Instituto Juan March
de Estudios e Investigaciones

141 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

The Structure of the Cortical Microcircuit

Organized by

R. Yuste, E. M. Callaway and H. Markram

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The lectures summarized in this publication were presented by their authors at a workshop held on the 17th through the 19th of June 2002, at the Instituto Juan March.

Depósito legal: M-32.494/2002
Impresión: Ediciones Peninsular. Tomelloso, 27. 28026 Madrid.

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Introduction

R. Yuste, E. M. Callaway and H. Markram

As frequently stated by Ramon y Cajal, understanding how the cortex works is arguably the most important problem in Neuroscience and one of the major challenges of modern Biology. Although some investigators argue that the function of the cortex could be understood without knowing its "wiring diagram", a sufficiently deep understanding of cortical function seems impossible without understanding the structure and basic function of the cortical microcircuit. In spite of over a century of research, the precise microcircuitry of the cerebral cortex, defined as the inter- and intralaminar connections found in any given cortical area, is still basically unknown. Indeed, it is still not even clear how many different classes of neurons exist in the cortical circuits. Widely different views coexist currently among researchers interested in cortical circuits. Some investigators argue that most cortical connections are essentially random, and the cortex is a gigantic neural network whose purpose is to shuffle and mix information in order to enable associations among any possible stimuli. Other investigators argue that the cortex is an ancient machine composed of scores of extremely precise circuits, each implementing a particular function.

While most investigators of cortical function study correlations between the activity of cortical neurons and behavioral or receptive field processing *in vivo*, there is a host of laboratories around the world whose main goal is to describe and reconstruct cortical microcircuits. These laboratories have traditionally used anatomical techniques. In the last decade, however, many new approaches have been introduced, such as dual recordings from synaptically connected neurons in slices, computerized photostimulation of specific cortical locations, optical probing of connectivity, multivariate analysis of morphological data and expression analysis of dozens of different genetic markers. These new techniques are providing exciting new information and have the potential to revolutionize the study of cortical circuits.

This workshop brought together representatives from these novel lines of work with structural researchers that represented and encompassed more traditional approaches. Our goal was to enable the extensive and, at the same time, relaxed, exchange of detailed information in order to facilitate cross-fertilization among a worldwide team of experts in different aspects of cortical microcircuitry. Such a cross fertilization appears necessary to formulate a comprehensive theory of microcircuit function. The workshop did not include the large area of *in vivo* cortical physiology, but remained focused on the architectural principles of the neocortical microcircuit. The meeting was organized into five sessions around three interrelated topics: (1) Excitation, (2) Inhibition and (3) Computation. As the reader can note from the collection of abstracts that follow, the recurrent themes, highlighted in many of the presentation as well as in the discussions that followed, were the description of different cellular types of cortical neurons, particularly among the interneurons, as well as the characterization of what appear to be very precise connections. The more ambitious goal of linking the specific circuits to particular computations was also touched upon by several presentations, although at the same time, recognized as premature at this stage. The overall sense of most participants was one of excitement, since the effort to decipher cortical circuits appears not only fruitful as an experimental program in itself, but also, increasingly related to the understanding of the "heart" of the neocortical computation. We hope the reader is able to share this excitement with us.

Rafa Yuste, Ed Callaway and Henry Markram

Session 1: Microcircuitry-Excitation 1
Chair: Kevan A. C. Martin

Properties of intra- and interlaminar synaptic connections in adult neocortex

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Dual and triple intracellular recordings with biocytin-labelling of recorded neurones, in slices of adult neocortex have been used to study intra- and interlaminar synaptic connections. The excitatory projections from layer 4 spiny cells targeted lower layer 3 pyramidal cells with a probability of a connection for each pair of cells tested that was approximately equal to the intra-laminar connectivity between excitatory cells in these two layers. Inhibitory interneurones were relatively more rarely innervated by this 'forward' projection, however. The one such layer 3 interneurone identified in cat as postsynaptic to layer 4 spiny cells, an unusually large 'basket-like' cell, was however excited by all 3 cells so tested. A minority of layer 3 interneurones are therefore activated from layer 4, but this minority appears to be densely innervated. In the return direction, from layer 3 to layer 4, no spiny excitatory cells were innervated by layer 3 pyramidal cells. This is despite the presence of pyramidal cells in layer 4 whose apical dendrites extend through layer 3. Layer 4 interneurones, particularly those in the upper part of the layer, were however frequently innervated by layer 3 pyramidal cells. Many of these interneurones innervated both the upper half of layer 4 and the lower part of layer 3, though few extended beyond layer 3. Several of these interneurones were immuno-positive for parvalbumin, suggesting that they may also have received direct thalamo-cortical input and thus be able to integrate primary sensory information with activity in both layers. (Thomson and Bannister, 2002)

A similar picture emerged from studies of connections between layers 3 and 5. Large, burst firing pyramidal cells in upper layer 5 received an extremely dense input from layer 3 pyramidal cells whose somata were within 100μm of their apical dendrites, although smaller layer 5 pyramidal cells rarely if ever received this descending input. This indicates that two parallel streams of information are being processed in layer 5, one that has direct access to inputs in layer 1 via the apical tufts of the large pyramidal cells as well as via their inputs from layer 3 pyramids, the other cannot access this information in either way. Although axon collaterals of layer 5 pyramidal cells extend to the superficial layers, they rarely innervate pyramidal cells there (Thomson and Bannister, 1998). Instead, they appear to innervate the distal dendrites of other layer 5 cells and interneurones with regular spiking behaviour (Dantzker and Callaway, 2000). Thus, 'forward' projections (from layer 4 to layer 3, and from layer 3 to 5) target pyramidal cells and some interneurones, while 'back' projections (from 5 to 3 and from 3 to 4) almost exclusively target inhibitory interneurones. These findings correlate well observations made in visual cortex *in vivo*. The response properties of neurones in each of these layers to sensory stimuli become further removed from those of thalamic relay neurones and indicative of additional levels of integration from layer 4 to 3 and from 3 to 5 (Hirsch *et al*, 1998). The influence that layer 5 pyramidal cells will have on the response properties of layer 3 and layer 4 cells would therefore be expected to be subtle and these studies suggest, act via inhibition alone.

Dual recording techniques also allow the properties of the synaptic connections involving morphologically identified neurones to be studied in detail. Pyramidal axons contact many different types of postsynaptic target in the neocortex and each class appears to receive its own unique

transform of the presynaptic spike code. This transformation occurs at the synaptic terminals at which the many different mechanisms that control the release of transmitter are differentially expressed (Thomson, 2000, for review). We do not know how the postsynaptic target cell signals its identity to the presynaptic terminal, we can only observe the outcome of this signal. To date, 5 different mechanisms that reduce the amount of transmitter released during a second or subsequent action potential and 3 that increase that release have been described. Some of these mechanisms are expressed at all terminals studied, but sometimes their presence is obscured by the functional dominance of others. Others can only be demonstrated at particular types of synapse. At some connections, therefore one or a few of these mechanisms dominate the control of release and the relationship between presynaptic firing rate and transmitter release is relatively simple. At others, combinations of these mechanisms can result in very complex relationships between the presynaptic firing rate and pattern and release, particularly at high firing frequencies. These patterns of release and the properties of the postsynaptic neurones together determine when, or whether it will fire in response to a give synaptic volley.

The more carefully the cortical circuitry is studied and the more detail that is recorded, the more impressive does the fine tuning of the connections appear.

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Imaging cortical microcircuits with single cell resolution: precision in synaptic connections and in intrinsic spontaneous dynamics

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The microcircuitry of the mammalian neocortex remains largely unknown. Although the neocortex could be composed of scores of precise circuits, an alternative possibility is that local connectivity is probabilistic or even random.

To examine the precision and degree of determinism in the neocortical microcircuitry, we used optical probing to reconstruct microcircuits in layer 5 from mouse primary visual cortex. We stimulated "trigger" cells, isolated from a homogenous population of corticotectal pyramidal neurons, while optically detecting "follower" neurons directly driven by the triggers. Followers belonged to a few selective anatomical classes with stereotyped physiological and synaptic responses. Moreover, even the position of the followers appeared determined across animals. Our data reveal precisely organized cortical microcircuits.

In a second study, we investigate the flow of spontaneous activity in the cortical microcircuitry. We use calcium imaging to reconstruct, with millisecond and single-cell resolution, the spontaneous activity of populations of neurons in unstimulated slices from mouse visual cortex. We find spontaneous activity correlated among networks of layer 5 neurons from slices of mouse primary visual layer 5 pyramidal cells. Synchronous ensembles occupy overlapping territories, often share neurons, and are repeatedly activated. Sets of neurons are also sequentially activated numerous times. Network synchronizations are blocked by glutamatergic antagonists, even though spontaneous firing persists in many "autonomously active" neurons. This autonomous activity is periodic and depends on hyperpolarization-activated cationic (H) and persistent sodium (Nap) currents. We conclude that the isolated neocortical microcircuit generates spontaneous activity, mediated by a combination of intrinsic and circuit mechanisms, and that this activity can be temporally precise.

