dendritic single synapses we measured EPSCs from the site of synaptic input. As with EPSPs, mean EPSC amplitude increased from ~7 pA for synaptic locations near 50 µm to ~28 pA for synapses around 325 µm from the soma. The kinetics of dendritic and somatic EPSP/Cs were also compared for both the actual synaptic input and EPSC-shaped current injections. In conclusion the spatial normalization of synaptic weight appears to be the result of a progressive increase in the strength of individual synapses with distance from the soma. This normalization and its proposed mechanism have important implications for the processing and storage of synaptic information.

(supported by NS35865 and NS39458 and the Alfred P. Sloan Foundation.)

Initiation zone interactions in pyramidal neurons

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Why do layer 5 neocortical pyramidal neurons have 2 sites of action potential initiation? We examine the possibility that they integrate different types of synaptic inputs and produce different firing patterns. We investigated the circumstances leading to the generation of dendritic calcium action potentials (Ca2+-APs) and the consequences for the generation of axonic sodium action potentials (Na+-APs) in vitro. Regenerative activity in the dendrite could be generated with 4 different stimulus paradigms: 1) direct current injection at the dendritic site of Ca2+-AP initiation using a dendritic patch pipette. 2) Combination of a back-propagating Na+-AP with reduced dendritic current injection. 3) A critical frequency of back-propagating Na+-APs generated at the soma. 4) Sub-critical frequency back-propagating Na+-APs coupled with small dendritic current injection. Each stimulus paradigm was designed to correspond to a different pattern of synaptic input arriving at either the tuft or the basal dendrites. Neurons could also respond to such regenerative dendritic activity with additional axonal action potentials. These results imply that the exact location and timing of input spread over the dendritic arbor will determine the firing pattern of the neuron in a highly complex manner. We propose that the two initiation sites of L5 pyramidal neurons allow the neuron to associate classes of inputs based on their laminal position in the cortex. Dendritic Ca2+-APs tend to produce bursts. The activity of the output neurons of the cortex thus reflects the distribution of synaptic activity within a given column.

Modeling Active Dendritic Integration: Some Consequences for Theory and Experiment

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The dendrites of many neuron types contain voltage-dependent channels which can support the generation and propagation of action potentials. Though a dendritic spike-generating mechanism would seem likely to produce strong superlinear interactions among nearby excitatory synapses, one recent study using dual glutamate pipettes (Cash & Yuste, *Neuron*, 1999, 22:383) failed to find prominent superlinear effects, finding instead that summation of EPSP's in response to activation of both pipettes was most often linear or sublinear; similar results were obtained for synaptic stimulation using extracellular electrodes (see also Urban & Barrionuevo, *PNAS*, 1998, 95:11450). This type of experimental result leads to pressing questions regarding the functional role of active dendritic channels in synaptic integration.

We have used detailed compartmental simulations of CA1 and neocortical pyramidal cells to explore aspects of synaptic integration in active dendritic trees. In this paper we report three main results.

First, we find that the precise experimental protocol used to assess the linearity or nonlinearity of synaptic integration is of critical importance. For example, a pair of synaptic inputs may sum sublinearly when activated with single test pulses—the standard protocol used in such experiments—but sum superlinearly when the same synapses are activated with high frequency trains. Furthermore, the same current, for example the transient A-type potassium current, may enhance both the sublinear and superlinear facets of synaptic integration depending on the stimulus.

Second, we find that the dendritic cable structure of hippocampal and neocortical pyramidal cells is ideally suited to create a large number of quasi-isolated dendritic subunits, whose outputs are summed at the cell body. Thus, spikes may be generated in thin branches, but given the unfavorable boundary conditions at branch points with large apical trunks or the cell body, these spikes may fail to actively propagate to other parts of the cell. We find, for example, that full blown spikes in thin branches may be seen as EPSP-like depolarizations of one or a few millivolts when measured at the cell body.

Third, we find that superlinear synaptic interactions in pyramidal cell dendrites, if they exist, could have important consequences for several computations to which these cells are known to contribute. For example, we show that active dendrites could account for the nonlinear subunit structure underlying a variety of classical and extraclassical receptive field properties of neuron in the mammalian visual system, including (i) phase-invariant orientation tuning and binocular disparity tuning in complex cells, (ii) competitive interactions among stimuli presented simultaneously within a cell's receptive field, (iii) attentional gain fields, and (iv) extraclassical receptive field effects such as are involved in contour completion.

In addition, we show that active dendrites could contribute greatly to the memory capacity of neural tissue. Using mathematical analysis and computer simulations we find that a Hebb-type learning rule driving the stabilization of synaptic contacts onto an active dendritic neuron of realistic size supports a memory capacity 1-2 orders of magnitude in excess of that available to a size-matched linear neuron.

SYNERGISTIC RELEASE OF CALCIUM FROM IP_3-SENSITIVE STORES IN PYRAMIDAL NEURON DENDRITES EVOKED BY ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS PAIRED WITH BACKPROPAGATING ACTION POTENTIALS.

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Although several mechanisms can raise [Ca^{2+}] in pyramidal neurons, most attention has focussed on Ca^{2+} entry through the NMDA receptor since this pathway is Hebbian, requiring both presynaptic stimulation to release glutamate and postsynaptic depolarization. In these experiments, we show that synaptically activated Ca^{2+} release from internal stores is also Hebbian. We measured [Ca^{2+}] changes with a high-speed camera in bis-fura-2-filled neurons patched in transverse hippocampal slices from the rat. Ionotropic receptors were blocked with APV and CNQX. Under these conditions, repetitive synaptic activation, paired with backpropagating action potentials, caused large wavelike increases in [Ca^{2+}] predominantly in restricted regions of the apical dendrites and soma. In some experiments, action potentials were not necessary. The increases were blocked by MCPG implicating mGluR. Synaptic activation could be replaced by bath-applied t-ACPD, DHPG, or CHPG, and could be blocked by MPEP, AIDA, and 4-CPG, implicating $mGluR_5$. The increases were blocked by CPA, ryanodine, and intracellular heparin. Release was not blocked by Ruthenium Red although Ruthenium Red blocked the effect of ryanodine. Together these experiments indicate that regenerative [Ca^{2+}] increases are caused by the synergistic effect of mGluR-generated IP_3 and spike-evoked Ca^{2+} entry acting on the IP_3 receptor. Experiments with low-affinity indicators indicate that the magnitude of the increase in the proximal apical dendrites is 2-6 μM, much higher than the increase caused by trains of action potentials alone.

Imaging of Dendritic Calcium Dynamics in the Intact Neocortex

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Dendritic integration in cortical pyramidal neurons depends on the intrinsic properties of the cells as well as the activation pattern of synaptic inputs. Both of of these might be modulated during different behavioral states. Measurements of dendritic activity in the intact cortex and eventually in awake, behaving animals therefore are essential.

We used two-photon laser-scanning microscopy to measure calcium dynamics in the apical dendrites of pyramidal neurons in layers 2/3 and layer 5 in the barrel cortex of anesthetized rats. Dendrites could be imaged down to about 500 μm below the brain surface. In layer 2/3 neurons, calcium transients evoked by whisker deflections were correlated with fast sodium action potentials and were restricted to the proximal dendrite. No indication of dendritic calcium action potentials upon sensory stimulation was observed. In contrast, large calcium transients were observed in the distal dendrites of layer 5 neurons consistent with the occurrence of dendritic calcium spikes. These transients occurred spontaneously during intrinsic bursts of action potentials and were also evoked by whisker deflections. Whisker-evoked calcium transients sometimes did not coincide with a somatic burst but were accompanied by only a single action potential, suggesting a partial decoupling of distal dendrite and soma. Our results suggest that supra- and infragranular pyramidal neurons differ in their ability to generate dendritic calcium spikes.

As a step towards similar experiments in awake, behaving rats, we have developed a miniature two-photon microscope based on fiber optics. It consists of a small microscope headpiece which can be carried by a rat. The headpiece contains epifluorescence optics and a small fiber-scanning unit. Images of layer 2/3 neurons and their dendrites in the somatosensory cortex of anesthetized rats will be presented. This novel technique thus may enable high-resolution imaging in the cortex of awake, freely moving animals.

(Supported by Lucent Technologies, Whitehall Foundation, and the Human Frontier Science Program)

Session 2: Calcium and plasticity

Chair: Tobias Bonhoeffer

Activity dependent plasticity: Neurotrophins and morphological changes at the synaptic level

Tobias Bonhoeffer

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Hippocampal long-term potentiation (LTP) is generally thought to be induced by concurrent pre- and postsynaptic activity leading to elevated calcium levels in the postsynaptic cell. How the subsequent synaptic enhancement is achieved and later maintained and which molecules are important in this process is much less clear.

I will present evidence for brain-derived neurotrophic factor (BDNF) being one crucial important component in this system. BDNF-knockout animals show severely compromised LTP and long-lasting LTP is even completely abolished. It can be rescued by locally infecting cells with an adenovirus vector containing the BDNF gene, indicating that the BDNF protein is required at the time of LTP-induction. Moreover thetaburst-induced LTP is substantially diminished in the presence of a function-blocking BDNF-antibody. Interestingly the attenuation with the BDNF-antibody is as strong as with a trkB receptor-body which blocks both BDNF and NT4 action. This implies that BDNF but not NT4 is involved in hippocampal LTP.

Since it is well known that BDNF can influence the morphology of neurons, it is attractive to speculate that this molecule might provide a link between functional and morphological aspects of synaptic plasticity.
We and others have recently also been able to show that, indeed, there are morphological changes that occur when functional changes in synaptic strength occur. For some tens of year it had been difficult to prove this old idea, mainly because it has not been easy to pin-point the location of the synapses which are expected to change. We have now tackled this problem by combining two-photon imaging with a local superfusion technique thereby confining the region on the postsynaptic dendrite where the synaptic changes could occur.

We were able to show that local LTP induction in such a restricted region of the dendrite reliably led to the appearance of new spines in this area. These novel spines remained stable in shape and position for the whole period of observation, which lasted up to 24 hours. We found furthermore that the disappearance of spines was not, as is the case in LTP and spine growth, controlled in a specific and activity-dependent manner but it rather occurred more or less randomly in time and space.

The most attractive explanation for the formation of additional spines is a concurrent emergence of new synapses on these structures. Although more experiments on the precise nature of these changes are necessary, our data provide strong evidence that in the mammalian hippocampus not only physiological but also structural changes play an important role when neurons change the efficacy of their connections.

Plasticity mechanisms: from silence to heavy traffic

Roberto Malinow

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Brief periods of strong neuronal activity induce long-lasting changes in synaptic function. This synaptic plasticity is thought to play important roles in learning and memory. One example, long-term potentation in CA1 hippocampus, has been studied extensively, with conflicting views regarding underlying mechanisms. Another form of plasticity is that which occurs during synapse maturation in early postnatal development. While both forms of plasticity appear to be triggered by neuronal activity, they appear to use different molecules to execute modifications. Here we discuss recent findings, regarding properties of glutamate receptor trafficking, that provide a new model of long-term plasticity.

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From form to function: calcium compartmentalization in dendritic spines

Rafael Yuste

Dendritic spines can compartmentalize calcium and this is likely to be related to their specific function. I will review recent experimental work that has examined the mechanisms regulating spine calcium dynamics. Calcium influx into spines is mediated by voltage-sensitive calcium channels, NMDA and AMPA receptors and is followed by fast (msec) diffusional equilibration within the spine head. Calcium decay kinetics are then mediated by slower (tens of msec) diffusion through the spine neck and by calcium pumps located at the spine. Calcium release by different mechanisms occurs in spines, although its exact role is controversial. Finally, little is known about the nature and properties of endogenous calcium buffers in spines, which might play an essential role in determining spine calcium dynamics. Our studies confirm that spines are calcium compartments because of their morphological features and their local influx and extrusion mechanisms and highlight the richness of pathways that regulate calcium accumulations in spines. Our data illustrate how the form and function of the spines are exquisitely intertwined.

