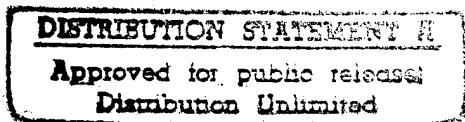


**PROGRESS REPORT FOR ONR CONTRACT
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During the period between January and December 94 our work has concentrated in 2 different areas: (i) understanding how dendrites process their synaptic inputs and (ii) characterizing the circuit diagram of the neocortex and its development. Both projects have been approached with a combination of techniques: optical imaging of calcium- or voltage-sensitive indicators, intra- and extracellular electrophysiology, and computer simulations. They have resulted in the publication of 3 manuscripts (enclosed), while another 3 manuscripts are in preparation or under review. The results have also been presented as 7 abstracts in 4 meetings. Following is a brief description of the rationale, results and conclusions for each study. A final paragraph reviews future plans.

A- Dendritic Processing in pyramidal neurons.

A1- Calcium accumulations in dendrites of neocortical pyramidal neurons: An apical band and evidence for two functional compartments. Rafael Yuste¹, Michael J. Gutnick², Drorit Saar², Kerry R. Delaney³ and David W. Tank¹. (1) Biological Computation Research Dept., AT&T Bell Laboratories, Murray Hill, NJ 07974, USA, (2) Dept. Physiology and Center for Brain Research, Faculty of Health Sciences, Ben Gurion University of the Negev, Beersheva, 84105 Israel, and (3) Dept. Biological Science, Simon Fraser University, Burnaby, B.C., V5A 1S6 Canada.

Apical dendrites constitute a prominent feature of the microcircuitry in neocortex, but their function is poorly understood. Using fura-2 imaging of layer 5 pyramidal neurons from slices of rat somatosensory cortex, we have investigated the calcium influx into dendrites under intracellular, antidromic, synaptic and receptor-agonist stimulation. We find three spatial patterns of calcium accumulations: an "apical band" in the apical dendrite approximately 500 mm from the soma, an accumulation restricted to the basal dendrites, soma and proximal apical dendrite, and the combination of both of these. We show that the apical band can be activated antidromically and synaptically and that, under blocked sodium and potassium conductances, it generates calcium spikes. Thus, the apical band may serve as a dendritic trigger zone for regenerative calcium spikes, or as a current amplifier for distal synaptic events. Our results suggest that the distal apical dendrite should be considered a separate functional compartment from the rest of the cell.

Abstract: Two abstracts presented at the Society for Neuroscience Annual Meeting, 1993.

Publication: Neuron 13:7, 23-34, 1994.

A2- Coincidence detection of synaptic inputs and spikes via supralinear calcium accumulation in single spines. Rafael Yuste and Winfried Denk. Biological Computation Research Department, AT&T Bell Laboratories, Murray Hill, NJ 07974.

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Dendritic spines are basic units of synaptic integration but their functional properties are poorly understood due to experimental difficulties resulting from their small size ($\sim 1 \mu\text{m}^3$). By taking advantage of the improved ability of two-photon microscopy to image fluorescence with high resolution in strongly scattering tissue we have measured calcium accumulations in spines from CA1 pyramidal neurons filled with calcium indicators in slices of rat hippocampus. Focal subthreshold synaptic stimulation and spontaneous synaptic events produced calcium accumulations localized to isolated spines and abolished by postsynaptic blockers. Synaptically-induced calcium responses showed stochastic failures. Single somatic spikes produced fast-peaking calcium accumulations in spines throughout the cell demonstrating antidromic invasion of the spines by the action potential and the existence of voltage-sensitive calcium channels in the spine heads. Pairing of spikes with synaptic stimulation was cooperative, i.e. results in supralinear calcium accumulation. Our results demonstrate that individual spines are discrete calcium compartments that can individually detect the coincidence of pre- and post-synaptic activity via their calcium concentration. This mechanism may underlie associative changes in synaptic transmission. Abstract: Presented at the Biophysical Society Annual Meeting, 1995.

Main Publication: Submitted to Nature. Related Publication: Denk, W., Delaney, K. R., Gelperin, A., Kleinfeld, D., Strowbridge, B. W., Tank, D. W. and Yuste, R. Anatomical and functional imaging of neurons using 2-photon laser scanning microscopy. J. Neuroscience Methods 54, 151-162, 1994.

A3- Numerical simulations of calcium electrogenesis in apical dendrites from neocortical pyramidal neurons: generation of intrinsic oscillations by an axial current. Rafael Yuste, Abdelkarim Elaagouby* and David W. Tank. Biological Computation Research Dept., AT&T Bell Laboratories, Murray Hill, NJ 07974 and *Div. Biology, California Institute of Technology, Pasadena, CA 90210.