Cortical dynamics

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Adult primary visual cortex performs a high level analysis of the visual scene, mediating contour integration and surface segmentation. These properties reflect the geometric regularities present in visual scenes, and there is a remarkable consonance between the receptive field properties of neurons in primary visual cortex (V1) and the statistics of natural scenes. Cells in V1 can encode a rich variety of complex incentive configurations, and even in V1 cells integrate information over large areas of visual space. The specificity for complex shapes is represented systematically across the cortical surface, and the functional architecture for complex stimulus configurations is related to the architecture of simple stimulus attributes. The mechanism underlying the representation of complex stimuli is found in the relationship between cortical connections and cortical functional architecture. The functional properties of primary visual cortex are subject to learning. It is capable of encoding information about stimuli that subjects are trained to recognize. We find that adult neurons, rather than having fixed functional properties, are dynamically tuned, changing their specificities according to sensory experience. We have shown further that the cortex continually changes its functional role according to the influence of attention, expectation, and perceptual task. This suggests that the properties of any cortical area, even in adults, are dynamic, being experience dependent and subject to top-down influences.

The mechanisms of experience dependent cortical plasticity can be observed at several levels – its perceptual characteristics, alterations in the representation of information across multiple cortical areas, the changes in the functional properties of neuronal ensembles, changes in local cortical circuits and changes in gene expression. The finding that plasticity of primary visual cortex are associated with both functional recovery following lesions of the CNS and with perceptual learning creates the opportunity for using it as a model for the study of the mechanisms of learning in general. It offers distinct advantages in this regard, because of our knowledge of the circuitry, receptive field properties and functional architecture of this area. Plasticity of V1 reflects an ongoing process, beginning with our early experience of the regularities of the world and continuing throughout our lives, to assimilate the specific patterns to which we become familiar.

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Functional correlation maps in neocortical microcircuits

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The neocortex consists of a diverse set of neurons intricately and precisely interconnected to form a recurrent microcircuit. The anatomy and physiology of the individual neurons and synaptic connections have been studied extensively, but the manner in which these neurons are active with respect to each other (their functional relationship) is however, not known. We developed an approach based on correlated sub-threshold synaptic activity to explore these functional relationships. Simultaneous multiple neuron whole-cell recordings were obtained from different neurons in rat neocortical slices and synaptic input was recorded before, during and after the network was stimulated with a solution containing high [K^+] and lowered divalent ions. Cross correlations of the sub-threshold membrane potential during network excitation were used to represent functional relationship between neurons. The electrophysiological identity of the different neurons was obtained from a detailed electrophysiological investigation and the anatomical identity was obtained after loading cells with biocytin and 3D anatomical reconstructions. The study reveals characteristic functional correlations between neurons depending on the type of neurons involved and the underlying synaptic architecture. We found that the functional correlations can also be used to predict detailed microcircuit properties. We conclude that this approach offers new potential insight into the functional structure of the neocortical microcircuit during activation.

Repeating templates of spontaneous activity recorded intracellularly and optically from visual cortex *in vitro*

Gloster Aaron and Rafael Yuste

Various models of cortical function have presented the neocortex as an exquisitely organized network of distributed feedback circuits (Hebb, 1949; Hopfield and Tank, 1986). These models imply that activity in the neocortex in the absence of peripheral stimulation should reflect the organization of this architecture as opposed to a purely random process. Indeed, isolated neocortical slices can generate persistent activity that can display oscillations similar to those found in intact preparations (Sanchez-Vives and McCormick, 2000), suggesting that the locus of the organization required for these oscillations is located in the cortex itself.

Results from a previous study have shown that pyramidal neurons from isolated visual cortical slices display repeating patterns of correlated activity in that sets of neurons will either fire at the same time or fire at specific time intervals relative to each other (Mao et al., 2001). These results are consistent with the existence of "synfire chains" (Abeles, 1991).

If sets of neurons spontaneously and periodically fire coordinated patterns of activity relative to each other in the isolated cortex, then this activity may be discernable to almost any single neuron, since a single neuron is synaptically connected to a large array of other neurons. Our project tested this hypothesis by recording spontaneous PSPs from single whole-cell patched layer 5 neurons in coronal slices of mouse V1 cortex and then examining these PSP records for repeated patterns of activities. From this examination we found that most neurons did indeed show 2-5 second long epochs of activity ("templates") that repeated with remarkable precision. These templates were not found when the PSP trace was randomly shuffled, nor were they found when the quality of the electrophysiological recording deteriorated. In some experiments, optical recording of activity from many neurons simultaneous with the recording of PSPs from a single neuron showed that the surrounding cortex displayed a specific pattern of activity during the template discovered in the single neuron, suggesting that the template in the single neuron was formed by a network phenomenon. Future experiments will attempt to patch those neurons participating in the templates. These results suggest that "synfire chains" may be discovered in isolated cortical slices and can be detected with the aid of a single neuron.

Session 2: Microcircuitry-Excitation 2
Chair: Patricia S. Goldman-Rakic

Scales for computational elements in the cortex

M. Abeles

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The classical parcellation of cortex is based on cyto- (myelo-) architectonics. Each area is expected to have a distinct function. This is a fundamental parcellation of function till our days. The same notion is taken also to mean that activity is induced by inputs to each area, the input information is processed for a while in the area and then outputted to another area.

In the last few decades the regional nature of function was refined by further parcellation of cytoarchitectonic areas into patches (Columns, Blobs, Barrels) with unified function. Within each patch the neurons share some specific common function (orientation, whisker). The idea of input—processing—output holds as well for the patchy cortical architecture. It has been known for long time that cortico-spinal neurons exist not only in primary motor cortex (area 4), but also in premotor (area 6) and somatosensory (area 3) cortices. But this did not disturb the basic dogma.

Most neurophysiological studies of single neurons in the cortex assume that each neuron has a distinct function. Experiments in which repetitive stimuli of the same kind are given for many times (or the same motor action is executed over and over again) support this notion. However adjacent neurons may have very different functions. This is stressed by the fact that cross correlations between spike trains of next neighbors is often flat or very week. When recording in association cortices while an animal behaves along a fixed behavioral paradigm one find that approximately one third of the neurons show "task related activity". But even this fraction may typically be further fractionated among different roles (epochs) within the task. Thus the special dimension of unity of function may be reduced to a single neuron.

However, in behaving animals, with paradigms that allow diversity one often observes periods in which the same neuron takes part in different functions. Such periods may last 0.1-1 sec. This has been shown by our group for the prefrontal and posterior parietal cortices long ago, and recently for the motor cortex. Such functional time-multiplexing is in accordance with the findings that cross-correlations between a pair of cells are dynamically changing on a time scale of a fraction of a second.

Extrapolating from the above one may ask is it possible that every spike participates in a different function?

On the theoretical ground this is quite possible. Synfire chains clearly posses the ability to multiplex functions on a spike-by-spike basis, and to readout such multiplexed processes without confusion. Experimental results on precise firing sequences show that it is possible to sort-out spikes which are part of a given process from spikes which are not.

The above will be discussed with examples from our experimental results using multi-electrode recordings in behaving monkeys.

Supported in part by grants from GIF and ISF.

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Microcircuitry of memory function in prefrontal cortex

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The stimulus-independent sustained activation of neurons in the prefrontal cortex and their content- specific coding of information constitute the fundamental cellular basis of the brain's working memory functions. The two properties – persistent neuronal firing that outlasts a stimulus by seconds; and preferential coding of stimulus parameters derive both from biophysical properties and network dynamics. My laboratory is interested in elucidating these mechanisms as they pertain to and underpin working memory capacity in primates, including humans. The nonhuman primate is an unexcelled model for in vivo examination of persistent activity as well as coding and we have used primarily ferret prefrontal cortex for complementary in vitro investigations of cellular mechanisms.

A first order hypothesis for understanding persistent activity in prefrontal cortex is that it is mediated, in part, by recurrent excitation among pyramidal neurons. This is an attractive hypothesis in that prefrontal neurons have been shown to possess a higher density of spines per neuron than in other cortices (Elston et al., 2001) and hence a possibly higher density of local excitatory synaptic connections . We have used two methods to examine recurrent excitatory interactions. Simultaneous recording of multiple neurons in monkeys trained on working memory tasks is the approach we have used to address questions of functional interactions between pairs of pyramidal cells. Our findings from diverse studies have established that microcircuits exist for memory phenomena and that they are constrained both by location in the cortex and by content, just as they are for sensory processes (Constantinidis et al.,2001). Moreover, in vitro analysis of pyramidal-pyramidal interactions has allowed us to examine the modulation of these recurrent circuits by neurotransmitters such as dopamine (Gao et al.., 2001). We have shown both in vivo and in vitro that dopamine depresses excitatory transmission between pairs of pyramidal neurons. The implications of these findings for understanding disorders of dopamine regulation, such as schizophrenia, will be discussed.

A cell's tuning function can be assumed to be critical for the accuracy of memory and comprehension. If it is sharp, memory may be razor-sharp; if blunted, memory may be inaccurate and faulty. Spike width and firing rates have been used to discriminate putative pyramidal and non-pyramidal partners as they are engaged during performance of spatial working memory task. We have shown the memory fields of prefrontal neurons are highly dependent on inhibitory input from both isodirectionally and cross-directionally tuned interneurons (Rao et al, 1999; 2000). Dopamine modulates these relationships as well both by presynaptic and postsynaptic mechanisms (Gao and Goldman-Rakic,submitted).