Neurotrophin-evoked signaling in dendritic spines

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Neurotrophins (NTs) represent a family of structurally related proteins that regulate the survival, differentiation, and maintenance of function of different neuronal populations. More recently, it became apparent that they also play an essential role in the modulation of activity-dependent neuronal plasticity. By using whole-cell recordings from visually-identified neurons in brain slices, we found that NTs elicit action potential firing in central neurons (Kafitz et al., 1999). Already at concentrations of 0.5 - 2.0 nM, brain-derived neurotrophic factor (BDNF) excited neurons in the hippocampus, cortex, and cerebellum. We found that BDNF and neurotrophin-4/5 (NT-4/5) depolarized neurons just as rapidly as the neurotransmitter glutamate, even though at a more than thousand-fold lower concentration. Neurotrophin-3 (NT-3) produced much smaller responses, while nerve growth factor (NGF) was ineffective. Ratiometric Na^+ imaging experiments and reversal potential measurements of the NT-evoked currents indicated that the NT-induced depolarization resulted from the activation of a Na^+-conductance. This conductance was reversibly blocked by K-252a, a protein kinase blocker with preference for Trk receptor tyrosine kinases. The NT-evoked responses persisted in Ca^{2+}-free perfusion medium. Also the glutamate receptor antagonists CNQX and APV had no effect on the NT-evoked inward current. By using confocal and two-photon imaging, we recently analyzed NT-evoked signaling in the apical spiny dendrites of hippocampal CA1 pyramidal neurons and in the dendrites of dentate granule cells. We found that BDNF applications caused local accumulations of intracellular Na^+ ions. These signals were restricted to a dendritic segment and had the largest amplitudes in the spines located near the tip of the BDNF application pipette. In a further series of experiments we performed Ca^{2+} imaging while applying BDNF focally to dendritic spines. Brief, pulse-like BDNF applications produced Ca^{2+} transients that were detected in dendritic domains that were smaller than those found for the Na^+ signals. Such Ca^{2+} transients were often restricted to individual spines. Both, BDNF-evoked Ca^{2+} and Na^+ signals in spiny dendrites were always associated with a depolarization recorded at the level of the cell body. Taken together, these results indicate that a presumed TrkB receptor/Na^+ channel complex, underlying the BDNF-evoked excitation, is expressed at high densities in dendritic spines, suggesting a postsynaptic site of induction of BDNF-mediated long-term potentiation.

Optical quantal analysis of synaptic transmission and plasticity in the hippocampus

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We have used laser-scanning confocal microscopy to study synaptically evoked calcium transients in single spines at two kinds of hippocampal synapse: Schaffer-commissural synapses at CA1 and CA3 pyramidal cells, and mossy fibre synapses at thorny excrescences of CA3 cells. Experiments were performed on pyramidal cells in organotypic hippocampal cultures (prepared from rat pups at 8-10d, and maintained in culture for 7-21d before use). Cells were filled with the Ca^{2+} indicator Oregon Green 488 BAPTA-1. Stimuli were delivered via an electrode placed in stratum radiatum (to activate Schaffer-commissural fibres) or in the dentate granule cell layer (to activate mossy fibres). A search was then made for a responding spine. Once a spine was identified, the scanning protocol was switched to line scan mode, and images through the spine obtained with 2 msec resolution. At Schaffer-commissural synapses, we find that the synaptically evoked calcium transient is NMDA-receptor dependent, and is effectively abolished by drugs that inhibit release of calcium from intracellular stores. In contrast, synaptically evoked calcium transients in thorny excrescences are only slightly depressed by NMDAR antagonists and not at all by drugs affecting release from stores; the main source of the calcium transient in thorny excrescences appears to be calcium entry through voltage-dependent calcium channels. We have also examined the action potential-evoked calcium transient at Schaffer-commissural boutons; here, calcium stores make a substantial contribution to the calcium transient, and play a significant role in paired-pulse facilitation and in the spontaneous quantal release of transmitter.

Quantal analysis of LTP has proved problematic in the hippocampus because the large number of synapses on pyramidal cells makes it difficult to match somatically recorded responses with individual synapses. We have analysed changes in the calcium transients at individual dendritic spines following the induction of LTP by tetanic stimulation. Specifically, we have looked for changes in the probability of a response (a measure of the probability of release, p_r), and in the amplitude of the calcium transient. Estimates of p_r were obtained before and 30 min after the induction of LTP. In a majority of spines, LTP was accompanied by an increase in p_r. An increase in the amplitude of the calcium transient was also common. In the presence of AP5 (50μM), neither LTP nor changes in optical quantal parameters were observed. These observations indicate a presynaptic component to the early phase of LTP, and do not exclude an additional postsynaptic component.

Session 3: Receptor localization

Chair: Mary B. Kennedy

GABAergic inputs subdivide the neuronal surface

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Cortical pyramidal cells receive multiple GABAergic inputs from interconnected networks of local circuit interneurons (Somogyi et al., Brain Res. Rev., 1998. 26. 113-135). The GABAergic innervation is stereotyped throughhout the cortical mantle including the hippocampal formation. Only one type of cell, the axo-axonic cell, innervates the axon initial segment. The soma and proximal dendrites are innervated by at least two types of basket cells, one of them expressing parvalbumin the other one cholecystokinin and vasoactive intestinal polypeptide. In contrast to only 3 sources of GABA providing a dense somatic and perisomatic innervation, the dendritic arbour of pyramidal cells is supplied by a large variety of GABAergic cells, each variety providing sparse innervation on their own, but collectively providing the majority of GABAergic synapses. For example, in the CA1 area of the hippocampus, where a relatively homogeneous population of postsynaptic pyramidal cells allows a clear definition of the input/output relationship of each interneuron class, at least 10 types of interneurons can be defined innervating the dendritic field. For many, but not all of these cells, the GABAergic axons co-segregate with the major glutamatergic inputs to the pyramidal cells. The expansion in the variety of dendritically terminating GABAergic cells in the cerebral cortex and the conjoint termination with excitatory inputs suggest that local interactions through the dendrites provide a great opportunity to improve signal processing. Traditionally, the action of synaptically released GABA has been thought of as opposing excitation. However, in the dendritic domain, GABAergic events might co-operate with excitatory inputs, for example through the precise timing of EPSPs or the phasing of dendritic oscillations as well as through the deinactivation of voltage sensitive cation channels.

Hippocampal pyramidal cells express at least 12 subunits of the pentameric $GABA_A$ receptor, providing the opportunity to supply a rich variety of receptors to the synpses received from 13 distinct types of GABAergic neurons. The subunit composition of the receptor determines channel kinetics, regulation and pharmacological properties. For instance, α_2 subunit-containing receptors show a 10-fold higher affinity for GABA compared to α_1-containing receptors. (see Sieghart, Pharmacol. Rev. 1995. 47. 181-234).

Do all synapses on pyramidal cells have similar receptor populations? Does the subunit composition of synaptic receptors correlate with the source of innervation? As a frist step towards defining molecular differences amongst GABAergic syanpses on pyramidal cells we have investigated the subunit composition of receptors in perisomatic synapses innervated by identified classes of presynaptic cells. We used subunit specific antibodies and postembedding, quantitative, electron microscopic immunogold labelling. The results show that, even on the same subcellular domain, pyramidal cells are able to target GABAA receptor channels of a given subunit composition preferentially to synapses receiving GABA from a distinct class of interneuron. The selective targeting of receptors to distinct inputs allows the fine-tuning of the time course and regulation of GABAergic effects depending on the interneuron type releasing GABA.

Synapse assembly and glutamate receptor targeting in hippocampal neurons

Ann Marie Craig

We study cellular mechanisms of synapse formation and plasticity between central neurons using a primary hippocampal culture system. Our focus is assembly of molecular components of synaptic connections, particularly synaptic clustering of neurotransmitter receptors. Excitatory AMPA and NMDA type glutamate receptors and associated scaffolding proteins such as PSD-95 cluster selectively opposite glutamatergic terminals, whereas inhibitory $GABA_A$ receptors and gephyrin cluster selectively opposite GABAergic terminals. Other receptors such as the metabotropic glutamate receptor mGluR7 function at presynaptic sites. Our recent research has focussed on four major topics: the basis of selectivity in sites of receptor clustering between glutamate and GABA synapses; modes of anchoring and cytoskeletal association of postsynaptic components; activity modulation of synaptic targeting of NMDA receptors; and molecular mechanisms of axonal targeting and presynaptic clustering of mGluR7.

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Direct interaction between the R1 subunit of $GABA_B$ receptors and the transcription factor CREB2

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γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the vertebrate CNS and it can mediate its cellular actions through both ionotropic ($GABA_A$ and $GABA_C$) and G-protein-coupled, metabotropic ($GABA_B$) receptors. $GABA_B$ receptors have both pre- and postsynaptic localisations and they have been shown to play an important role in synaptic transmission and plasticity and pharmaceutical manipulation of these receptors may prove useful for a number of diseases [1]. Recently, cDNAs encoding $GABA_B$R1 and R2 subunits have been cloned and it has been proposed that these receptors are only surface expressed and functional as R1/R2 heterodimers [2].

The CREB family of transcriptional regulatory proteins are members of the basic leucine zipper (bZIP) group of DNA binding proteins which homo- or heterodimerise via the zipper domain and then interact with defined sequences of DNA through the basic region [3]. Interestingly, members of the CREB protein family have been proposed as important facilitators and suppressers of long-term memory [4]. For example, CREB has been proposed as a signalling molecule from synapse to nucleus [5] and it has been shown to have a postsynaptic localisation.

We have shown a robust, direct and specific interaction between the C-terminal coiled-coil domain of the $GABA_B$R1 subunit and the rat homologue of the transcription factor CREB2. Neither $GABA_B$R2 nor any of the related metabotropic glutamate receptors (mGluR1-5, 7) interact with CREB2. Native CREB2 displays a nuclear and extranuclear cellular distribution in cultured rat hippocampal neurones $GABA_B$R1 and CREB2 are co-localised in discrete puncta throughout the dendrites. In both clonal cell lines and cultured rat hippocampal neurones activation of $GABA_B$ receptors leads to a marked redistribution of CREB2 within the cells. The demonstration that a transcription factor can bind directly to a neurotransmitter receptor and that both co-localise outside the nucleus suggests a novel neuronal signalling mechanism. We propose that one part of such a signalling pathway is that CREB2 may act to prevent dimerisation of the $GABA_B$ receptor subunits and thereby regulate surface expression of functional $GABA_B$ receptors.

