202.7

To investigate the conductances underlying the two different types of excitatory input to Purkinje cells we used whole-cell recording in thin (100-250 μm thick) sagittal slices of fetal rat cerebellum. Cells were visualized directly using Nomarski optics, and debris overlying the soma was removed with a large "pipette" (Edwards et al., 1980, J. Physiol., 312:400). Recording pipettes contained (mM): 140 CsCl, 10 NaCl, 10 HEPES, 10 TTX.

Electrical stimulation of the white matter caused an all-or-none excitatory post-synaptic potential (EPSP) followed by a prolonged postsynaptic depression. Stimulation of the molecular layer, on the other hand, resulted in a gradual EPSC that exhibited paired-pulse facilitation. Both EPSCs showed recovery potentials around 0 mV, and both were blocked by application of the non-NMDA receptor antagonist CNQX (10 μM). When the cell was voltage clamped at -40 mV and then depolarized to 0 mV, both types of EPSC were blocked by the non-NMDA receptor antagonist DL-APV (10 μM). We conclude that both types of EPSC are mediated entirely by non-NMDA receptors.

Both application of NMDA (100 μM) did not evoke an inward current, even in Mg2+-free Ringer or at a holding potential of -40 mV. Superfusion of an agonist caused an inward current, but this was blocked by 10 μM CNQX. Non-NMDA receptors thus appear to mediate all the electrophysiological effects of excitatory amino acids on Purkinje cells.

202.8

CI1 pyramidal neurons receive two anatomically distinct types of synaptic input that exhibit different forms of long term potentiation (LTP): an associational/commissural (ac) input from CA1 pyramidal neurons and a mossy fiber (mf) input from the dentate gyrus. The ac receptor binding is low in the mf synaptic region compared to the ac region, however, little is known concerning the contributions of non-NMDA and NMDA receptors at these synapses.

To address this question, synaptic currents in CA3 pyramidal neurons were studied using the method of "blind" whole-cell recording (Blanton, M. J., et al., J. Neurosci., 18: 30, 1988) in thick (500 μm) hippocampal slices from adult guinea pigs. Ac excitatory post-synaptic currents (EPSC) were elicited by a non-repetitive stimulating electrode placed in stratum radiatum. To eliminate the contribution of contaminating non-mf inputs which can be activated by conventional stimulating electrodes, "pure" mf EPSCs were evoked by the direct stimulation of granule cells with a glutaime-filled lontophoretic electrode positioned in the dentate gyrus. EPSCs evoked from stimulation of axon fibers displayed a slow component during depolarizing holding potentials (> 40 mV). The slow component to the EPSC was blocked by the addition of APV to the perfusing medium.

Glutamate-evoked mf responses, presumably reflecting the activation of single fibers, often yielded EPSCs ranging in amplitude from 200 to 1400 μA at a holding potential of 80 mV. These EPSCs also contained a small, slow depolarized membrane potential which was blocked by APV. A large APV-sensitive component to mf EPSCs was observed in slices pulsed with Mg2+-free Ringer.

These results provide support for the idea that in CA3 pyramidal neurons activate NMDA and non-NMDA receptors. The functional role of NMDA receptors at the mf-CAL synapse remains to be elucidated.

202.9
ARE SPINES AND DENDRITES DYNAMICALLY DISTINCT CALCIUM COMPARTMENTS? Messiah Segal, Peter Gutirrez, and Stanley B. Kater. The Program in Neuronal Growth Development and the Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Dendritic spines are likely to be a primary site for neural plasticity. Intracellular calcium is thought to be the prime tool for regulating neural plasticity. Despite extensive interest, little is known about calcium's role in the regulation of synaptic plasticity. Of particular interest is the role of calcium in regulating spine morphology.

Intracellular calcium imaging is a powerful tool for examining intracellular calcium dynamics. We have injected pyramidal neurons in hippocampal slices from adult rats with the fluorescent calcium indicator, aequorin. We used the method of "blind" whole-cell recording to monitor calcium transients within individual spines during agonist-induced excitation and afterward.

Calcium transients in spines were following 

202.10

31P- and 1H- nuclear magnetic resonance measurements of 3-10 day-old rat cortical slices were used to monitor evolution of intracellular ATP, phosphocreatine (PCR), lactate and pH following addition of excitatory amino acids agonists. PCR was dephosphorylated during agonist-induced neuronal excitation and after washout of the agonist, and related to the amount of agonist applied (threshold agonist concentration = 1μM).

Reversal was abolished by mitochondrial inhibitors (cyanide, antimycin A). The ratio of PCR/ATP was reduced in the presence of NMDA, kainate, and quisqualate, which together induced LTP in slices. These results validate the use of 31P-MRS in brain slice preparations.