Finally, both excitatory and inhibitory mechanisms are intertwined in the online operations that underly the conscious thought process that is disturbed in numerous disorders (Constantinidis et al., 2002).

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Interaction of spontaneous activity with sensory responses in layer 2/3 barrel cortex

Carl Petersen and Bert Sakmann

The primary somatosensory neocortex of urethane anesthetised rats displays slow large amplitude spontaneous electrical activity observed in EEG recordings which are tightly correlated with changes in membrane potential of individual layer 2/3 pyramidal neurons observed through whole-cell recordings. These spontaneous events can be imaged with voltage-sensitive dye revealing propagating waves of activity in layer 2/3. The membrane potential of layer 2/3 pyramidal neurons is defined by two distinct states: a DOWN state at resting membrane potential and a noisy UP state depolarised by ~20mV from which spontaneous action potentials can occur. Local pharmacological blockade of ionotropic glutamatergic synaptic transmission prevents spontaneous activity, suggesting that local excitatory synaptic transmission through recurrent feedback excitation mediates these waves of excitation. Sensory responses evoked by deflection of whiskers show large trial-to-trial variability which is almost entirely accounted for by interaction with spontaneous activity. Responses during UP state were small, brief and confined to a small cortical region relative to DOWN state responses. The spontaneous activity thus appears to compete with sensory evoked responses and in some cases spontaneous events appear in fact to be a replay of recently evoked sensory experience.

Synaptic responses to sound in visual areas from visually deprived cats

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Our objective has been to determine if there is cross-modal neuronal reorganization between auditory and visual areas in visually deprived cats and if there is, to characterize it at the cellular level by means of intracellular recordings. Cats were deprived of visual stimulation by keeping them in a dark room, where they were supervised with an infrared sensitive video camera. Four different pure tone sounds between 2 and 20 KHz at 60 -70 dB were used to generate sound stimulation during 2-7 hours/day, apart from the usual noises at the animal facility. Intracellular recordings were obtained from the visual cortex of anesthetized and paralyzed cats held to the stereotaxic by hollow ear-bars containing loudspeakers inside. Auditory stimulation was generated by 150 ms to 2 s pulses of pure frequencies (1-20KHz) or white noise. Our recordings from area 17 (n=17) did not show noticeable responses to sound stimulation. However, some (n=5) of the recordings obtained at the border 17 / 18 did show clear synaptic responses to auditory stimulation. We conclude that under visual deprivation and sound stimulation, the auditory-visual innervation is functionally active and induces synaptic potentials in cortical visual neurons.

Key words: plasticity, cross-modal, *in vivo*, auditory

Supported by: GV00-138-3 and 2002FR0030.

Session 3: Microcircuitry-Inhibition 1
Chair: Jennifer S. Lund

Interplay of excitation and inhibition in the sculpting of anatomical cortical columns in macaque primary visual cortex

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The concept of a cortical column will be re-examined in macaque primary visual cortex, to consider to what extent a functionally defined column reflects any sort of anatomical entity that subdivides cortical territory. Functional studies have shown that columns relating to different response properties are mapped in cortex at different spatial scales. We suggest that these properties first emerge in mid-layer 4C through a combination of thalamic afferent inputs and local intracortical circuitry, and are then transferred to other layers in a columnar fashion, via interlaminar relays, where additional processing occurs. However, not all properties are strictly columnar since they do not appear in all cortical layers. In contrast to the functional column, an anatomically-based cortical column is defined most clearly in terms of the reciprocal connections it makes, both via intrinsic intra-areal lateral connections and via inter-areal feedback/ feedforward pathways (Angelucci et al., 2002; Lund et al., 2002). We suggest that the anatomical column boundaries are reinforced by interplay between lateral inhibition spreading beyond the column boundary and disinhibition within the column. The anatomical column acts as a functionally tuned unit and point of information collation from laterally offset regions and feedback pathways. Thalamic inputs provide the high contrast receptive field sizes of the column's neurons, intrinsic intra-areal lateral connections provide their low contrast summation field sizes, and feedback pathways provide surround modulation of column receptive fields responses. We suggest that these various excitatory puts sum and engender local inhibition within the column that modulates the activity of its output neurons.

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Quantitative classification of cortical GABA cells by the axonal morphology

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It remained to clarified how many classes of GABAergic interneurons exist in the cortical circuit. Depending on the assumption that cortical cells could be defined morphologically, chemically and physiologically, we continued their grouping into several classes by the qualitative descriptions. Recently, we've started to quantify morphological parameters of GABA cells. In the present study, we analyzed their morphological characteristics quantitatively by the 3D-reconstructions and the principal component analysis (PCA) of the morphological parameters.

In the rat frontal cortex, we divided GABAergic non-pyramidal cells into 3 groups, that is, fast-spiking (FS) cells, late-spiking (LS) cells, and non-FS cells, based on their firing characteristics. Non-pyramidal cells in layer II/III and V of frontal cortex were physiologically identified and loaded with biocytin by whole cell recording in slices from young rats (19 - 23 days postnatal). After fixation, the recorded cells were histochemically stained and embedded in Epon. Some cells were also identified immunohistochemically. Axon collaterals, boutons, somata, and dendritic branches were reconstructed using Neurolucida.

We measured following 8 parameters for the axon branching pattern and bouton distribution,: (1) number of branching points (nodes); (2) the highest order of branching; (3) branch length corresponding to the 33.3% of their cumulative plot; (4) number of boutons; (5) bouton percentage below the soma along the radial axis; (6) bouton percentage within the 200 μm vertical column; (7) bouton percentage within the 400 μm vertical column; (8) bouton percentage within the 200 μm wide horizontal slab (100 μm from soma to white matter and pial side, respectively). We also measured the bouton percentage attaching to somata.

By PCA of these 8 parameters, we obtained several principal components (PCs). We mapped nonpyramidal cells on the plane made by PC1 and PC2. This mapping revealed some clusterings on the PC1-PC2 plane. This map was purely made from axonal morphology parameters, but the clusterings on the PC plane were correlated with firing characteristics, chemical content and basket terminal formation to some extent.

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Principles of neocortical interneuron recruitment

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GABAergic interneurons constitute only a minor fraction (20-30%) of neocortical cells, but are essential for normal brain function. Recent studies have expanded our knowledge of how this small and highly diverse population of cells, inhibit neighboring neurons, but the properties of their recruitment are still largely unknown. We therefore obtained simultaneous whole-cell patch clamp recordings (n>120) from several anatomical and electrophysiological distinct types of interneurons receiving convergent inputs from up to three presynaptic pyramidal cells (PCs) in layers 2-4 of rat neocortical slices. Glutamatergic synapses formed by PCs onto interneurons were diverse in their dynamic properties, being either facilitating or depressing. Morphologically distinct interneuron types were found to receive both types of synapses. However, distinct electrophysiological subclasses of a given morphological interneuron type invariably received either only facilitating or depressing synapses (synapse mapping principle). Indeed, inputs from several PCs converging onto a single interneuron target all form synapses of the same type (n>25), and single pyramidal neurons innervating different electrophysiological interneuron subclasses (divergence) differed in their temporal dynamics (differential synaptic transmission). Facilitating synapses varied widely in their underlying kinetic properties and synaptic strengths: some target interneurons could be discharged by a single presynaptic PC, whereas others could not be discharged by even three convergent PC-inputs. Interneurons, therefore, differ in their thresholds for recruitment, alluding to functionally unique positions within the neocortical microcircuitry during network activity. Finally, in many cases PCs were found to be reciprocally connected to their target interneurons indicating that many interneurons may directly affect the population of PCs responsible for their initial recruitment. Our findings show that innervation of interneurons by PCs follows distinct organization

Keywords: neocortex, interneurons, pyramidal cells, recruitment

Basic cortical microcircuits

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The neocortex might be conceived as composed of multiple, small vertical units of information processing. Each vertical unit is subdivided across layers into different subunits of processing, these subunits being composed of multiple repeating microcircuit elements. Most researchers on cortical microantomy would agree that the skeleton of the basic microcircuit is formed by a pyramidal cell and its input-output connections, which shows the same basic pattern of synaptic connectivity in all cortical areas and species. That is excitatory inputs arrive only to the dendritic arbor and originate from extrinsic afferent systems and spiny cells (which include other pyramidal cells and spiny stellate cells). Inhibitory inputs, which mostly originate from GABAergic interneurons, terminate on the dendrites, soma and axon initial segment. These interneurons are interconnected between themselves, with the exception of chandelier cells, which only form synapses with the axon initial segment of pyramidal cells. However, the neuronal elements that made up the basic microcircuit are differentiated into subtypes, some of which are lacking or display great modification in different cortical areas of the same or different species. For example, the size, number of bifurcations, and spine density of the basal dendritic arbors of pyramidal cells (the principal neuron of the cerebral cortex) differ considerably between cortical areas within the same or different species. Furthermore, the number of neurons contained in a discrete vertical cylinder of cortical tissue is highly different in various species. Similarly, double bouquet cells, which constitute a widespread microcolumnar inhibitory system in humans and monkeys, in other species like in rats this microcolumnar organization is lacking. Thus, it is impossible to draw a sufficiently complete basic diagram of cortical circuitry that may be valid for all species. In conclusion, cortical microanatomy in different areas (within the same or different species) differs considerably among themselves and, therefore, data obtained in one area are not necessarily applicable in another. Assuming that the functional signatures of neurons in different cortical areas are determined, in part, by microanatomy and intraareal circuitry, the differences in microcircuitry are likely to be instrumental in determining function throughout the cortical areas. Therefore, it is of great interest to discern what are the basic or fundamental bricks of cortical microcircuits which are common to all cortical areas and species and what are the specific variations in a given cortical area and species.