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Regulation of glutamate receptor function and synaptic plasticity

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Neurotransmitter receptors mediate signal transduction at the postsynaptic membrane of synaptic connections between neurons in both the central and peripheral nervous systems. We have been studying the molecular mechanisms in the regulation of neurotransmitter receptor function. Recently we have focused on glutamate receptors, the major excitatory receptors in the brain. Glutamate receptors can be divided into two major classes: non-NMDA and NMDA receptors. Non-NMDA receptors mediate rapid excitatory synaptic transmission while NMDA receptors play important roles in neuronal plasticity and development. Studies in our laboratory have found that both non-NMDA and NMDA receptors are multiply phosphorylated by a variety of protein kinases. Phosphorylation regulates several functional properties of these receptors including conductance and membrane targeting. For example, phosphorylation of the GluR1 subunit of non-NMDA receptors by multiple kinases including PKA, PKC and CaM kinase II regulates its ion channel function. Recent studies have demonstrated that the phosphorylation of AMPA receptors is regulated during cellular models of learning and memory such as long term potentiation (LTP) and long term depression (LTD). We have also been examining the mechanisms of the subcellular targeting and clustering of glutamate receptors at synapses. We have recently identified a variety of proteins that directly or indirectly interact with non-NMDA and NMDA receptors. We have found a novel family of proteins that we call GRIPs (Glutamate Receptor Interacting Proteins) that directly bind to the C-termini of the GluR2/3 subunits of non-NMDA receptors. GRIPs contain seven PDZ domains, protein-protein interaction motifs, which appear to crosslink non-NMDA receptors or link them to other proteins. In addition, we have recently found that the C-termini of GluR2 also interacts with the PDZ domain of PICK1, a protein kinase C-binding protein that is found at excitatory synapses. Finally, the GluR2 subunit also interacts with the NSF protein, a protein involved in the regulation of membrane fusion events. These non-NMDA receptor interacting proteins appear to be involved in the proper subcellular targeting and synaptic clustering of these receptors. In addition to these studies on non-NMDA receptors, we have been characterizing a separate NMDA receptor associated protein complex that appears to be involved in synaptic targeting and downstream signaling of NMDA receptors. We have recently identified an excitatory synapse specific rasGAP, which we call synGAP, that appears to regulate synaptic ras signaling. In summary, we have examined the molecular mechanisms underlying the regulation of glutamate receptor function. These studies have suggested that regulation of glutamate receptor function may be a major mechanism for the regulation of synaptic plasticity in the nervous system.

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Molecular Mechanisms Controlling Dendrite Morphogensis in *Drosophila*

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Signaling between neurons requires highly specialized subcellular structures, including dendrites and axons. Dendrites of different neurons exhibit diverse morphologies yet little is known about the mechanisms controlling dendrite formation *in vivo*. We have established an assay system to visualize dendritic morphogenesis of sensory neurons in living *Drosophila* embryos. Time lapse analysis reveals that dendritic branching is a highly dynamic process, however, the branching pattern in a particular hemisegment is fairly invariant from embryo to embryo, suggesting a genetic program controlling the process. Laser ablation and genetic studies indicate that the dendritic branching pattern of each sensory neuron is largely independent of its neighboring neurons in developing embryos. Dendrite development is altered in *prospero* mutants and in transgenic embryos expressing a constitutively active form of the small GTPase *cdc42*. From a genetic screen we have identified several genes which control different aspects of dendrite development including dendritic outgrowth, branching and routing. These genes include *kakapo*, a large cytoskeletal protein related to plectin and dystrophin; *flamingo*, a seven transmembrane protein containing cadherin-like repeats; *enabled*, a substrate of the tyrosine kinase *Abl*; and 9 potentially novel loci. Molecular and genetic analysis of some dendritic mutants will be presented.

Compartmental calmodulin dynamics in neurons

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Calmodulin is a key transducer of intracellular Ca^{2+} signals. However, nothing is known about calmodulin mobility nor its availability in neurons. We successfully utilized MPFPR and MPFCS to analyze the diffusion of fluorescently labeled calmodulin in neurons. The use of both techniques for diffusion measurements allows for a range of probe concentrations to be used. This can provide information about *in vivo* diffusion under conditions where calmodulin is either highly buffered or less hindered by buffering interactions (i.e. saturated buffers). Use of multiphoton excitation was necessary to minimize out-of-focal plane autofluorescence and photobleaching while spatially confining measurements to femtoliter volumes. Our initial results indicate that an anomalous diffusion model best describes calmodulin diffusion inside neurons in culture. In anomalous diffusion the mean squared distance traveled is proportional to time raised to a fractional power ($\langle r^2 \rangle = \Gamma t^{\alpha}$), rather than being linearly proportional ($\langle r^2 \rangle = 6Dt$). We find differences in the measured diffusion of calmodulin in different regions of the cell body and in the dendrites. This information imparts novel constraints on how neuronal calmodulin-dependent enzymes are activated and poses implications on the role of calmodulin as a calcium detection system to regulate cellular processes.

Session 4: Proteins and RNA Targeting

Chair: Gary Banker

Visualizing protein transport in living nerve cells

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The targeting of proteins to different neuronal domains is thought to begin with the segregation of proteins into distinct populations of carrier vesicles. Downstream of this event, little is known about the mechanisms which ensure that the sorted proteins reach only the correct membrane. One possibility is that microtubule-based transport, which is required to move proteins the long distances from their site of synthesis in the soma to the plasma membrane of dendrites and axons, is selectiveBi.e., carrier vesicles are delivered to the correct domain and excluded from the incorrect domain. Alternatively, the selectivity may occur downstream of transport, via selective fusion with or retention in the plasma membrane. To examine these possibilities, we prepared GFP-tagged chimeras of the dendritic protein transferrin receptor and the axonal protein NgCAM and monitored the transport of carrier vesicles containing these proteins in mature cultured hippocampal neurons. Our results show that selective microtubule-based transport does occur and is sufficient to account for the polarized distribution of transferrin receptor. In contrast, NgCAM is transported into both dendrites and axons; thus mechanisms downstream of transport are required to account for polarization of this protein at the axonal plasma membrane. These data demonstrate that neurons utilize two distinct mechanisms for the targeting of polarized membrane proteins, one (for dendritic proteins) based on selective transport, the other (for axonal proteins) based on a selectivity "filter" that occurs downstream of transport.

Targeting of recently-synthesized mRNA to active postsynaptic sites on neuronal dendrites

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Long-lasting forms of synaptic plasticity require gene expression, and there is increasing evidence that the newly-synthesized gene products play a role in modifying synapses that have experienced particular patterns of activity. One mechanism that may underlie synapse-specific modifications is synapse-specific gene expression. Synapse-specific gene expression refers to the capability that neurons have for transporting particular mRNAs to synaptic sites on dendrites where the mRNAs can be locally translated. This presentation will review of the nature of the protein synthetic machinery that is present at synaptic sites, and provide an update regarding mRNAs that are localized in dendrites (and thus potentially present at SPRCs). I will summarize recent findings regarding the trafficking of the mRNA for the immediate early gene *Arc*. Arc is induced by synaptic activity, the newly-synthesized mRNA is transported into dendrites, and the mRNA localizes selectively at synaptic sites that have recently been activated. The synapse-specific localization process depends on NMDA receptor activation. Thus, neurons possess a mechanism that allows a precise targeting of recently synthesized mRNA to synapses that are undergoing activity-dependent modification. I will review evidence that local translation of certain mRNAs may be regulated by synaptic actvity, and then consider how these pieces might fit together to suggest a mechanism through which gene expression at the synapse mediates long-term synaptic modifications induced by activity.

The role of cytoplasmic polyadenylation in dendritic mRNA translation

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Activity-driven protein synthesis in dendrites is thought to play an important role in long-term changes synaptic plasticity. However, the mechanisms regulating the translation of dendritic mRNAs are poorly understood. In maturing oocytes, cytoplasmic polyadenylation of a set of dormant mRNAs is an essential step in their translational activation. This process requires a U-rich element in the 3' UTR of the message, the CPE (cytoplasmic polyadenylation element) and its cognate binding protein CPEB. We find that CPEB is also expressed in the brain and is localized to synapses. Further, at least one dendritically-localized mRNA, that encoding alpha-CaMKII, contains two CPEs and binds to CPEB. Visual experience induces polyadenylation of this mRNA in the visual cortex and synthesis of CaMKII protein. Pretreatment of the animal with the NMDAR antagonist CPP blocks this synthesis. To test the role of the CPE in this process we transfected cultured neurons with GFP-reporter constructs bearing wild type or CPE-mutant CaMKII 3' UTRs. We show that translation is induced by synaptic activation and requires the presence of intact CPEs. Further, NMDA receptor activation is necessary for this stimulation. Thus, CPE-regulated mRNA translation is activated in neurons in an NMDAR-dependent fashion. These results reveal a pathway between synaptic activation and translation of specific dendritic mRNAs.

Targeting of CaMKIIα mRNA and Polyribosomes to Dendrites and Spines of Hippocampal Neurons is Stimulated by BDNF and NT-3

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The regulated localization of specific mRNAs and polyribosomes to postsynaptic sites, such as dendritic spines, is believed to be an important mechanism to influence synaptogenesis and synaptic plasticity. However, no direct evidence exists for the subsynaptic localization of specific mRNAs which encode proteins that mediate synaptic plasticity. Part of the difficulty has been the lack of suitable methods to visualize the spatial organization of specific mRNAs within dendrites at high resolution. We have developed fluorescence *in situ* hybridization and digital imaging methods to localize specific mRNAs within dendrites of cultured hippocampal neurons. The mRNA encoding calcium/calmodulin dependent protein kinase IIα (CaMKIIα), a key mediator of synaptic plasticity, was distributed throughout the dendritic length within RNA granules. The abundance and distribution of CaMKIIα mRNA within dendrites and dendritic structures was distinct from other examined mRNAs. CaMKIIα mRNA granules localized within dendritic spines and colocalized with synapsin at synaptic contacts. Two neurotrophins, BDNF and NT-3, were shown to promote localization of CaMKIIα mRNA and polyribosomes into dendrites and spines. CaMKIIα mRNA levels within dendrites were increased by brain derived neurotrophic factor (BDNF), which also promoted an increase in the number of synaptic contacts. NT-3 stimulated the formation of dendritic spines and synaptic contacts. Ultrastructural analysis indicated that NT-3 stimulated the targeting of polyribosomes into dendritic spines. These results provide the first direct visual evidence for the identification of specific mRNAs which are localized to dendritic spines. Neurotrophin signals may influence synaptic architecture and plasticity by targeting specific mRNAs to sites of synapse development and/or remodelling.

Dendritic transport of RNA-containing Staufen-GFP granules in living hippocampal neurons

Martin Koehrmann

Dendritic mRNA transport and local translation at individual potentiated synapses may represent an elegant way to form synaptic memory (Martin et al., 1997). Recently, we characterized Staufen, a double-stranded RNA-binding protein, in rat hippocampal neurons and showed its presence in large RNA-containing granules which colocalize with microtubules in dendrites (Kiebler et al., 1999). In this paper, we transiently transfect hippocampal neurons with human Staufen-GFP and find fluorescent granules in the somatodendritic domain of these cells. hStau-GFP granules show the same cellular distribution, size and do also contain RNA as already shown for the endogenous Stau particles. In time lapse videomicroscopy, we show the bidirectional movement of these Staufen-GFP labeled granules from the cell body into dendrites and vice versa. The average speed of these particles was 6.4 μm/min with a maximum velocity of 24.3 μm/min. Moreover, we demonstrate that the observed assembly into granules and their subsequent dendritic movement is microtubule-dependent. Taken together, we have characterized a novel, non-vesicular, microtubule-dependent transport pathway involving RNA-containing granules with Staufen as a core component. This is the first demonstration in living neurons of movement of an essential protein constituent of the mRNA transport machinery.

The intrinsic electroresponsiveness of dendrites

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Beyond the integration of synaptic inputs, dendrites are known to have intrinsic electroresponsive phenotypes. Such phenotypes define the metaconnective character of neuronal circuit dynamics, and the resonant properties for particular classes of re-entry loops. Dendrites of different cell types tend to respond differently to synaptic inputs depending on the temporal pattern and dendritic membrane potential. Thus, dendritic electroresponsiveness is deeply modulated by the immediate past history of synaptic input activity reflecting the dynamics of channel distribution, density and level of modulation. These properties tend to facilitate the acquisition of particular ensemble activation and the ability to reset the collective properties quite rapidly. Examples at the olivocerebellar and thalamocortical levels will be discussed.