Active conductances exist in dendrites from mammalian neurons, but their function still remains unknown. In previous work, using intracellular recording and calcium imaging, we discovered an electrogenic band of calcium channels in the apical dendrites of layer 5 pyramidal neurons. To explore the functional consequences of this distal electrogenic area in the intrinsic electrophysiology of those neurons we have now carried out numerical simulations of multicompartmental models of the calcium imaging and electrophysiological data obtained from one such neuron, recorded under conditions of blocked sodium and potassium conductances. We find that our data can be fitted by a variety of models with an increased functional density of calcium channels in the apical dendrite. Importantly, in all models a significant axial current flows from the apical dendrite into the somatic region. This axial current depolarizes the soma and generates oscillations similar to those seen in the electrophysiological data. Thus, one role of this

distal electrogenic area in apical dendrites may be to sustain an oscillatory dynamics in pyramidal neurons.

Abstract: Presented at the Society for Neuroscience Annual Meeting, 1994.

Publication: Journal of Computation Neuroscience, in preparation

B- Studies of the cortical circuit diagram.

B1- Functional characterization of the cortical microcircuit with voltage-sensitive dye imaging of neocortical slices. Rafael Yuste, David Kleinfeld and David W. Tank. Biological Computation Research Department, AT&T Bell laboratories, Murray Hill, NJ 07974.

In addition to the pattern of inputs, the internal dynamics of cortical circuits may be essential to the computation they perform. As a first step to characterize these functional dynamics we have used optical imaging of signals produced by the voltage-sensitive dye di-4-ANEPPS in slices from rat primary visual cortex. Two types of signals are produced: a fast signal which seems directly related to changes in membrane potential, and a slow signal which appears related to changes in scattering properties of the slice and non-electrochromic effects of the dye. Imaging of the fast signal in response to small electrical stimulations clearly shows all major interlaminar projections and a system of clustered horizontal projections in the upper layers. Finally, imaging of the fast signals during spontaneous activation of the disinhibited circuit reveals generalized responses, without evidence for modular activation under our imaging conditions.

Abstract: Presented at the Society for Neuroscience Annual Meeting, 1994

Publication: Journal of Neuroscience, in preparation

B2- Neuronal domains in developing neocortex: mechanisms of co-activation. Rafael Yuste, Darin A. Nelson*, William W. Rubin* and Lawrence C. Katz*. Biological Computation Research Dept., AT&T Bell Laboratories, Murray Hill, NJ 07974 and (*) Dept. of Neurobiology, Box 3209, Duke University Medical Center, Durham, NC 27710.

The mammalian neocortex consists of columnar circuits. The developmental rules that define this microcircuitry remain poorly understood and are thought to be controlled by patterns of spontaneous activity during early cortical development. The existence of columnar domains of spontaneously co-active neurons was previously described using optical imaging with the calcium-sensitive dye fura-2 in slices of developing rat neocortex. To investigate the cellular mechanisms responsible for the co-activation of these domains, spontaneous and evoked domains were examined using faster optical recordings and pharmacological manipulations. The activation of a

domain starts in cells located near its center and spreads at speeds of approximately 100 $\mu\text{m}/\text{sec}$. Domains occur in the presence of the sodium channel blocker tetrodotoxin (TTX) but are blocked by the gap junction blockers halothane and octanol. Finally, combined intracellular recording and optical imaging from dye-coupled cells revealed functional coupling between developing neocortical neurons. These results support the hypothesis that a neuronal domain results from the spontaneous excitation of one or a few "trigger" neurons that subsequently activate, either electrically or biochemically, the rest of the cells via gap-junctions.

Abstract: Presented at the Society for Neuroscience Annual Meeting, 1992.

Publication: Neuron 14:1, 7-17, 1995.

Future Plans.

Our main goal for the next year is to perform optical recordings with single-cell resolution of a population of neurons in the adult cortical slice. These experiments did not work initially because of the failure of calcium indicators to label populations of adult neurons. Nevertheless, preliminary experiments by us and a number of groups suggest that extracellular injections of dextrans coupled to calcium indicators can be used to label populations of neurons in a variety of systems. Other possible approaches are the use of calcium indicators with less acetoxymethyl esters or the use of a new generation of voltage-sensitive dyes that can be transported retrogradely or that have much larger signals than previous ones. These different strategies appear to us very promising solutions to the problem.

The question we will study with this approach is the detailed description of the spontaneous patterns of activity present in the cortical microcircuitry. The first order question is if the activity is organized in multicellular modular units or not. A related question is the role of single cells in the activity of the network. These issues are of great interest because they will help clarify if there is a simple algorithm implemented in the cortical circuit.