A kinetic signature for hippocampal interneurons

R. Cossart, C. Dinocourt, J.C. Hirsch, Y. Ben-Ari, M. Esclapez, C. Bernard

Inhibitory g-Amino butyric acid (GABA) containing interneurons are a major component of most neuronal networks in the brain. They play multiple functions, ranging from the control of signal integration, action potential firing, synaptic plasticity of their postsynaptic targets to network oscillations and epileptic synchronization. Since interneuronal discharge is instrumental to insure these functions, it is important to characterize the parameters which control the firing of action potentials, and hence the properties of the synaptic inputs that these interneurons receive. Such study is difficult because of the extreme diversity of interneurons at the morphological (somatic location, dendritic arborization and axonal projection field), neurochemical and physiological levels. Since the pre- and post-synaptic properties of neurotransmission depend on both the source and the target each morphological class of interneurons could have a unique, specific repertoire of synaptic properties. Our understanding of the physiological properties of synaptic currents in interneurons in relationship to their morphology is incomplete. To examine this question, we have analyzed the kinetics of inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs) recorded in various morphological classes of CA1 hippocampal interneurons. Surprisingly, there turns out to be a strict correlation between the repertoire of kinetics of excitatory and inhibitory currents recorded in CA1 interneurons and the hippocampal layers that their axons innervate. In contrast, the repertoire of kinetics of synaptic events that excite or inhibit a given interneuron does not depend upon its somatic location or dendritic arborization. This reveals a physiological signature of interneurons and a new rule of inhibitory network organization.

Session 4: Microcircuitry-Inhibition 2
Chair: Peter Jonas

Cell type specificity of neural circuits in visual cortex

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We have studied the organization of local circuits within the primary visual cortex (V1) to better understand how neural circuits give rise to the visual response properties of cortical neurons and to cortical function in general. Intracellular labeling and reconstruction of axonal and dendritic arbors of individual neurons has revealed the cell types present within the various layers of V1 and how they might be interconnected. Relating the axonal projection patterns to the laminar and columnar functional architecture of V1 provides insight into the functional influence of the various connections. We have used "Photostimulation" to identify which of the possible connections, inferred from anatomical overlap of dendritic and axonal arbors, in fact exist. In these experiments, light is used to release caged glutamate and thus "photostimulate" small populations of neurons while recording from a single cell. This results in the identification of the locations of neurons presynaptic to the recorded cell. Analysis of functional input to individual neurons reveals that anatomically and physiologically distinct types of inhibitory and excitatory neurons typically receive input from distinctly different sources, even amongst neurons located in the same cortical layer. Thus, functional connectivity cannot be predicted from the spatial overlap of axons and dendrites. Cell type specificity confers an even finer level of organization of functional microcircuits than the laminar and columnar cortical organization. This specificity implies that future studies of relationships between circuits and function must match this level of organization. For example, because differences in functional input are correlated with morphological differences, this provides the possibility of correlating circuits with single cell receptive fields in future studies. We are also developing methods using viruses and cell type specific promoters to allow quickly reversible inactivation of selected cell types. These methods will allow *in vivo* tests of the role of particular cell types within the functioning cortical network.

Networks of GABAergic neurons in the neocortex

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Ramon y Cajal proposed a hundred years ago that interneurons are important in organizing local circuits in the neocortex. These ideas, which preceded the concept of inhibition, were raised because neocortical inhibitory neurons have strictly local axons with projection fields that are cell type specific. This suggests that different classes of interneurons may define different functional domains within the cortex. In the last 10-20 years experimental work ***in vivo*** and ***in vitro*** suggested that interneurons are not only acting in balancing excitation but could also form inhibitory networks controlling timing in the cortex. Furthermore, it has been suggested that the properties of the interactions among interneurons play a critical role in coordinating the activity of a group of interneurons. However, the properties of synaptic interactions among neocortical interneurons and how inhibitory networks may generate synchrony are only poorly understood.

We have used whole-cell recordings to study the properties of synaptic interactions among pairs of neocortical inhibitory neurons in vitro. We recorded simultaneously from pairs of fast-spiking (FS) cells in layer 5 and from pairs of latespiking (LS) cells in layer 1. The FS cells were immunoreactive to parvalbumin and had local and horizontal axon projections. The LS cells had dense horizontally oriented axons restricted mostly to layer 1.

We have found that both FS and LS cells form GABAergic chemical synapses among themselves as well as with other interneurons and pyramidal cells. We also found that both FS and LS cells form electrical synapses among themselves with high probability of occurrence (60-80% of pairs). However, FS cells did not form electrical synapses with non-FS cells and LS cells did not form electrical synapses with non-LS cells. These data demonstrate directly the presence of electrical synapses among GABAergic neurons and support the hypothesis that electrical synapses define networks of inhibitory neurons embedded within the neocortex.

Next we asked how both electrical and GABAergic connections among inhibitory neurons may allow groups of interneurons to be sensitive to synchronous excitatory inputs. First we showed that the EPSP-to-spike transformation of local excitatory inputs to FS cells is temporally precise. Moreover, this property allowed groups of FS cells interconnected by electrical and GABAergic synapses to detect the relative timing of their excitatory inputs.

Taken together, our results suggest that electrical synaptic interaction occurs among GABAergic neurons belonging to the same class, and that the electrical and GABAergic connections within groups of interneurons may play a role in the detection and promotion of synchronous activity.

Interactions between convergent unitary inputs in neocortical neurons

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The temporal relationship between input and output events appears crucial in cortical operation: important findings showed that correlated neural activity is a potential neural code underlying sensory processing in the brain. How cortical neurons integrate inputs and how neuronal output reflects input activity are key questions for the explanation of cortical function. Theoretical studies predict that the modes of integration of coincident inputs depend on their location and timing.

To test these models experimentally, we simultaneously recorded from three neocortical neurons in vitro and investigated the effect of the subcellular position of two convergent inputs on the response summation in the common postsynaptic cell. When scattered over the somatodendritic surface, combination of coincident excitatory and/or inhibitory synaptic potentials summed linearly in postsynaptic cells. Slightly sublinear summation with connection specific kinetics was observed when convergent inputs targeted closely placed sites on the postsynaptic neuron. Moderate sublinearity of summation was maintained between inputs targeting cellular compartments of relatively limited volume and also when a significant portion of inputs targeting the same compartment was activated. The degree of linearity of summation also depended on the type of connection, the relative timing of inputs, on the activation state of I_h and on the level of background synaptic activity. Furthermore, we recorded the effects of convergent, unitary inputs on postsynaptic firing and found that synchronization entrained by individual inputs was enhanced during simultaneous activation.

These results suggest that, when few inputs are active, the majority of afferent permutations undergo linear integration, maintaining the importance of individual inputs. However, compartment and connection specific nonlinear interactions between synapses located close to each other could increase the computational power of individual neurons in a cell type specific manner.

Molecular determinants in GABAergic interneurones for network synchrony and oscillatory activity

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Synchronous neuronal activity underlies a number of higher brain functions including plasticity and complex cognitive tasks. Synchronous neuronal activity at different frequencies has been found and characterized in various brain regions during development and in the adult and has been associated with distinct functions. One major aim of our research is directed towards the identification of the 'key players' involved in the generation and modulation of synchronous network activity. Thus, there is increasing evidence that GABAergic interneurones, in addition to being the main source of inhibition in the adult brain, are critically involved in the generation of synchronous activity and oscillatory activity in large networks of pyramidal neurones. Our experimental efforts, which include the use of gene-manipulated mice, are directed towards addressing the following questions: 1. Which are the critical players regarding neurochemical transmission in GABAergic interneurones that are important for synchrony and oscillations? 2. To which extent is electrical neurotransmission via gap junctions important, in which cell types does it occur and which brain rhythms are affected? 3. Which are the GABAergic interneurone subtypes that control brain rhythms at different frequencies? 4. How does developmental change of receptor and gap junction expression affect network oscillatory activity? The presentation will address the importance of differential AMPA receptor expression in GABAergic interneurones, the role of Cx36 expression in interneurones and the scientific potential residing in the technique of *in vivo* labelling of different types of GABAergic interneurones. The latter is a tremendous aid for functional and anatomical characterization of identified neuronal subtypes in the acute brain slice but also *in vivo*.