Session 5: Development

Chair: Lawrence C. Katz

Dendritic filopodia and the generation of precision and diversity during synapto genesis and neural circuit formation

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Our laboratory has used various physiological optical methods and electron microscopy to study the roles of dendritic filopodia in developmental synapse formation. Recently, we have used fluorescent labeling and time-lapse microscopy to observe early stages in the formation of an obligatory synapse between pairs of identified neurons in zebrafish embryos. We observed the Mauthner cell growth cone as it descended down the spinal cord, as well as the serially-repeating primary motoneurons, the Mauthner cell's post-synaptic targets. Upon reaching successive motoneurons, the Mauthner growth cone pauses briefly before continuing along its path. Varicosities formed at regular intervals, and were preferentially associated with the target regions of the primary motoneurons. In addition, the post-synaptic motoneurons exhibited highly dynamic filopodia, which interacted transiently with both the growth cones and the axons. Our observations indicate that both the Mauthner cell and motoneurons are highly active and each exhibit motility that is sufficient to initiate synaptogenesis. We suggest that such apparent redundancy of motility mechanisms may represent an adaptation to assure the establishment of contact between a precisely specified pair of individual neurons.

In another study, we transfected cultured rat hippocampal neurons with VAMP-GFP cDNA, a construct expressing as a fluorescently tagged synaptic vesicle protein. This method allowed the visualization of transport packets containing varied vesicular and tubulovesiclular membrane structures, α1a subunits of calcium channels, the synaptic vesicle protein SV2, synapsin Ia, and amphiphysin I in addition to VAMP. Individual transport packets range up to 1 μm in size and include membrane and protein components in amounts comparable to entire nascent active zones. After transport along developing axons at rates up to 0.5 μm/min, packets were observed to stabilize at new sites of cell-cell contact and to become competent of evoked vesicle recycling within less than 1 hour. In some cases, functional presynaptic specializations of this type were observed at sites of cell-cell contact which were initiated by protrusion of dendritic filopodia. Such observations reinforce earlier suggestions that dendritic filopodia may represent a mechanism for the generation of diversity in nascent synaptic interconnections.

The presentation will include an epigrammatic review of earlier observations of filopodial dynamics from our laboratory, including work by Paul Forscher, Craig Jahr, Monica Cooper, Michael Dailey and Noam Ziv.

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Activity-dependent mechanisms controlling dendritic arbor development

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The brain changes in response to experience. This occurs during learning in the adult and it occurs during brain development. The research in my lab is directed toward understanding how experience causes the brain to change and what exactly changes as a result of experience. We know that this is an important issue because animals raised without sensory experience, say in the dark, are never able to see normally, even if they are brought out into the light at a later point in their lives. What does the sensory stimulation do that is so essential for brain development? One valuable way to address this question is to actually look at the structure of cells in the part of the brain that processes visual information and see if they change their shape in response to visual stimulation. We can do this using state of the art imaging techniques that allow us to take three-dimensional images of single neurons in the brain of an intact living animal [1]. We have found that if we stimulate the visual system with light, then the neurons in the brain that process visual information, grow larger and more elaborate arbors compared the neurons in the brains of animals that stayed in the dark. We also found that the visual stimulation can turn on certain genes in the neurons. One of the genes which is turned on by visual activity is *candidate plasticity gene 15* (*cpg15*). Expression of the CPG15 protein makes the arbors of optic tectal neurons grow larger than normal [2]. The same protein also increases the number of contacts between neurons and therefore the communication between neurons. This indicates that there is a positive feedback loop: brain activity turns on a gene that increases the growth of the neurons and increases brain activity. When will it stop? Will the brain cells grow and grow? Much to our relief we also discovered a separate protein which works to stabilize the size of the neuron's arbor once it has grown to a certain size. This protein, CaMKII, is also sensitive to brain activity. It acts to sense how much input the neuron has coming from other neurons. The bigger the neuron, the more inputs. When the inputs reach a certain level or strength, then this protein acts to prevent the neuron from growing any larger [3,4]. We are continuing to carry out experiments designed to discover the genes and proteins that control brain development and learning.

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Control of cortical dendrites by neurotrophins

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The architecture of the dendritic arbor of cortical pyramidal neurons results from the addition, loss and selective stabilization of specific dendritic branches and spines. In numerous systems, including the cortex, the growth and stability of dendrites and spines is influenced by the levels and patterns of neuronal activity. This suggests that molecular factors must exist for transducing activity into control of dendritic growth.

One attractive set of candidate molecules are the neurotrophins, a small family of growth factors whose expression is modulated by overall levels of activity. For a number of years we have been investigating the potential roles of neurotrophins in regulating the initial outgrowth of dendrites. As a model system, we use an *in vitro* preparation of the visual cortex in which individual neurons are visualized by transfection with marker proteins (via particle-mediated gene transfer, or biolistics). Previous work in the lab established that the initial growth of basal dendrites was specifically regulated by ligands of TrkB and TrkC receptors; bath applied brain-derived neurotrophic factor (BDNF) had an especially potent effect, increasing the number of dendritic branches and spines. Interestingly, these effects could only be manifested when neurons were active, indicating a co-requirement for activity and Trk receptor activation.

Prior experiments, however, were subject to two significant limitations: they depended on a rather non-physiological presentation of neurotrophin (i.e. bath application) and all data were derived from population averages. To overcome these limitations, we initiated a study of dendritic stability in which the neurons themselves were the source of BDNF, and in which it was possible to directly assay the stability and growth of individual cells over time. To accomplish these goals, biolistics was used to co-transfect neurons with cDNAs encoding myc-tagged BDNF and green fluorescent protein (GFP). The growth and stability of neurons in postnatal day 25 ferret slices was then monitored directly using time-lapse two-photon microscopy for up to 24 hours.

In control neurons, dendrites and spines were remarkably stable. Over a day, there was virtually no gain or loss of basal dendrites, although significant elongation of dendrites was detected, along with some addition of secondary dendrites. Dendritic spines were considerably less stable: over a 16 hour time frame, about 10% of spines were lost and 15% of the original number added. In marked contrast, neurons cotransfected with BDNF showed dramatic enhancement in the growth of basal dendrites. Within 24 hours post-transfection, the neurons formed "haloes" with roughly 3-fold more dendrites than controls. Unlike control dendrites, these were highly unstable, with numerous dendrites being gained or lost over 24 hours. Even more dramatic were the effects on dendritic spines: cotransfected neurons had almost no spines, and the few remaining ones were very unstable.

Taken together, these findings suggest that BDNF serves to regulate the stability of dendrites and spines. We were next interested to determine the spatial scale over which these interactions can take place. To determine this, we performed "dual gold" experiments, in which one population (the "reporter cells") was transfected with GFP only, and a second population co-transfected with a combination of myc-BDNF and red fluorescent protein (RFP) ("secreting cells"). Through appropriate modification of our two-photon microscope, we were able to simultaneously image both populations. In the control situation, dendrites from RFP and GFP labeled cells showed no modifications even when crossing within micrometers of each other. In contrast, when GPF labeled dendrites from reporter cells crossed near the dendrites of BDNF expressing RFP cells, they formed obvious modifications, including thickenings, enhanced branches, and changes in direction, presumably as a result of BDNF originating from the secretor cells. However, these effects were remarkably local. Although still in progress, we estimate that the range over which such secreted BDNF exerts its effects must be less than 10 micrometers.

Thus, BDNF can locally modify dendrites and branches, in part by increasing growth and in part by modulating stability. Using a more natural way of delivering BDNF (i.e. by transfection into individual cells) the actions of BDNF appear to be highly local, rather than a diffuse effect on a large region of the cortex.

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Purkinje cell dendrite and spine differentiation: Granule cell influences and dynamics

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Our work aims to understand how afferent axons contact specific target cells, form synapses on target cells, and in turn, how these interactions lead to target cell differentiation, in the developing rodent cerebellum. This presentation will focus on granule cell influences on Purkinje cell dendrite and spine development.

Molecular basis of afferent-induced target cell differentiation

For the Purkinje cell, the role of climbing versus parallel fiber afferents in formation and maintenance of Purkinje dendrites and spines has been much debated. To dissect extrinsic and intrinsic influences on the development of this important cell, we have developed methods to purify one of the synaptic targets in the cerebellum, the Purkinje cell (Baptista et al., 1994). Coculture of purified granule and Purkinje cells shows unequivocally that the granule cell is a potent regulator of Purkinje cell dendritogenesis. This effect requires contact between Purkinje and granule cells. It is not clear, however, whether granule cells actually elicit spine formation directly or stimulate Purkinje cells to undergo an intrinsic program of spine differentiation.

Neurotrophins and spine development: Neurotrophins are candidates for mediating granule-Purkinje cell interactions leading to Purkinje dendritic differentiation. Both granule and Purkinje neurons express neurotrophins and their receptors (see Morrison and Mason, 1998). The culture model based on purified Purkinje cells enabled us to analyze the role of growth factors in Purkinje cell survival and differentiation (Morrison and Mason, 1998). A critical balance of neurotrophin and neural activity is required for Purkinje cell survival in cocultures of purified granule and Purkinje cells. In this setting, neurotrophins modulate the number of Purkinje dendritic spines (Shimada et al., 1998). BDNF dramatically increases the density of Purkinje cell dendritic spines without causing a shift in the proportions of headed and filopodia-like spines. Spine increase is blocked by adding TrkB-IgG, and TrkB-IgG alone yields spines with longer necks than those in control cultures. None of the treatments alter Purkinje cell dendritic parameters. These data implicate TrkB signaling in modulating spine development, consistent with reported effects of neurotrophins on synaptic function. Experiments are in progress to determine whether BDNF acts on the granule cell or directly on the Purkinje cell to elicit these effects.

DCC is expressed by granule cells and regulates Purkinje dendrite development: Because neither BDNF nor other factors tested signaled dendrite development in Purkinje cells in isolation, or affected dendritogenesis in granule-Purkinje cocultures, we sought other molecular factors that were expressed in granule cells that would affect Purkinje dendrite development. DCC (Deleted in Colorectal Cancer) is a receptor involved in cell proliferation and differentiation. DCC is also a receptor for netrin-1, an axon guidance factor. Moreover,

DCC is expressed postnatally on granule cell parallel fibers, an appropriate spatiotemporal pattern for interaction with emerging Purkinje cell dendrites.

Function-blocking anti-DCC antibodies added to granule-Purkinje cocultures stunt Purkinje cell dendritic growth. DCC perturbation does not affect granule cell proliferation, survival, or neurite outgrowth, but does impair granule cell association with Purkinje cells. Because granule cell influence is thought to be contact-mediated, signaling for dendritogenesis would be diminished if fewer granule cells were in contact with Purkinje cells. A panel of markers that are granule cell stage-specific is being utilized to resolve whether block of DCC function leads to disruption in granule cell surface proteins or transcription factors. This would in turn, disallow Purkinje cell-granule cell contacts that would otherwise induce Purkinje dendritic development. As with studies on BDNF, mix-and-match cultures of cells from wildtype and knockout mice will also resolve whether DCC effects are via the granule cell directly.

Thus, with the distinct morphology of Purkinje cell dendrites and spines as icons, the in vitro approach allows tests of molecular regulators that direct Purkinje cell spine, dendrite and synapse development.

Purkinje cell spine dynamics

New dyes and imaging advances have led to a renaissance in the study of dendrite dynamics, interactions with synaptic partners and influences of neural activity in a more intact context. Because of our interest in cell-cell interactions, we aimed to chronicle cell behavior as a prelude to identifying molecular mediators of cell contacts, adhesion, and subsequent synaptogenesis, and to provide another assay for regulation of these events.

Purkinje spines are motile: Even though granule-Purkinje cell cocultures provide an unparalleled representation of Purkinje cell dendritic and spine development, in the living state spines are difficult to discern. In contrast, slices of mid-postnatal cerebellum allow visualization of spine dynamics: Purkinje cells are labeled through expression of green fluorescent protein delivered by biolistic gene transfer, and spine dynamics can be recorded by two-photon microscopy. This approach allowed us to witness rapid motility in Purkinje cell dendritic spines (Dunaevsky et al., 1999). Included in the motile behaviors are morphogenetic shape changes of existing spines, filopodial emergence from dendrites or spines, and spine emergence or retraction. These morphological rearrangements take place over the course of minutes, and are actin-dependent. Moreover, spine motility is more prevalent in slices from immature brain, and is unaffected by many perturbations of neural activity.