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Synaptic transmission in hippocampal interneuron networks

Peter Jonas

Mutual synaptic interactions between GABAergic interneurons are thought to be of critical importance for the generation of network oscillations and for the temporal encoding of information in the hippocampus. The functional properties of inhibitory synaptic transmission between hippocampal interneurons are, however, largely unknown. To address this issue, we have made paired recordings from putative basket cells (BCs) of rat and mouse hippocampal slices. BCs were identified either on the basis of their morphological properties by infrared differential interference videomicroscopy (in the dentate gyrus) or by enhanced green fluorescent protein (EGFP) labelling in transgenic mice that expressed EGFP under the control of the parvalbumin promoter (in the CA3 and CA1 region).

Unitary $GABA_A$ receptor-mediated IPSCs at BC-BC synapses in all hippocampal subfields showed a fast rise and decay, with a mean amplitude-weighted average decay time constant of 1 - 3 msec (at 32 - 34°C). IPSCs were approximately 2-fold faster at BC-BC synapses than at BC-principal neuron synapses in the same subfield. Synaptic transmission at BC-BC synapses showed paired-pulse depression (PPD) and multiple-pulse depression during repetitive stimulation. Electrical coupling was observed in a subset of BC-BC pairs in all subfields. Thus, fast postsynaptic conductance change, PPD, and electrical coupling appear to be general principles of synaptic transmission at interneuron-interneuron synapses in the entire hippocampal formation.

To examine the consequences of the fast postsynaptic conductance change for the generation of oscillatory activity, we developed a computational model of an interneuron network based on realistic assumptions about synaptic properties and network structure. Interneurons were represented as single compartments and endowed with interneuron-specific active conductances. 200 neurons were arranged in a ring-like structure, and were coupled by inhibitory synaptic connections and gap junctions. In comparison to an interneuron network model based on slow inhibitory postsynaptic conductance changes, the quasi-realistic model showed (1) higher coherence values, (2) higher robustness against both heterogeneity of the excitatory drive and sparseness of the connectivity, and (3) coherent oscillations over a much wider range of frequencies. A tonic excitatory drive applied to all cells resulted in the generation of high-frequency coherent oscillations over the entire gamma frequency band. However, a tonic excitatory drive applied to a subset of closely spaced interneurons evoked coherent oscillations in the ripple frequency. Thus, interneuron networks could act as robust generators of coherent oscillations, with a preferred oscillation frequency that is dependent on the spatiotemporal structure of the excitatory drive.

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Session 5: Microcircuitry-Computation
Chair: Moshe Abeles

Chance or design? The structure of axons in cat visual cortex

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Discovering the rules by which neurons interconnect has significant consequences for model of cortical microcircuits. Despite the effort devoted to studying cortical connectivity, we are still far from understanding these rules. While it is clear that the formation of any particular synapse requires that an axon grows to its target and there forms a synapse, the literature on the branching patterns of axons is largely anecdotal. Similarly, the actual placement of boutons along individual axon collaterals has been little studied. Fragments of Golgi-stained pyramidal cell axons in the rodent neocortex have provided the major source of data and the major impetus for theories of how cortex is wired. The rather surprising result from the rodent cortex is that boutons are distributed randomly along the axon. This led Braitenberg and Schuz (Anatomy of the Cortex, 1991) to propose that the cortical wiring is unspecific. Our explorations with complete axons of different types of cortical neurons from cat visual cortex indicate that the bouton distributions are skewed as in the rodent, although the spacing of the boutons are cell type specific. These long-tailed distributions, however, are consistent with diffuse and specific models of neuronal connections. We show that quite simple rules can explain these distributions and resolve the dichotomy between chance or design in cortical microcircuits.

Diversity of inhibitory input tuning and its contribution to the genesis of orientation and direction selectivity in primary visual cortex

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This quantitative investigation of the functional role of the balance between excitation and inhibition and its dynamics during sensory processing gives insight into the emergence of orientation and direction selectivity in cat primary visual cortex. Using Sharp and Patch electrodes, the visually evoked synaptic activity was measured intracellularly in a large population of cells. Various steps of postsynaptic integration in the cortical response were analyzed and compared, based on integral measures of the evoked spiking rate, of the subthreshold membrane potential changes and of input conductances. This analysis provided three indirect estimates of the presence of visually evoked inhibition: spike suppression, hyperpolarization and reduction of trial-to-trial variability of membrane potential. In addition, two methods were used to quantify the input more directly, by increasing the driving force of inhibition during spike inactivation protocols, and by monitoring the dynamics of excitatory and inhibitory conductances, in continuous mode in voltage clamp and current clamp. The results suggest three different patterns of interaction between excitation and inhibition that underlie the genesis of orientation and direction selectivity. In a first scheme, (P-P: 62% of cells), excitatory and inhibitory inputs are both selective and demonstrate the same preference as the spike. In a second scheme (P-NP: 19% of cells), excitation has the same preference as the spike, and inhibition is the strongest for non-preferred stimuli. In the last scheme (NP-NP: 19% of cells), the excitatory and inhibitory inputs are both optimal for non-preferred stimuli. In all cases, and particularly for the P-P scheme, the evoked increases in excitatory and inhibitory conductances have a tendency towards a greater temporal overlap for the non-preferred stimulus (in-phase) than for the preferred one (anti-phase).

We propose that the diversity of input combinations found in different cells reflects anatomical non-homogeneities in the pattern of intracortical lateral connectivity. This diversity may be predicted in part by neighborhood relationships derived from the topological lay-out of the orientation and direction preference map in the plane of the cortical layers. It is thought to be the result of up- and down-regulation of intracortical connectivity by correlation-based activity-dependent processes. We conclude that there exists for each individual cell a variable interaction scheme between excitation and inhibition depending on its spatial location in the global orientation map, whose diversity conditions the substrate for orientation adaptation and plasticity.

This work was supported by grants from the CNRS (Bioinformatique) and HFSP RG-103-98 to Y.F.

Differential functional roles of three inhibitory cell types in a recurrent cortical network model of working memory

Xiao-Jing Wang

It is well known that inhibitory interneurons in the cortex can be subdivided into different classes, according to their distinct physiological, morphological, and wiring properties. However, the functional implications of these interneuron types are basically unknown. Here, we propose that in a local cortical network, stimulus-selective sustained neural activity is generated by recurrent circuit mechanisms in which three major interneuron types play distinct roles. Our conductance-based network model is designed for persistent activity during working memory.

There are four cell populations: pyramidal neurons, parvalbumin-containing, calbintin-containing and calretinin-containing interneurons. The firing properties of these cell types and the synaptic connections in a microcircuit are calibrated from the anatomical and physiological data. We show that stimulus tuning of mnemonic persistent activity arises from concerted action of broadly projecting inhibition mediated by parvalbumin-containing interneurons, and localized disinhibition of pyramidal cells via calretinin-containing interneurons. Moreover, resistance against distracting stimuli (a fundamental feature of robust working memory maintenance) is subserved by calbintin-containing interneurons which inhibit dendrites of those pyramidal cells not engaged in encoding the stored stimulus. We discuss experimental evidence in support of our model, testable predictions, and implications for dopamine modulation of working memory process in prefrontal cortex.

Our model provides a theoretical framework for understanding division of labor and cooperation among different inhibitory cell types in a reverberatory cortical circuit.

Rate and timing in cortical plasticity

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Debate has raged over the last few years as to whether cortical neurons transmit information primarily in their average firing rates or in the precise timing of their spikes. I will address the related question of which features of spike trains control plasticity at cortical synapses. Using paired recording in slices we have developed a quantitative and predictive description of the joint dependence of cortical plasticity on the rate and relative timing of pre- and postsynaptic firing. The results hold important implications for which parts of the neural code are most readily stored for later retrieval. In addition we have examined the complimentary question of how plasticity changes the coding properties of cortical synapses. Prior work suggested that LTP in neocortex acts mainly by changing short-term plasticity, which changes the way cortical spike trains are read out by their postsynaptic targets. In contrast, work in the hippocampus suggests that LTP affects mainly the gain of transmission, without altering synaptic dynamics. We find that neocortical LTP has mixed effects, altering both short-term plasticity and and the overall gain of transmission. In contrast, LTD has essentially pure effects on response dynamics. Finally, we have identified the signalling pathways required for induction of spike-timing-dependent LTD. Suprisingly, this form of plasticity appears to require retrograde signalling by endogenous cannabinoids.

Molecular basis of electrophysiological diversity of neocortical interneurons

Toledo-Rodríguez M., Blumenfeld B., Wu C.Z., Luo J.Y., Mae S.L., Markram H.

Neocortical GABAergic neurons exhibit a daunting heterogeneity in electrophysiological properties. At least 15 major electrophysiological subclasses have been identified. This electrophysiological diversity is due to different constellations K^+, Ca^{2+}, and non-specific ion channels. Studies investigating the ion channel basis of the electrophysiological behavior have been limited to a few channels. Recently we have develop a series of single cell multiplex RT-PCR protocols that allow the simultaneous investigation for the expression of over 30 voltage activated ion channel alpha and beta subunits at the single cell level. We have included virtually every channel subunit that may play a role in shaping the neurons electrophysiological behavior. Whole-cell patch clamp recordings were obtained from interneurons in neocortical slices, a detailed electrophysiological analysis carried out in which over 140 parameters of the passive and active properties of the neurons was obtained and cytoplasm was aspirated for subsequent single cell multiplex RT-PCR. During recording, neurons were also loaded with biocytin in order to allow subsequent 3D anatomical computer reconstructions, morphometric analysis and objective anatomical classification of interneurons. We will present the results of detailed correlations between mRNA profiles of ion channels and the different electrophysiological features of the cell as well as correlations between expression patterns and anatomically defied neurons.

POSTERS

Two populations of tyrosine hydroxylase immunoreactive neurons and fibers in the human temporal cortex as defined by the differential expression of nitric oxide synthase

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In the mammalian neocortex, neurons containing tyrosine hydroxylase (TH), the rate limiting synthesizing enzyme for catecholamines, constitute an enigmatic and ill-defined group of aspiny non-pyramidal cells. In the human they are mostly found in layers V-VI, where another conspicuous group of non-pyramidal cells can be labeled by NADPH-diaphorase histochemistry, and by immunocytochemistry for nitric oxide synthase (nNOS). The main goal of the present work was to determine the extent to which the populations of neurons and fibers containing TH, NADPHd or nNOS overlap in the human temporal cortex, using single and double-labeling techniques. We found that 26% of TH neurons contained nNOS and that 31% of labeled nNOS neurons also expressed TH. In layer I TH and nNOS did not co-localize in horizontally orientated fibers, indicating that TH and nNOS containing fibers constitute two separate populations in this layer. In contrast, co-expression of these markers was observed in some fibers of layers II-VI. Previous studies have shown that in the human neocortex, only 50% of TH neurons contain GABA, whereas nitrergic neurons are GABAergic and contain a variety of other peptides. Therefore, it is likely that TH/nNOS neurons are those cells that express GABA and that contain a variety of peptides, while the TH neurons that do not express nNOS probably include those that do not contain GABA. Thus, these results suggest that TH neurons can be divided into at least two sub-populations of non-pyramidal cells, one characterized by greater neurochemical diversity and the other by the presence of TH alone.

(Supported by CAM 01/0782/2000 and DGCYT PM99-0105)

Reorganization of exuberant axonal arbors contributes to the development of laminar specificity in ferret visual cortex

Victor Borrell and Edward M. Callaway

Layer-specific cortical axons are believed to develop precisely from the outset without making exuberant branches in incorrect cortical layers. Recent in vitro studies, however, suggested the possibility that layer 2/3 pyramidal neurons might make incorrect branches during the initial formation of axonal arbors. This possibility was here tested directly by following the development of axonal arbors in ferret visual cortex. We began our studies at an earlier stage than previous studies and focused on layer 2/3 pyramidal neurons, which in the adult have dense axonal arbors in layers 2/3 and 5 and not in layer 4. At the earliest age examined (postnatal day 14) axonal arbors were very immature, with very few axonal branches. At P18 there were significant increases in axonal branches in both layer 4 ("incorrect" branches) and layer 5 ("correct" branches). At subsequent ages the number of incorrect branches in layer 4 decreased while there was extensive axonal growth specifically in layers 2/3 and 5. Analyses of axonal arbors growing in slice cultures showed that this developmental sequence can be mimicked in vitro. Axonal growth lacked normal specificity in slice cultures from P14 animals, but was layer-specific after P18. These studies suggest that the mechanisms that regulate layer-specific growth of layer 2/3 pyramidal cell axons do not mature prior to the growth of the first branches. Furthermore, there are likely to be mechanisms that can eliminate branches from incorrect layers.

Local functional inputs to deep layer neurons in primate V1

Farran Briggs and Edward M. Callaway

We are interested in understanding how the local circuitry involving specific neuronal cell types contributes to their cellular function. We have recently been studying neurons in the deepest layers of primate primary visual cortex (V1) because their anatomical diversity suggests they play significant roles in visual information processing.

Layer 5 of primate V1 contains three main types of excitatory neurons: non-projecting pyramidal cells, projecting pyramids, and large Meynert cells (Callaway and Wiser, 1996). Layer 6 contains eight morphologically distinct types of pyramidal neuron grouped into two main classes (Wiser and Callaway, 1996; Briggs and Callaway, 2001). Class I neurons are characterized by dense axonal and dendritic arborizations within the magnocellular (M) and/or parvocellular (P) subdivisions of the lateral geniculate nucleus (LGN) recipient layer 4C (Wiser and Callaway, 1996). Class II neurons avoid arborizing in layer 4C and instead extend axons to deep and/or more superficial cortical layers. Based on their axonal and dendritic morphologies, the excitatory neurons in layer 5 and 6 are hypothesized to play roles in functionally distinct types of local circuits (Callaway, 1998).

Close examination of the anatomy of the cell types in the deepest cortical layers reveals that these neurons have the capability to receive input from and provide output to neurons in every layer of V1. This observation illustrates the necessity of an assay of functional connections to discriminate the individual input patterns for each cell type (see Sawatari and Callaway, 1996; Dantzker and Callaway, 2000; Sawatari and Callaway, 2000; Yabuta et al, 2001, Briggs and Callaway, 2001). To identify functional connectivity, we employ scanning laser photostimulation and whole-cell voltage clamp recording to assay the laminar sources of functional excitatory input onto each type of deep layer pyramidal neuron. In order to target the rare Meynert cells for photostimulation, we will employ the use of an adeno-associated viral infection system in which adeno-associated virus (AAV) expressing GPF is injected into cortex where it infects Meynert cells causing them to fluoresce. Large fluorescing neurons are then targeted for recording.

We find that distinct cell types in layers 5 and 6 receive distinct patterns of local laminar inputs. An extensive study has been completed on pyramidal neurons in layer 6 (Briggs and Callaway, 2001). Contrary to our initial predictions, Class I neurons, regardless of their anatomical preference for the M or P subdivision of layer 4C, receive input from both subdivisions of layer 4C. M or P pathway specificity onto Class I neurons originates instead from the superficial target layers of 4Ca and 4Cb, layers 4B and 2/3 respectively. Class I neurons with dense anatomical projections to layer 4Ca receive M-dominant superficial layer input from layer 4B while Class I neurons with dense projections to layer 4Cb receive P-dominant superficial layer input from layer 2/3. Class II neurons received input from the same layers targeted by their local axonal projections, as predicted based on their anatomies.

Preliminary results from our study of input to layer 5 neurons suggest that layer 5 pyramids receive strong deep layer input. An emerging trend suggests that non-projecting layer 5 neurons receive stronger superficial layer input than projecting layer 5 neurons.

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Reelin immunoreactivity in the adult primate brain: Intracellular localization in most projection and local circuit neurons of the cerebral cortex, hippocampus, and subcortical structures

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Reelin is the protein defective both in the ataxic mutant mice reeler, and in a recessive form of human lissencephaly with cerebellar hypoplasia. Reelin has been shown to act as an intercellular signaling molecule of the extracellular matrix, regulating cell-to-cell interactions that lead to correct neuroblast positioning in development. In addition, Reelin is widely expressed in the brain of adult mammals, including humans, but its biological function/s in the adult brain remain largely unknown. To gain insight on which neuronal populations and specific circuits may be under the influence of Reelin in the adult, we have conducted an analysis of Reelin-immunoreactive neuron types in the cerebral cortex, and subcortical regions of macaques at the light and electron microscope levels. Results show that a large majority of brain neurons, including both interneurons and projection neurons, are immunoreactive for Reelin in adult macaques. The immunoreactive protein is located intracellularly, mainly in neuronal somata, dendrites. Reelin is also present in some long axonal pathways and their terminal arborizations, indicating that Reelin can be axonally transported over long distances. There is also a remarkable diversity of staining patterns of the labeled neurons. Compared to previously published reports, our data reveal a wider distribution of Reelin in adult brain of primates than in any other species investigated to date. Most brain circuits in the adult primate brain, therefore, may be under the direct influence of reelin function.

A network model of generation and propagation of slow (<1 Hz) oscillations in the cerebral cortex

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Cortical slow oscillations (<1Hz) that resemble those occuring *in vivo* during slow wave sleep (Steriade et al. J. Neurosci. 13:3252, 1993) were recorded from cortical slices *in vitro* (McCormick and Sanchez-Vives, Soc. Neurosci. Abs. 1999; 874.11). Bursts of synaptic activity (0.3 Hz) occurred in all cortical layers, originated in infragranular layers, propagated across the slice at ~10 mm/s and were followed by a silent period of 2-4 s. To understand better the mechanisms of generation and propagation of this cortical activity, we modeled it through a two-population network model of a cortical module consisting of excitatory and inhibitory units.

Neurons are modeled as conductance-based units with Hodgkin-Huxley spiking dynamics.

Excitatory neurons have a Ca-activated K current and a Na-activated K current contributing to spike-frequency adaptation at very different time scales. Neurons are interconnected with high probability if they lie within 200 microns of each other through realistic synaptic dynamics (mimicking AMPA, NMDA and GABA_A postsynaptic currents). When the neurons in the model are set to display spontaneous activity, we observe slow oscillations with active episodes at low rates followed by a slow afterhyperpolarization and a long time interval between episodes, consistent with experiments.