Do motile spines have synaptic contacts? The cytochalasin effects and lack of influence of neural activity argues for cell-intrinsic regulation of spine motility. Synaptic contacts have been proposed to dampen motility of immature spines and filopodia, but other new studies show that activity block depresses spine motility. To directly examine whether spines that are motile bear synapses, we analyzed identified spines imaged first by 2-photon microscopy, then, after immunostaining with antibodies to GFP, by electron microscopy. To date, our analyses reveal that there is no correlation between spine motility and the presence or absence of a synapse.

Dynamics of the presynaptic granule cell afferents and the postsynaptic Purkinje cell spines: Previous studies in vivo implicated afferent contacts in triggering target cell dendritic extension and spine formation, predicting a role for synaptic activity in spine and synapse development. Another issue we are addressing is whether granule cell afferent (parallel fiber) motility influences Purkinje cell spine dynamics. Methods have been developed for labeling the granule cell and for imaging the granule cells simultaneously with Purkinje cells. We have seen that the parallel fiber terminals are remarkably stable compared to the rapid shape changes in the spines. These observations suggest that even though a spine is in contact with a presynaptic element, spine shape can fluctuate, having important implications for synaptic function.

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Glutamate receptor regulation of actin-based dendritic spine plasticity

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Dendritic spines are rich in highly dynamic cytoplasmic actin. To investigate how this may influence spine morphology we have made time-lapse recordings from live hippocampal neurons using either actin-GFP to directly visualize the spine cytoskeleton or green fluorescent protein fused to a membrane targeting sequence from K-ras 4B (kr-GFP) to visualize the dendritic surface membrane. In either dispersed neurons or in slice cultures established from transgenic mice, both methods revealed changes in spine morphology occurring on a time-scale of seconds whereas overall dendrite morphology remained stable for up to 30 min. We also examined the influence of postsynaptic glutamate receptors on actin dynamics in dendritic spines. Actin motility was blocked by low concentrations of AMPA and this effect was insensitive to NMDA receptor antagonists indicating that inhibition depends on AMPA receptor activation. Blockade required extracellular Na^+, was mimicked by K^+-induced depolarization, and was itself blocked by low concentrations of Ni^{2+} suggesting the involvement of low voltage-activated Ca^{2+} channels. The effect was insensitive to tetrodotoxin, suggesting further that postsynaptic glutamate receptors regulate dendritic spine morphology via Ca^{2+}-dependent effects on the actin cytoskeleton.

Synapse-Specifc Facilitation and Local Protein Synthesis

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The requirement for transcription during long-lasting forms of synaptic plasticity has raised the question of whether the critical cellular unit of synaptic plasticity is the cell and its nucleus or the synapse; the presynaptic terminal and the postsynaptic dendrite. If it is the synapse, then there must be a set of mechanisms that allow the products of gene expression to alter synaptic strength at some synapses independently of others made by the same cell. To address this question, we have developed a new culture system consisting of a single *Aplysia* sensory neuron with a bifurcated neuron making synaptic contact with two spatially separated target motor neurons. By perfusing serotonin onto the synaptic connections made onto only one of the motor neurons, we have found that a single axonal branch is able to undergo both short-term and long-term synapse-specific facilitation. The long-term synapse-specific facilitation produced by repeated local exposure to serotonin is dependent on CREB-mediated transcription and involves the growth of new synaptic connections at the serotonin-treated, but not at the untreated, branch. Our studies show furthermore that the establishment of synapse-specific long-term facilitation requires rapamycin-independent local protein synthesis in the presynaptic, but not the postsynaptic, cell. This led us to examine protein synthesis in the presynaptic sensory neuron processes deprived of their cell bodies, where we found that basal protein synthesis was stimulated threefold by exposure to serotonin; part of this synthesis was rapamycin-dependent, part was rapamycin-independent.

In addition to the establishment of synapse-specific long-term facilitation produced by repeated local application of serotonin, this culture system demonstrates synaptic "capture"—the

ability to establish the long-term process at a second branch receiving a subthreshold stimulus once transcription-dependent facilitation has been established at another synapse in the same cell. Thus, when a single pulse of serotonin (which produces only transient, short-term facilitation) is applied to one synapse immediately following five pulses of serotonin to the other synapse, long-lasting facilitation occurs at both branches. Thus, the short-term process initiated by a single pulse of 5-HT has a double function. Acting alone, it produces a transient facilitation lasting minutes which contributes to short-term memory. Acting in conjunction with long-term facilitation produced either at another synapse or at the cell body, such a stimulus can mark and stabilize any other synapse of the neuron for long-term functional and structural changes by means of a covalent mark and rapamycin-sensitive local protein synthesis.

POSTERS

Calcium influx through NMDA receptors directly triggers neurotransmitter release from olfactory bulb dendritic spines

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In the mammalian olfactory bulb, signal processing is largely mediated by synaptic interaction between dendrites. Glutamate released from mitral cell dendrites excites dendritic spines of granule cells, which in turn release GABA back onto the mitral cell dendrites, forming a pair of dendrodendritic reciprocal synapses. Such a reciprocal connection makes the granule cell spines not only receive synaptic input but also produce synaptic output. How these dendritic spines perform a direct input-to-output transformation is the focus of this presentation.

It has recently been demonstrated that feedback inhibition through the reciprocal synapses is mainly mediated by NMDA receptors whereas non-NMDA receptors make little direct contribution, although both types of receptors are found to be co-localized on the granule cell spines (Chen and Shepherd, 1998; Isaacson and Strowbridge, 1998; Schoppa et al., 1998). Such a situation contrasts with a classical concept that synaptic transmission is mostly mediated by non-NMDA receptors while NMDA receptors play a modulatory role. In order to understand the mechanisms underlying these different actions of NMDA and non-NMDA receptors at the reciprocal synapses, we have hypothesized that the action of NMDA receptors is due to a direct role of Ca^{2+} influx through these receptors in triggering GABA release from the granule cell spines, whereas non-NMDA receptor-mediated EPSP might be too small to evoke sodium spikes and activate high-threshold Ca^{2+} channels, which are required for classical neurotransmitter release. To test this hypothesis, we applied caged Ca^{2+} compounds to the mitral cell dendrites to trigger glutamate release by UV flash, a way independent of voltage-gated Ca^{2+} channels. Glutamate released by photo-uncaging activated the reciprocal synapses and evoked a feedback IPSP in the recorded mitral cell. Like the spike-evoked feedback IPSP, the uncaging-evoked feedback IPSP was sensitive to extracellular Mg^{2+} ions and was blocked by APV, an NMDA receptor antagonist. In contrast, non-NMDA receptor blocker, CNQX, had little effect. A mixture of Ca^{2+}-channel blockers, at doses high enough to block both low- and high-threshold Ca^{2+} channels, only slightly suppressed the uncaging-evoked feedback IPSP. The possible involvement of Ca^{2+}-induced Ca^{2+} release from internal stores was ruled out by experiments with thapsigargin and ryanodine. Taken together, these results indicate that NMDA receptors on the granule cell dendritic spines can build up a Ca^{2+} concentration high enough to trigger neurotransmitter release directly.

Supported by NIH grants DC03918 (Wei R. Chen), DC00086 and DC/NS03972 (Gordon M. Shepherd), and by a Whitehall Foundation Research Award (Wei R. Chen).

Pyramidal cells of the human neocortex: systematic heterogeneity of dendritic arbors

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Recent studies have shown substantial, and systematic, differences in the morphology of cortical pyramidal neurones in functionally related cortical areas of monkeys (Elston and Rosa 1997, 1998; Elston, et al. 1999a-c; Lund, et al. 1993). For example, layer III pyramidal neurones in the superior temporal polysensory area (STP), which contains neurones that integrate visual, somatosensory and auditory inputs, are 6 times larger, have 4 times more dendritic branches, and 13 fold more dendritic spines, than those in the primary visual area (V1). Here we present data from the human cortex which extends these previous findings. There are significant differences in the morphology of pyramidal in areas 18, 20 and 10 of man. These results suggest that the morphological differences in pyramidal neurones results in unique circuitry in different cortical areas, and may facilitate the progressive reconstruction of inputs through areas. Moreover, these results suggst that pyramidal cells in different cortical areas have differing electromorphological properties, and itegrate inputs in different ways. Further investigations are required to determine whether these morphological differences are determined through functional interactions during development, or if there are genetic determinants of cell size.

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Principles of organization of excitatory and inhibitory synaptic inputs on hippocampal CA1 area inhibitory and pyramidal cells.

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The geometry of the cell and the ratio of excitatory vs. inhibitory inputs as well as the total number and laminar distribution of afferents influence the activity of a neuron. The geometry and afferents of functionally different subsets of inhibitory neurons and pyramidal cells were quantified in the CA1 area of the rat hippocampus. First a database containing data on geometry of 3D reconstructed parvalbumin (PV), calbindin (CB) and calretinin (CR) - containing interneurons and pyramidal cells were built. The number of excitatory and inhibitory synapses per unit length was then measured on serial EM sections.

Pyramidal cells possessed the largest dendritic arbor, ~12,000 μm. Among the interneurons PV cells had the longest dendritic tree (~4500μm). CB and CR cells had ~3500μm and 2500μm dendrites. The number of synaptic inputs per unit dendrite length on average is highest on pyramidal cells and PV cells (~300-700 and ~480/100μm respectively) and much lower on CB or CR cells (112.3 and 103.7/100μm, respectively). The total number of synaptic inputs is significantly different among the 4 neuron populations: pyramidal cells receive ~30000 synapses, PV cells ~17000 while CB and CR cells only ~4000 and ~2500, respectively.

We found common principles in the organization of synaptic inputs among the different cell populations as well as differences. For all neuron populations the proportion of inhibitory terminals increased toward the proximal dendritic/perisomatic region, thus resulting that the majority of inhibitory inputs concentrated in the perisomatic region. The total ratio of inhibitory inputs were different however for the 4 cell types. The ratio of inhibitory terminals on distal dendrites were rather low on pyramidal cells (~3%) and on PV cells (~7%), medium on CR cells (~15%) and high on CB cells (~30%). We also found differences in the ratio of inhibitory terminals in different layers. Thus pyramidal cells and PV neurons received higher amount of inhibitory terminals in str. lacunosum-moleculare then in strata radiatum and oriens.

These results predict profound differences in the integration and processing of synaptic inputs among the 4 examined cell types and in their dendrites in distinct layers.

Computer reproduction of *in vivo* CA1 population spikes along the somatodendritic axis: dendritic initiation versus backpropagation

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Synchronous action potential (AP) firing of CA1 pyramidal cells originates an extracellular population spike (PS) along the somatodendritic axis whose precise spatiotemporal map results from the addition of all unitary somatic and dendritic transmembrane currents (I_m). Recently, we reported a notable and variable contribution of backpropagating APs to the experimental PS. We also have reproduced the PS recorded *in vivo* using an architectonically realistic model of the CA1 region immersed in a volume conductor. Earlier, we used and checked the available experimental data on subcellular and macroscopic parameters to reproduce a single antidromic PS. The model was tuned so as to fit both unitary and aggregate main features, including precise AP and PS parameters and backpropagation. We now present some experimental and model results comparing anti- and orthodromic activation and repetitive activation. For a constant number of firing cells, the PS amplitude depended only slightly on firing synchronization within the experimental range, but critically on the activation mode (the anti-PS is 30-50 % larger than the ortho-PS), the initial locus for the action potential (initial segment or apical dendritic shaft), the length of actively invaded dendrites, the presence of prior inhibition (increase in the somatic anti-PS and decrease in both dendritic fields due to reduced backpropagation), factors easily altered during experimental manipulations. Even with low Na+ channel densities, moderate synaptic activation of apical dendrites initiated local APs that sequentially propagated toward the soma and axon, matching the experimental results obtained *in vivo*, but not *in vitro*. The modulation of transmembrane currents during repetitive antidromic APs was made by intracellular recordings and current-source density (CSD) analysis *in vivo*. Unitary a-APs followed short trains of up to 300 Hz. Backpropagation of the basal dendritic tree ceased after only 3-4 APs (at 300 Hz), whereas apical invasion required much longer trains. Repetitive orthodromic activation (3-5 Hz) caused a somatopetal shift of the AP initiation locus due to inactivating depolarization of synaptically activated far dendritic regions. Confluent results indicate that synaptic initiation of APs in dendrites of CA1 pyramidal cells has been largely underestimated. While backpropagation could still be the preferred mode during spontaneous firing *in vivo*, a conclusive evidence is still missing.