Oscillations propagate along the network at ~10 mm/s. We analyzed how the oscillation is generated and propagates as a result of the interplay between intrinsic membrane properties (adaptation currents) and synaptic mechanisms (spatial range and dynamics of recurrent currents).

Sponsored by NSF (IBN-9733006), A.P. Sloan Foundation and MCyT (Plan Ramon y Cajal)

Synaptic connectivity among parvalbumin-fast spiking inhibitory interneurons in the neocortex of adult mice

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Networks of GABAergic interneurons connected via electrical and chemical synapses are thought to play an important role detecting and promoting synchronous activity in the cerebral cortex (Jefferys et al., 1996; Galarreta and Hestrin, 2001b). Although networks of inhibitory interneurons are thought to operate in adult animals, the synaptic interactions among these cells have only been studied systematically in juvenile animals (Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamás et al., 2000; Venance et al., 2000; Bartos et al., 2001; Galarreta and Hestrin, 2001a; Szabadics et al., 2001). Here, we have used transgenic mice expressing the enhanced green fluorescent protein (EGFP) in cells containing parvalbumin (PV) (Meyer and Monyer, 1999) to study the synaptic connectivity among fast-spiking (FS) cells in slices from adult animals (2-7 month old). By recording simultaneously from pairs of PV-FS cells, we have found that the majority of them were electrically coupled (61 %, 14 out of 23 pairs). In addition, 78 % of the pairs were connected via GABAergic chemical synapses, often reciprocally. The average coupling coefficient for step injections was 1.5 % (n=14). GABA mediated IPSCs and IPSPs decayed with exponential time constants of 2.6 and 5.9 ms, respectively, and showed paired-pulse depression (50 ms interval). These results indicate that PV-FS cells are highly interconnected in the adult cerebral cortex by both electrical and chemical synapses, establishing networks that can have important implications for coordinating activity in cortical circuits.

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Cortical pyramidal neurons as non-linear oscillators: experiment and theory

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Cortical neurons are capable of generating repetitive discharge in response to constant current injection. These cells thus can be considered as non-linear oscillators. We indentify the underlying dynamics that produce the oscillation by probing the neuron with sinusoid inputs of different frequencies. The details of the response then yield the signatures of different dynamical mechanisms. Here we present experimental results from in vitro recordings alongside of theoretical work. The experiments show that the pyramidal neurons are capable of following the input up to a critical frequency above which period skipping and 1-to-x phase locking occurs. These cells show clear "devils staircase" behavior. The critical frequencies can be influenced by the input parameters (amplitude; the dc offset) and/or correlated with intrinsic cell characteristics (spike width). We propose that the latter can be related to the dynamics of the spike generating currents. Our thesis is that the experimental data can be explained by a canonical theory of membrane excitability and spike generation. We show that this canonical model shows the full 'devils staircase" behavior, and reproduce the critical frequency dependence on the dynamics of the spike generating mechanism.

Simulating stereotyped topology in neocortical microcircuits

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A fundamental, yet controversial, question among anatomists and theoreticians alike is to what degree the microcircuitry that interconnects neocortical neurons is random or precise. Given that neocortical tissue comprises hundreds of neuronal types, and since each neuron makes and receives thousands of connections, tracing this microcircuitry has proven daunting throughout the past century. By starting at an identified point in the in the neocortical microcircuit and employing a novel method for tracing connections optically, we observed a highly precise pattern of connectivity between individual neocortical neurons. Not only did we observe the same types of neurons targeted for connections across different animals, but even the position of these targets relative to the starting point were conserved. Our results suggest a highly conserved sub-millimeter topology of circuit elements across different individuals, indicating robust developmental control over microcircuit formation and function. Furthermore, our results indicate that a simulation infrastructure is needed for studying cortical microcircuits that will allow specification of complex, yet stereotyped patterns of connectivity between large number of computational units.

Contributing authors: [•]Cortical microcirctuit topology, [†]Specification architecture for microcircuit simulations.

Organization and hormone sensitivity of associational and callosal cortical connections in rats

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The cerebral cortex orchestrates highest order sensory, motor and cognitive functions. Behavioral studies in humans, primates and rats suggest that the maturation of some of these functions, e.g., language, motor, or spatial skills, is sensitive to gonadal steroid stimulation. The studies presented here explored anatomical endpoints of cortical circuit organization that may be relevant to this influence.

Specifically, studies paired classical hormone manipulations with microcircuit tract tracing to address hypotheses concerning hormonal shaping of the three-dimensional structure of associational and callosal connections of identified layers of representative sensory, motor, and association areas of the cerebral cortex in male rats. Studies focused separately on the supragranular and infragranular layers of the primary motor and primary visual cortex, and on the infragranular layers of the dorsal anterior cingulate cortex. The connections of each of these compartments were revealed by localized nanoliter volume injections of the retrograde tracer cholera toxin (inactive B fragment conjugated to colloidal gold), followed by reconstructions of resultant labeling with respect to cytoarchitechtonic areas and lamination. Qualitative and quantitative comparisons of this labeling among hormonally manipulated and intact animals revealed a selective effect of postnatal hormone stimulation on the lateral reach of ipsilateral labeling in primary motor cortex; in this area alone, small, but statistically significant decreases in the horizontal spread of associational labeling were revealed that were attenuated by replacing hormone-deprived rats with exogenous steroid (Venkatesan and Kritzer, 1999). Otherwise, there was a general insensitivity of the connections examined to postnatal changes in the hormone environment. The data obtained in control animals utilized in these comparisons did, however, provide extremely precise and novel detail about regional and laminar specializations of cortical circuit structure in rats, including systematic differences that seem as a rule to distinguish associational from callosal connections. Thus, in addition to being significantly foreshortened in the mediolateral plane, callosal labeling is also often distinguished from corresponding associational circuits by selective reductions in connections with laterally situated cortical zones that are typically of disparate function. For example, whereas the associational connections of primary motor cortex extended well into the medially adjacent premotor cortex and laterally adjacent somatosensory area Par1, in the contralateral hemisphere labeling extended into premotor cortex, but invaded the sensory region to a significantly lesser extent. Driven by the links between gonadal hormones and cortical function on the one hand, and between cortical structure and function on the other, these and future studies examining for example the influence of prenatal hormone exposure on the sculpting of cortical circuit organization may provide insight into the biology of sex differences in acquisition and performance of cortically mediated skills. Insights may also be gleaned regarding cortical deficits in disorders such as schizophrenia, dyslexia, and autism which are often accompanied by sex differences in illness incidence or outcome. Along the way, however, useful and highly detailed information about the precise anatomical organization of cortical circuits should also continue to emerge.

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The influence of spike history on synaptic efficacy at different stages of cortical processing

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We are interested in how the cortical microcircuit shapes functional response properties. Here, we aimed to determine how spike history influences the efficacy of presynaptic spikes to evoke firing in postsynaptic cells located at different layers of cat primary visual cortex and, hence, at different stages of cortical processing. For this purpose, a matrix of seven electrodes was introduced in the medial bank of the cortex. Since each electrode could be moved independently and run parallel to the cortical surface, we could record simultaneously from cells within the same or nearby orientation columns at different cortical layers (from layer 4 to layer 5). We recorded from 166 pairs of cortical neurons with overlapping receptive fields (percentage of overlap > 50%). In more than half of the cases (n=92),cross-correlation analysis showed strong, statistically significant, peaks with latencies consistent with monosynaptic connections1. In those 92 cell pairs, 32 showed enough signal-to-noise and spike counts to allow further analysis. In 19 cases a simple cell in layer 4 was presynaptic to a complex cell in the superficial layers; in 7 cases a simple cell in layer 4 was monosynaptically connected to a complex cell in upper layer 5; finally, in 6 instances we could record form pairs of complex cells, the first one in layers 2+3 and the second one in layer 5, also monosynaptically connected.

Our results suggest that the influence of spike history on synaptic efficacy changes according to laminar location and cell type. When simple cells in layer 4 fired at low frequencies (below 20 Hz), their synapses onto layers 2+3 complex cells showed signs of synaptic facilitation or depression depending on whether the complex cell responded, or not, to static stimuli. The connection from layers 2+3 to layer 5, however, showed no trace of synaptic facilitation at low frequencies and rather, a tendency to synaptic depression was apparent on most pairs. Finally, some layer 4 cells sent a direct projection to layer 5 that underwent strong synaptic facilitation at low frequencies.

Thus, the flow of information through primary visual cortex appears to follow two parallel, alternative, pathways originating in layer 4 that are selected for on the basis of the input pattern determined by the visual stimulus. One of them, from layer 4 simple cells to layer 5 complex cells, via a relay in the superficial layers, that is mostly active when the cortical network is firing at high frequencies (above 20 Hz). The other, a divergent connection from layer 4 to a subset of cells in the superficial layers and layer 5 that is preferentially engaged when the network gain is low.

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Sponsored by NIH and HFSPO.

Molecular phenotype of D5-positive neurons in the striatum

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Within the basal ganglia complex, the striatum plays a pivotal role in the regulation of motor control, as well as cognitive and emotional processing. The integration of information within this neural system requires the precise coordination of the activities of striatal projection neurons and interneurons. Dopaminergic input from the substantia nigra plays a crucial role regulating the activity of all striatal neurons, including interneurons. Dopamine (DA) acts on specific G protein-coupled receptors, grouped in D1-class (D1 and D5 subtypes) and D2-class (D2, D3 and D4 subtypes) families, based on their biochemical, pharmacological and physiological profiles (Missale et al., 1998). Dopamine stimulates or inhibits the cortico-basal ganglia circuits by interacting with these receptors located on striatal neurons. In this study we demonstrate the presence of D5 receptor subtype in different populations of striatal neurons, projection neurons and interneurons. The overall abundance of this receptor subtype in the striatum is low, particularly, in striatal projection neurons of both, direct and indirect projection pathways, identified by the presence of the neuropeptides dynorphin or enkephalin respectively. However, the expression of D5 receptors in striatal interneurons identified by the presence of choline acetyltransferase or somatostatin is rather high. The expression intensity in other interneurons like parvalbumin- or calretinin-positive is low to moderate. Therefore our results demonstrate the presence of D5 receptors in all striatal cell populations described so far, although with different intensities in each. The fact that a lot of striatal neurons express D5 receptors subtype suggests that this receptor have an important function in the integrative information process of the striatum.

Electrophysiological evidence for the existence of a posterior cortical-prefrontal-basal forebrain circuitry in modulating sensory responses in visual and somatosensory cortical areas

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The prefrontal cortex receives input from all sensory neocortical regions and send projections to the basal forebrain. The present study in rats tested the possibility that pathways from sensory cortical regions via the prefrontal cortex and basal forebrain back to specific sensory cortical areas could modulate sensory responses. Two prefrontal areas that responded to stimulation of the primary somatosensory and visual cortices were delineated: (1) an area encompassing the rostro-dorsal part of the cingulate cortex responded to stimulation of the visual cortex, (2) a region dorso-lateral to the first in the precentral-motor association area reacted to stimulation of the primary somatosensory cortex. Basal forebrain neurons responded to electrical stimulation of the prefrontal cortex. They were located in the ventral pallidum, substantia innominata and dorsal part of the horizontal limb of the diagonal band areas. Of the responsive neurons, 42% reacted only to 'visual' prefrontal, 33% responded only to the 'somatosensory' prefrontal stimulation and the remaining neurons (25%) reacted to both prefrontal cortical areas. The effect of basal forebrain and prefrontal cortex stimulations on tactile and visual evoked potentials was tested. Basal forebrain stimulation increased the amplitude of both somatosensory and visual evoked potentials. However, stimulation of the somatosensory prefrontal area increased only somatosensory evoked potentials (90% of the cases) while stimulation of the visual prefrontal area increased only visual evoked potentials (83%). Atropine blocked both facilitatory effects.

The proposed cortico-prefronto-basalo-cortical circuitry might be important in cortical plasticity and selective attention.

Functional and anatomic compartmentalization of primary auditory cortex (AI)

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The pattern of layer III horizontal connections reflects topographic organization of acoustic feature representation in the primary auditory cortex. A uni-modal gradient for characteristic frequency (CF) at threshold sound pressure levels (SPL) is observed along the cochlear epithelium and conserved in the caudal-rostral axis of the primary auditory cortex (AI)of the cat. In species such as the cat that have an extended iso-frequency representation in AI a modular organization of level-dependent bandwidth has been described (Read et al., PNAS 2001; Cheung et al., J. Neurophysiol. 1999). Anatomic connections between layer III neurons observe this functional modularity so that connections are most prevalent between subdomains with common bandwidth properties. Ventral division neurons of the Medial Geniculate Body (MGBv) project to layers III and IV forming a patchy anisotropic pattern that is also aligned to the iso-frequency axis of AI. Thalamocortical neuron pairs exhibiting fast cross-correlation functions can have precisely aligned CF's or show convergence of up to 1/3 of an octave (Miller et al., 2000). Thalamocortical neuron pairs can have extreme disparity in their temporal following rates as characterized with reverse correlation of dynamic noise envelops and neural spiking. Thus, it would appear that several receptive field properties converge in the transition from MGBv to AI. We describe the nature of this convergence with respect to functional compartmentalization in AI.

Domain-specific summation of unitary EPSPs and IPSPs in neocortical cells

János Szabadics and Gábor Tamás

We have shown experimentally that summation properties of IPSP-IPSP and EPSP-EPSP interactions depend on the subcellular position of the inputs. Here we address the summation of convergent, unitary EPSPs and IPSPs in neocortical cells using simultaneous triple whole-cell recordings in layers 2/3. EPSPs evoked by local pyramidal cells were paired with IPSPs evoked by electrophysiologically identified interneurons innervating separate domains of the postsynaptic cells.

Out of 24 experiments, the experimental sum 10 unitary EPSP-IPSP input combinations was similar to the calculated sum of individual responses. In these linearly summating combinations, presumably perisomatic IPSPs (n=7) were more frequent than dendritic IPSPs (n=3). Moderately non-linear (14-3 %) input summation was observed in 14 triplets and non-linearity was due to IPSP (n=11) or EPSP (n=3) dominance. Dendritically evoked IPSPs (n=8) were more effective in dominating non-linear summation than perisomatic IPSPs (n=3) when recorded at –50 mV membrane potential or at the reversal potential of the IPSP. Summation properties were not significantly correlated to the amplitude of unitary PSPs.

These results suggest that unitary dendritic IPSPs are more effective in controlling coincident excitation than perisomatic GABAergic inputs. The spatial preference of linear and non-linear input processing increase the computational power and selectivity of cortical neurons.

Excitatory synaptic plasticity in visual cortex interneurons

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Activity-dependent synaptic plasticity is important for neocortical development and sensory processing. Hebbian pairing of pre- and postsynaptic firing induces long-term potentiation (LTP) or -depression (LTD) of excitatory postsynaptic potentials (EPSPs), in neocortical and hippocampal pyramidal neurons. Also, some interneuron subtypes in hippocampus exhibit excitatory synaptic plasticity, while others do not. Here we studied for the first time excitatory synaptic plasticity in different subtypes of visual cortex interneurons.

Whole-cell patch-clamp recordings were obtained from layer 2/3 interneurons in visual cortex slices (4-week-old rats). Several classes of interneurons were identified by quantitative electrophysiological parameters (firing patterns and action potential properties) and morphological features after neurobiotin labeling and confocal fluorescence imaging.

Stimulation electrodes in layer 4 or in layer 2/3 evoked EPSPs in the recorded interneurons. The induction protocol consisted of 500 EPSP- action potential (AP) pairings at 20 Hz on a depolarizing step pulse or 100 EPSP-AP pairings at 40 Hz on membrane oscillation peaks. These pairings of coincident pre- and postsynaptic activity induced homosynaptic LTP or LTD in the paired pathway in several subtypes of interneurons and heterosynaptic plasticity in a non-stimulated control pathway.

These results indicate, that several subtypes of visual cortex interneurons exhibit homosynaptic LTP or LTD and heterosynaptic plasticity. These properties are relevant for the activity-dependent network dynamics of neocortical processing.

Supported by DFG, Humboldt Foundation, and Max-Planck Society.

Action potentials in distal apical dendrites of layer 2/3 neocortical pyramidal neurones studied *in vitro* and *in vivo*

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The dendritic trees of layer 2/3 (L2/3) and L5 pyramidal neurones receive inputs in both L1 and deeper layers of the neocortex (Cauller & Connors (1994) J. Neurosci. 14, 751-762). A cellular mechanism for associating superficial- and deeper-layer inputs has been described in L5 neurones (Larkum, Zhu & Sakmann (1999) Nature 398, 338-341). This results from two cellular properties: active backpropagation of a somatically initiated action potential (AP) and a second AP initiation zone in the apical tuft. Here we consider whether L2/3 neurones have comparable dendritic properties. We used whole-cell recording and calcium imaging to study apical dendrites of L2/3 pyramidal neurones in primary somatosensory cortex in acute slices (CCD camera) and urethane-anaesthetized rats (2-photon microscope).

Both *in vitro* and *in vivo*, action potential (AP) amplitude declined gradually from the soma (to half the somatic amplitude at approx 250mm from the soma *in vitro*). Calcium transients induced by single somatic APs were visible at (and often distal to) the principal bifurcation in all neurones, both *in vitro* and *in vivo*, but rarely in far distal L1 branches. Active AP backpropagation was confirmed *in vitro* by blocking sodium channels with TTX, which decreased both dendritic depolarization and the resulting calcium transient following injection of an AP-like waveform at the soma (under voltage clamp).

L2/3 neurones also have a distal initiation zone since a dendritically initiated regenerative potential could be induced in the majority of neurones by dendritic current injection *in vitro*. A substantial dendritic calcium transient accompanied this dendritically-initiated event. Pairing of a somatic action potential with subthreshold dendritic depolarization could also trigger this distal event. These active dendritic properties therefore provide a mechanism whereby L2/3 neurones may associate superficial- and deeper-layer inputs.

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