Differences in calcium dynamics among dendritic spines in neurons from mouse primary visual cortex.

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Dendritic spines are targets of excitatory inputs in the CNS and are morphologically heterogeneous. To explore the functional diversity of dendritic spines in neocortical neurons we used two-photon calcium imaging of individual spines in coronal slices from the binocular region (V1B) of mouse primary visual cortex of P14-19 mice. Layer V pyramidal neurons were patched and filled with 200 μM Calcium Green. Calcium dynamics of spines were imaged with a custom-made two-photon microscope and were triggered with backpropagating action potentials.

We investigated if the location of the spine within the dendritic tree influences its intrinsic calcium dynamics. Although we detected no difference in onsets of calcium accumulations among different spines, we found two classes of spines with different offset kinetics. In one group of spines, present in secondary and tertiary branches of layer V pyramidal neurons, the decay of calcium kinetics was mono-exponential with a mean τ of 272±72 ms (n=10). In a second group of spines, located in primary dendrites, the decay was double-exponential with a short τ of 180±38 ms and a long τ of 1078±125 ms (n=4). The decays of the first class of spines and the slow decays of the second class were linearly correlated to the decays of the adjacent dendritic shaft. The decay of the dendrite was also linearly correlated with its diameter, suggesting that a plasma membrane calcium pump or a pump that scales linearly with the diameter control its decay kinetics.

We conclude that the diameter of a dendrite influences the calcium kinetics of its spines during action potentials. Because the calcium dynamics are crucial for specific activation of conductances and enzymes, and the timing of AP-induced calcium accumulations regulates synaptic plasticity, the location of spines on the dendritic tree may therefore influence their calcium-driven electrophysiological or biochemical events.

(Supported by the NEI, the HFSP and the EJLB Foundation)

Dendro-dendritic contact is cell type specific during retinal development

Christian Lohmann

Both intrinsic and extrinsic factors contribute towards shaping the dendritic arbors of neurons. The shaping of retinal ganglion cell (RGC) dendrites includes two concurrent processes: 1. The expansion of the dendritic field. 2. The stratification of dendrites of ON and OFF RGCs into two different sublaminas of the inner plexiform layer (IPL). In the mature animal ON RGCs are depolarized and OFF RGCs are hyperpolarized by light onset. RGCs can adjust the growth of their dendrites to local changes in cell density to maintain uniform dendritic coverage. It was hypothesized that contact mediated inhibition between RGCs of the same class and sign (ON/OFF) regulates dendritic growth to obtain and maintain an optimal sampling of visual space. However, cell class or sign specific contacts between RGCs have not been described so far. We therefore examined the spatial relationship between the dendrites of alpha RGCs in the developing ferret retina from the age when cell classes can be identified (P2/3) until the age when dendrites are stratified (P17-P23). We used multiphoton microscopy to image and reconstruct the arbors of pairs of neighboring RGCs, which were intracellularly filled with two different dyes. Our results show that the stratification of alpha RGC dendrites in ferrets begins before birth and is completed by the third postnatal week. Dendrites of neighboring cells of the same sign (ON/ON or OFF/OFF) displayed conspicuous "contacts" between each other: Segments of each cell's dendrite were apposed and fasciculated for at least a distance of 10 µm up to 44 µm. Even before stratification was complete (at P2-9) dendrites of neighboring alpha RGCs displayed these fasciculations, if they were of the same sign. Fasciculation of dendrites was never observed between different sign cells although the proximity of their dendrites at these ages could make contact possible. Interestingly, the relative overlap of the dendritic fields and the relative position of dendritic fasciculations between same sign alpha RGCs remained constant in spite of the substantial expansion of the dendritic fields during the investigated period. These results suggest that cell class and sign specific dendro-dendritic contacts between alpha RGCs may regulate the growth of their dendrites and may be important for the accomplishment and maintenance of uniform dendritic coverage.

Regulation of spine calcium compartmentalization by rapid spine motility

Ania Majewska

Dendritic spines receive most excitatory inputs in the CNS and compartmentalize calcium. Although the mechanisms of calcium influx into spines have been explored, it is unknown what determines the calcium decay kinetics in spines. With two-photon microscopy we investigate action-potential-induced calcium dynamics in spines from rat CA1 pyramidal neurons in slices. The $[Ca^{2+}]_i$ in most spines shows two decay kinetics: an initial fast component, during which $[Ca^{2+}]_i$ in spines decays to dendritic levels, followed by a slower decay phase where the spine follows dendritic kinetics. The correlation between $[Ca^{2+}]_i$ in spine and dendrite at the breakpoint of the decay kinetics demonstrates diffusional equilibration between spine and dendrite during the slower component. To explain the faster initial decay we rule out saturation or kinetic effects of endogenous or exogenous buffers and focus instead on: (i) active calcium extrusion and (ii) buffered diffusion of calcium from spine to dendrite. The presence of an undershoot in most spines indicates that extrusion mechanisms can be intrinsic to the spine. Supporting the two mechanisms, pharmacological blockade of SERCA pumps and the length of the spine neck affect spine decay kinetics. Using a mathematical model we find that the contribution of calcium pumps and diffusion varies from spine to spine. We conclude that dendritic spines have calcium pumps and that their density and kinetics, together with the morphology of the spine neck, determine the time during which the spine compartmentalizes calcium.

Measuring dendritic calcium concentration and regulation with a new single-wavelength method

Miguel Maravall and Karel Svoboda

We present a new method for determining intracellular free calcium concentration ([Ca2+]) from single-wavelength fluorescence signals. Contrary to previous single-wavelength approaches, this method does not require independent estimates of resting [Ca2+] but relies on measuring fluorescence close to indicator saturation. Consequently, it works well with [Ca2+] indicators that can reach saturation under physiological conditions. In addition the method requires that the indicators have large dynamic ranges. We show that the popular indicators Oregon Green BAPTA-1 (OGB-1) and Fluo-3 fulfill these conditions. As a test of the method, we have measured dendritic calcium concentration and regulation in pyramidal neurons from slices of rat cortex and hippocampus, using OGB-1 and two-photon laser scanning microscopy. In the apical dendrites of CA1 pyramidal cells, resting [Ca2+] was 32-59 nM (mean=47 nM, n=12). The method also enables estimates of peak [Ca2+] changes and of buffering capacity. For CA1 pyramidal neurons (n=7), these quantities' values were respectively in the ranges 178-312 nM (mean=245 nM) and 44-80 (mean=61). Results from young animals (postnatal days (P) 14-17) were comparable to previous ratiometric estimates (Helmchen et al., 1996). Cells from older rats (P 24-28) showed no significant differences in mean; however, dendritic buffering capacity showed more variability across neurons from older animals. We expect this method to be widely applicable to measurements of [Ca2+] and [Ca2+]-dependent processes in small neuronal compartments, particularly in many situations where wavelength ratio imaging is not possible.

Ca^{2+} CURRENTS IN DISSOCIATED AND *IN VITRO* MOUSE SYMPATHETIC NEURONS. MECHANISMS OF ACTIVATION OF Ca^{2+} DEPENDENT K^+ AND Cl^- CONDUCTANCES.

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In mouse sympathetic neurons of the superior cervical ganglion (SCG), Ca^{2+} entry during spike firing activates Cl^- (depolarizing) and K^+ (hyperpolarizing) conductances. We have investigated the types of Ca^{2+} channels that are present in dissociated SCG neurons using the perforated-patch technique and in intact neurons using sharp microelecrodes in an *in vitro* preparation of the SCG. In addition, we have studied if Ca^{2+} entry through particular types of channels activates preferentially Cl^- or K^+ channels.

Voltage-clamp recordings of Ca^{2+} currents using selective pharmacological blockade revealed the presence of L-, N-, P/Q- and R-type Ca^{2+} channels both in cultivated and intact cells.

In intact cells, blockade of L- and P-type Ca^{2+} channels both decreased the Ca^{2+}-activated Cl^- current generated by an action potential by about 70%. In contrast, blockade of N-type Ca^{2+} channels reduced the apamin-sensitive Ca^{2+}-activated K^+ current. Furthermore, ryanodine (20μM) reduced the Ca^{2+}-activated Cl^- current following an action potential by 75% but on average did not affect the Ca^{2+}- activated K^+ current.

We conclude that: a) a number of Ca^{2+} channels must be in the dendrites and hence the process of dissociation affects the proportion of the different Ca^{2+} currents. b) Ca^{2+} entering through L- and P-type Ca^{2+} channels preferentially activates the Cl^- current in mouse sympathetic neurones, predominantly through Ca^{2+}-induced Ca^{2+} release, whereas Ca^{2+} that activates the K^+ channels enters predominantly through N-type channels. The data can be explained by the selective association of each type of Ca^{2+} channel with a particular type of Ca^{2+}-activated channel and/or with intracellular Ca^{2+} stores, probably at discrete sites on the soma and dendrites. (DGICYT grants: PM95-0107and PM98-0102-C02-01, Spain).

Mapping somatic and dendritic GABA-evoked depolarization and calcium transients from developing visual cortex *in-vitro*

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The depolarizing effect of GABA has been attributed to a cytoplasmic accumulation of chloride during GABAa receptor activation. Alternatively, it could be due to a specific effect mediated by a different type of GABA receptor. GABA has been reported to cause an increase in internal calcium concentration both in neocortical and hippocampal pyramidal cells. We therefore further investigated the spatial and temporal characteristics of GABA-induced depolarizations and their possible involvement in internal calcium increases.

Here, we investigate GABA-evoked depolarization by recording in whole-cell and perforated-patch configurations whilst GABA applications (10 mM) were performed along the soma and proximal apical dendrite. In both configurations the application of GABA induced a clear somatic depolarization, being of 28 ± 6 mV and 12 ± 3 mV in whole-cell and perforated-patch respectively. We explored this effect by mapping the amplitude of somatic GABA-evoked depolarization in response to GABA application along the apical dendrite. Amplitude of somatic GABA-evoked depolarizations decreased in whole-cell configuration in response to GABA application up to 100-150 μm distance from the soma. For applications at 100 μm along apical dendrite, somatic GABA-evoked depolarization was 22 % less compared to somatic application of GABA. In perforated-patch configuration smaller somatic depolarizations were observed for GABA application up to 80-100 μm along apical dendrites (10 ± 1 mV at 80 μm).

We also investigate the possibility that GABA-evoked depolarization triggers Ca^{2+} transients by loading cells with 100-150 μM Calcium-Green-1 and using 2-photon microscopy. Somatic and dendritic Ca^{2+} increases in layer V pyramidal neurons from rat visual cortex were recorded in response to voltage steps (from –70 mV to + 10 mV). Calcium responses consisted in transients of ~ 200 ms recorded at soma and proximal apical dendrites. Nevertheless, calcium transients were not observed in response to GABA application neither at somatic nor dendritic compartments (10 mM, n=5).

In summary, the somatic and dendritic application of GABA triggers depolarization in layer V pyramidal neurons from immature visual cortex both in whole-cell and perforated-patch configurations. Calcium transients triggered by such a depolarization failed to be detected.

L.M.P was supported by an EMBO fellowship.

Experience dependent reductions in spike amplitude attenuation: A role for back-propagating action potentials in the construction of spatial representations within the rodent hippocampus.

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Activity dependent attenuations in extracellular spike amplitude are known to correlate with a decrease in the effectiveness with which action potentials actively back-propagate into the dendritic arbor of hippocampal pyramidal cells (Buszaki et.al., 1996). As a rat runs through an environment hippocampal pyramidal cells fire within restricted regions of space known as "place" fields. Using tetrodes to monitor systematic changes in the extracellular amplitude of hippocampal spikes, we demonstrate that the amplitude of spikes produced as an animal enters a place field are significantly larger than spikes produced as the animal exits the field. Furthermore, the degree to which a cell's spikes show activity dependent attenuation in amplitude is reduced with an animal's experience in an environment. The observed experience dependent reductions in the degree of attenuation are input specific and are blocked by antagonists of the NMDA receptor. Our results suggest that, in the freely behaving animal, the effectiveness with which hippocampal spikes back-propagate in to a cell's dendrites can be dynamically modulate on both short time scales (i.e. a single pass through a place field) and on longer time scale (i.e. experience dependent changes in the degree of attenuation). In hippocampal slices, back-propagating action potentials play an important role in modulating synaptic strength (Magee and Johnston, 1997); consequently, systematic changes in amplitude attenuation may reflect the systematic activation of mechanisms of neural plasticity during behavior. The activation of these mechanisms of plasticity may serve to establish functional representations of an animal's environment as a consequence of experience.

Slow oscillations (< 1hz) in ferret visual cortex in vitro

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Slow oscillations (<1 Hz) occur in the cortex, thalamus and other brain structures of anesthetized animals and have been implicated in the global synchronization of brain activity during sleep (Steriade et al. J. Neurosci. 13:3252, 1993). This slow rhythm is characterized by a high degree of synchronization across long distances and between different structures, and appears to originate in the cortex. What are the cortical intrinsic and network properties that give rise to this slow oscillation? How does it synchronize large areas of the cortex? Some of these questions may be optimally answered by using in vitro preparations which allow pharmacological and other manipulations. However, to our knowledge, slow oscillations have never been recorded in cortical slices. Using an ACSF in which ionic concentrations were modified to more closely match that in situ, we have recorded slow oscillations (0.1-0.7 Hz) from slices of adult ferret visual cortex maintained in vitro. This slow rhythm resembles in vivo slow oscillations. When cells are hyperpolarized, the oscillations appear as recurrent series of PSPs of 0.4-1 s in duration. These episodes of synaptic activity are correlated with extracellularly recorded action potentials in other areas of the slice as far as 10 mm apart and across different layers. When the membrane potential is above threshold, the oscillation is recorded as a series of action potentials usually followed by a hyperpolarization that lasts several seconds. The origin and mechanisms of propagation of this oscillation will be considered.

Synchronization of cortical interneurons by perisomatic and dendritic mechanisms

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Neural networks in the cortex can engage in synchronous oscillations in the gamma frequency range, a coherent activity likely to subserve a code in neural information processing. Networks of GABAergic neurons are implicated in synchronizing cortical activity, but the cellular mechanisms underlying rhythmic interactions amongst interneurons remain to be defined.

Coronal slices were prepared from rat somatosensory cortex and we performed in vitro paired whole-cell recordings combined with light- and electron microscopic analysis of connected pairs of identified GABAergic neurons.

Three types of interactions were identified between basket cells targeting the perisomatic domain of postsynaptic neurons. The first type of basket cell connection was mediated by IPSPs showing paired pulse depression, blocked by bicuculline and therefore due to $GABA_A$ receptors. Presynaptic beta- and gamma-frequency (20-37 Hz) firing initially stopped spontaneous postsynaptic discharge, then phased postsynaptic activity in the theta frequency range. Similar results were obtained for basket cell to low-threshold spiking interneuron (LTS) and basket cell to pyramidal cell interactions. Connections mediated exclusively by electrical synapses were also identified between basket cells. Electron microscopic analysis defined the sites of interactions as one or two gap junctions between proximal dendrites and/or somata. Presynaptic 20-37 Hz spike trains elicited gap junctional potentials (GJPs) of stable amplitude and were either ineffective in modulating postsynaptic firing or entrained postsynaptic suprathreshold activity with a substantial (~10 ms) phase lag. The third class of basket cell-basket cell interactions was conjointly mediated by electrical and GABAergic synapses. Postsynaptic responses were comprised of GJPs followed by short-latency IPSPs mediated by $GABA_A$ receptors and showing paired pulse depression. IPSPs dramatically curtailed the decay of GJPs, as the subsequent application of bicuculline resulted in a more than three-fold increase in the half-width of GJPs. After the onset of presynaptic beta- or gamma-frequency firing, postsynaptic spikes were instantaneously synchronized. This initial precision of action potential timing decreased in parallel with the use-dependent depression of IPSPs.

Interactions between interneurons targeting the dendritic domain were also effective in the timing of postsynaptic firing. Based on random samples of postsynaptic target distribution, we have found that the output of LTS cells is preferentially directed towards dendritic shafts. The first class of connections between LTS cells was mediated by dendritically evoked IPSPs showing either facilitation or no change following paired presynaptic action potentials (60 ms interval). Presynaptic beta- or gamma-frequency firing either abolished spontaneous postsynaptic discharge completely or phased postsynaptic firing. At a presynaptic firing rate of 37 Hz maximal postsynaptic firing probability was observed in synchrony with the presynaptic spikes, whereas at a rate of 20 Hz, due to a fast rebound mechanism, most of the postsynaptic spikes occurred with a phase lag of ~30 ms. LTS cells were also interconnected by electrical synapses which were either ineffective in phasing postsynaptic firing or entrained postsynaptic spikes with a substantial phase lag. The third class of LTS cell to LTS cell interactions was conjointly mediated by electrical and GABAergic synapses. Due to the non-decrementing GABAergic component, which shortened the decay of GJPs, presynaptic beta- or gamma-frequency firing effectively synchronized postsynaptic spikes for the entire duration of presynaptic activity.

The results suggest that action potential timing is regulated by multiple subcellular domain and cell class specific mechanisms within tonically active interneuronal networks. Both the suppression and precise synchronization of postsynaptic firing can be achieved by perisomatic as well as dendritic inputs and/or mechanisms. The subcellular compartmentalization of communication within and across different classes of interneurons may allow the independent modulation of inputs arriving from spatially and temporally distinct sources.

INTRINSIC MOTILITY OF DENDRITIC SPINES FROM CORTICAL AND HIPPOCAMPAL PYRAMIDAL NEURONS IN BRAIN SLICES. A. Tashiro[1]*, A. Majewska[1], A. Dunaevsky[2], C. A. Mason[2] and R. Yuste[1]. [1]Depts. Biological Sciences, and [2]Pathology, Columbia University, New York, NY 10027.

Dendritic spines have been traditionally considered stable structures. Following Crick's suggestion that spines may "twitch", it has been recently shown that spines in cultured neurons are capable of rapid motility. We previously reported motility of Purkinje cell spines in cultured slices from P12 cerebellum (Dunaevsky et al., CSH Axon Guid. Abs. 223, 1998). We have now investigated if spines from pyramidal neurons are also motile using a custom-made two-photon microscope and time-lapse imaging of pyramidal neurons transfected with GFP constructs with a gene gun. We imaged neurons in acute and cultured slices from p8-p23 neocortex and hippocampus.

We find that, in both hippocampal and cortical pyramidal neurons, most spines exhibit motility with movements of ~1 spine head diameter over times as short as 3 minutes. This motility is not an imaging artifact or a consequence of Brownian motion, because it disappears when cells are incubated at 45°C. Spine motility is also abolished by bath applications of cytochalasin-D and does not occur in whole-cell recordings, indicating that is intrinsic to the neuron and that it requires actin-based biochemical pathways.

We investigated whether the developmental stage of the neurons affects this motility. Indeed we found that spines from older animal showed less motility, although at older ages (>p19) 40 to 60% of spines still moved. Within the same age this motility was not appreciably different between acute (8hr) and cultured (2-20 DIV) slices. We also investigated the role of activity in regulating this motility. No significant effects on motility were observed by either enhancing neuronal activity (KCl, NMDA, Mg^{2+}-free ACSF, or LTP cocktails) or blocking synaptic activity (Ca^{2+}-free media or APV/CNQX). We conclude that, as in Purkinje cells (see Dunaevsky et al., Soc. Neurosci. Abs. 99), spines from pyramidal neurons in slices are capable of actin-based intrinsic motility that appears activity-independent and is reduced as neurons mature. This motility could influence the function of developing cortical circuits. (Supported by the NEI, NINDS, HFSP and the EJLB Foundation)

Properties of ryanodine-sensitive intracellular calcium stores in rat neocortical pyramidal neurons

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Ryanodine receptors (RyRs) are abundantly expressed in different types of neurons, including pyramidal neurons of the cerebral cortex. Activation of RyRs releases Ca2+ from intracelular stores, elevating intracellular Ca2+ ([Ca2+]i). We combined digital fluorometric imaging with whole-cell patch-clamp recordings, to study the temporal and spatial dynamics of Ca2+ transients triggered by caffeine, a selective agonist of RyRs, in large layer V pyramidal neurons of the rat sensorimotor cortex. Brief, focal applications of caffeine onto cell somata caused transient elevations in [Ca2+]i. These [Ca2+]i signals were potentiated following a depolarization triggered by KCl or a voltage-step. The amplitude of [Ca2+]i transients to repeated, closely spaced, caffeine applications were attenuated by ryanodine and by the SERCA pump blocker cyclopiazonic acid. In current-clamp, caffeine-activated [Ca2+]i signals led to a transient hyperpolarization. Correspondingly, in cells voltage-clamped at -60 mV, caffeine caused a Cs+ and TEA-sensitive outward current, most probably due to activation of a Ca2+-activated K+ current. Focal applications of caffeine onto pyramidal cell somata and dendrites evoked highly localized [Ca2+]i transients in both subcompartments. Using high-speed confocal imaging, we established an uneven distribution of caffeine-activated [Ca2+]i responses in the dendrites. They were localized primarily on the main apical dendrite, with the largest [Ca2+]i responses centered near dendritic branch-points. Unlike [Ca2+]i responses evoked by single back-propagated action potentials, no caffeine-evoked [Ca2+]i responses could be detected at dendritic spines. In summary, the non-homogeneous distribution of caffeine-sensitive intracellular Ca2+ stores and the activation of K+ currents by elevated Ca2+ may control the propagation of electrical signals within the dendrites of cortical pyramidal neurons. (Supported by DFG/SFB 246)

A Role for Gangliosides in Pyramidal Neuron Dendritogenesis. S.U. Walkley, M. Zervas, M. Gondré, D.A. Siegel, and K. Dobrenis. Department of Neuroscience, R.F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, NY 10461 USA

During development of the cerebral cortex postmitotic neurons migrate to their final location within the maturing cortical plate and undergo a dramatic burst of dendrite outgrowth. Primary dendrites sprout prolifically from neuronal perikarya and from proximal apical processes and within days to weeks, depending on the species, individual neurons form characteristic dendritic arbors that persist for the lifetime of the cell. Distal branches of established dendrites on pyramidal neurons may slowly elongate in adult brain, but initiation of new primary dendrites is not believed to occur at this time. However, the discovery of dendritic sprouting in a group of rare genetic brain disorders – neuronal storage diseases – has revealed that mature cortical pyramidal neurons retain the capacity for new dendritogenesis under appropriate stimulation. New dendritic membrane is produced principally at the axon hillock, emerging either as a single dendritic spine-covered enlargement (meganeurite) or as a prolific tuft of new dendritic neurites. Over time ectopic dendrites become richly invested with spines and synapses and thus resemble normal adjacent basilar dendrites. In spite of their ectopic location (at the axon hillock or meganeurite), the new dendrites appear to be an integral part of the overall dendritic arbor since experimental reversal of the storage process does not necessarily lead to their disappearance. This phenomenon of ectopic dendritogenesis has been found to exhibit both neuron-type and species differences. Cortical pyramidal neurons and a few other select cell types (e.g., multipolar cells of amygdala and claustrum) consistently sprout new dendrites, whereas other types of neurons (e.g., cortical nonpyramidal cells and motoneurons) do not. The capacity for renewal of dendritogenesis in storage diseases also has been found to exhibit a species gradient with human > cat, dog, sheep > mouse. Pyramidal neurons in humans with storage diseases often exhibit massive production of new dendritic membrane, whereas the phenomenon is present but less extensive in carnivores and ungulates, and is essentially absent in mouse models of storage diseases.

A central question in the study of ectopic dendritogenesis has been whether there are common mechanisms underlying this disease-associated phenomenon and the sprouting of primary dendrites in *normal* developing brain. Studies examining cortical neurons undergoing normal and ectopic dendritic sprouting indicate that elevated expression of one specific glycosphingolipid – GM2 ganglioside – consistently links these two events. This particular ganglioside has been found elevated in all neuronal storage diseases with ectopic dendritogenesis whereas normal mature neurons and neurons in storage diseases not characterized by ectopic dendritogenesis do not exhibit significant expression of GM2 ganglioside. Furthermore, ectopic dendrites are most abundant in GM2 gangliosidosis, a disorder in which GM2 elevation is the direct result of a catabolic defect, and there is also evidence that intraneuronal elevation of GM2 ganglioside precedes the outgrowth of ectopic dendrites. These findings are consistent with GM2 elevation being a cause, rather than a consequence, of new dendritogenesis. In examining normal developing neurons in the same species (domesticated cat) used to document the correlation between GM2 ganglioside and ectopic dendritogenesis in storage diseases, it was found that a heightened expression of GM2 again correlated with dendrite outgrowth. That is, postmigratory cortical neurons expressed GM2 ganglioside coincident with normal dendritic sprouting and after dendritic maturation was complete in the early postnatal period, GM2 levels dropped to negligible levels. Subsequent studies in developing ferret and human brains documented a similar correlation. Ultrastructural studies of all three species revealed GM2 localized to vesicles in a manner consistent with Golgi synthesis and exocytic trafficking to the soma-dendritic plasmalemma. No other ganglioside has been identified that exhibits a similar correlation, although GD2 ganglioside exhibits a vesicular staining pattern in adult neurons essentially identical to that of GM2 in immature neurons.

The functions of gangliosides in neurons and other types of cells remain poorly understood. We propose that GM2 ganglioside functions in glycosphingolipid-enriched microdomains (lipid rafts) in the plasmalemma to promote dendritic initiation through modulation of specific membrane proteins and/or their associated second messenger cascades. (Supported by NS37027)

Rapid induction of dendritic development by BMP-7

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How is it that dendrites acquire their distinct shape and branching pattern? Live cell imaging, which has been applied to analyze axonal growth, is difficult to apply to dendritic growth and maturation because these events take place over days to weeks. Here we show that bone morphogenetic proteins greatly accelerate dendritic maturation, allowing many aspects of this process to be followed in living neurons.

Recently we demonstrated that bone morphogenetic protein-7 (BMP-7), a member of the TGF-β superfamily, selectively enhances dendritic development in cultured hippocampal neurons (Withers et al., Eur. J. Neurosci., 2000). When added at the time of plating, BMP-7 markedly stimulated the rate of dendritic development. Within one day, the dendritic length of BMP-7 -treated neurons was more than twice that of control cells, but axonal growth was not increased. By three days, the dendritic arbors of BMP-7 -treated neurons had attained a level of branching comparable to that of 2 week old neurons cultured under standard conditions. This rapid onset of dendritic growth suggests that BMP-7 may be used to visualize the formation of a dendritic arbor in a single recording session.

In time-lapse recordings that began immediately after treating hippocampal neurons with BMP-7 (within hours after placing them into culture), a dendritic arbor emerged, but there was little or no axon growth. This pattern of growth was quite different from that in control cultures at a comparable stage, where axonal outgrowth dominates and immature dendrites exhibit little or no net growth. The effects of BMP-7 on the pattern of growth occurred rapidly, as early as 2 hours in some cases. During the period of the recordings, which continued for 16 to 24 hours, we observed not only the growth of primary dendrites and appearance of dendritic taper, but also the formation of many higher order branches. Frequently, the higher order branches arose as collaterals from existing dendrites rather than by branching at the growth cone. Taken together, these data show that BMPS have a rapid and profound effect on neuronal development and offer a promising new approach for analyzing the cellular mechanisms that underlie dendritic arborization.

Supported by NS17112 and NS23094

Protein Kinase Modulation of Dendritic A Channels in Hippocampus

Li-Lian Yuan and Dan Johnston

Previous studies demonstrated that there is a very high density of transient, A-type K^+ channels in dendrites of hippocampal CA1 pyramidal neurons, and that channel activity can be downregulated by a broad range of protein kinases and 2nd messengers. These channels prevent action potential (AP) initiation in the dendrites, limit the back-propagation of AP into the dendrites, and reduce the amplitudes of excitatory synaptic events. The channels thus exert a powerful control over dendritic signal propagation and neuronal excitability. Kv4.2, the best candidate for the molecular identity of these dendritic A-channels, contains consensus sequences for PKA, PKC and MAPK phosphorylation, which raises the question about functional significance of these biochemical signal pathways. We investigated action potential back-propagation in dendrites and the modulation of dendritic AP amplitude by endogenous neurotransmitters and second messenger system in CA1 pyramidal neurons of adult rat using whole-cell dendritic recordings.

Isoproterenol, an agonist of β–adrenergic(NE) receptor, boosts the amplitudes of back-propagating action potentials on distal but not proximal dendrites in a PKA-dependent manner. This effect, however, can be blocked by U0126, a MAPK inhibitor. Moreover, direct addition of 8-Br-cAMP, the activator of PKA does not rescue U0126 inhibition, suggesting MAPK is downstream of PKA in the signaling pathway. This is consistent with the biochemical evidence that MAPK can be phosphorylated by PKA and PKC. We are currently investigating the relationship between PKC and MAPK in terms of downregulation of dendritic A-channels. Understanding the interplay of PKA, PKC, MAPK and dendritic A-channels should help illuminate the mechanisms of electrical signal propagation and synaptic integration in dendrites.

HIGH RESOLUTION VOLTAGE-SENSITIVE DYE IMAGING IN MITRAL NEURONS OF THE RAT OLFACTORY BULB. S. Antic[1], W. R. Chen[1], J. Wuskel[2], L. Loew[2] and D. Zecevic[1], [1]Yale Univ. Sch. Med., New Haven, CT and [2]Univ. Connecticut Health Center, CT.

The principles of information processing in the dendrites of single neurons can only be determined by studying specific neuronal types in different experimental models. Our present understanding of the functional organization of individual nerve cells is best described by computer simulations based on restricted experimental data obtained by low spatial resolution electrical measurements. Further refinement of the existing models would be facilitated if more detailed results from direct measurements are available. To obtain such a measurement one would, ideally, like to be able to monitor, at multiple sites, subthreshold events as they propagate from the sites of origin on dendritic processes and summate at particular locations to influence action potential initiation. It is essential to be able to perform these measurements in semi-intact neuronal structures (invertebrate ganglia or brain slices) in which specific regional electrical properties of individual neurons and characteristic synaptic connections are preserved. To approximate these ideal conditions it is necessary to turn from direct electrical recording to indirect, optical measurements using voltage-sensitive dyes.

We carried out multi site optical measurements on mitral neurons in slices from the olfactory bulb of P14-P20 rats. Individual neurons were stained intracellularly, by diffusion from a patch pipette of the voltage-sensitive styryl dye JPW3028. Optical signals associated with action potentials, expressed as fractional changes in fluorescent light intensity ($\Delta F/F$), were between 1 and 3% in recordings from neuronal processes. The signal-to-noise ratio was sufficiently large that action potential and synaptic potential signals could be monitored (NeuroCCD, RedShirtImaging LLC., Fairfield, CT) from multiple sites on dendritic processes, including the terminal arborization of the primary dendrite (tuft). This distal dendritic tuft is impossible to approach by patch electrodes.

The results show that the nerve impulse can be initiated in either the axo-somatic region or in the distal tuft of the primary dendrite. The initiation site is controlled by the interaction of excitatory synaptic inputs restricted to the distal tuft and by inhibitory synaptic inputs from granular neurons. The modifying effect of excitatory and inhibitory inputs on the site of spike initiation and on the pattern of spike propagation was examined by activating synaptic inputs selectively by two stimulating electrodes positioned on the olfactory nerve and in the external plexiform layer. The temporal and spatial aspects of the initiation and propagation of the spike were readily obtained from the optical data. Additionally, since it has been established earlier, by direct measurements, that the action potential of the rat mitral neuron has the same amplitude in the soma and along the whole length of the primary dendrite, it was possible to calibrate optical signals from multiple sites in terms of membrane potential using the action potential as a calibrating signal. In this way we derived, from optical recording, the shape and size of the excitatory synaptic potential at the site of origin (distal tuft of the primary dendrite) and analyzed the spatial and temporal dynamics of its spread toward the soma. The results of experimental measurements were then used to test the existing computer model of the rat mitral neuron.

Supported by NSF Grants IBN - 9604356 and IBN– 9812301.

Glutamate receptor trafficking in the dendrite during early development: formation of functional neuronal circuitry

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Spontaneous neural activity is a widespread phenomenon with little known function. Early in postnatal development such activity may be crucial for the proper formation of functional neuronal circuits. During this early period, many glutamatergic synapses contain only NMDA-receptors (Rs) and are electrophysiologically "silent". Combining recombinant DNA delivery, immunocytochemistry, two-photon laser scanning microscopy, electron microscopy and dual whole-cell recording techniques, we here show that spontaneous neural activity in the hippocampus potentiates transmission and makes "silent" synapses functional by selectively delivering GluR4-containing AMPA-Rs. This process differs from long-term potentiation seen in older animals, which delivers GluR1-containing AMPA-Rs and requires CaMKII activity. Consistent with this special role, GluR4 expression in the hippocampus is largely restricted to the first postnatal week. Once delivered by activity, synaptic GluR4-containing AMPA-Rs are exchanged with GluR2-containing AMPA-Rs in a manner that requires little neuronal activity and maintains the enhanced strength of the synapse for at least days. The synaptic delivery of receptors induced by spontaneous activity and their subsequent exchange represents a new form of activity-dependent long-term synaptic plasticity that may be critical for the establishment of functional neuronal circuitry.

Supported by the NIH, NARSAD, Alzheimer's Association.

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107 **Workshop on Biochemistry and Molecular Biology of Gibberellins.**
Organizers: P. Hedden and J. L. García-Martínez.

108 **Workshop on Integration of Transcriptional Regulation and Chromatin Structure.**
Organizers: J. T. Kadonaga, J. Ausió and E. Palacián.

109 **Workshop on Tumor Suppressor Networks.**
Organizers: J. Massagué and M. Serrano.

110 **Workshop on Regulated Exocytosis and the Vesicle Cycle.**
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The lectures summarized in this publication were presented by their authors at a workshop held on the 5th through the 7th of June, 2000, at Columbia University, New York (USA).

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