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Dendritic Spine Recovery and Time-lapse Neurite Formation

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Dendritic spines, found in the brain, are actin-rich protrusions of nerve terminals and house post-synaptic contacts. Current theory holds that these spines are strengthened when receiving repeated stimuli, and lost when they are inactive. This may represent the root changes found in the brain during memory and learning. Spines are effectively isolated from each other by a thin neck region, which may act as a diffusion barrier. In this way, spines are believed to play a neuroprotective role by sequestering ions away from the dendritic shaft [1, 2].

Receptors for the neurotransmitter glutamate are found in the head region of dendritic spines. Studies have demonstrated that over-activation of glutamate receptors *in vivo* can lead to neuronal cell death in 12-24 hours [3, 4]. Such excitotoxicity may lead to neuronal damage observed in seizure, ischemia, and trauma. This damage exhibits as collapse or loss of post-synaptic dendritic spines [5]. Loss or altered spine morphology is also associated with chronic neurodegenerative diseases, such as Huntington's and Creutzfeldt-Jakob diseases [6].

Excitotoxicity can be induced in cell culture by incubation with glutamate receptor agonists [7]. Spine collapse and loss occur within minutes, with ensuing cell death within 24 hours. Using fluorescence microscopy, we show that post-excitotoxic treatment with glutamate receptor inhibitors induced spine recovery to control levels. This suggests that pharmacological intervention may have therapeutic benefit after neuronal damage.

We have also been studying neurite outgrowth, the formation of dendrites *de novo*. Neuronal cells in culture first exhibit actin-rich lamellipodia around the cell body. Later, the lamellipodia condense and protrude to become growth cones on the tips of microtubule-containing neurites. Significant reorganization of the cytoskeleton is required, although little is known about the molecular events underlying this dynamic morphology change. One protein that may play a key role is the microtubule-associated protein, MAP2c, which can bind to both microtubules [8], and actin microfilaments [9]. Using time-lapse microscopy of CFP and YFP-labeled proteins, we show that MAP2c is sufficient for neurite initiation. A model is proposed for MAP2c to coordinate actin as well as microtubule rearrangements to establish growth cone-like structures.

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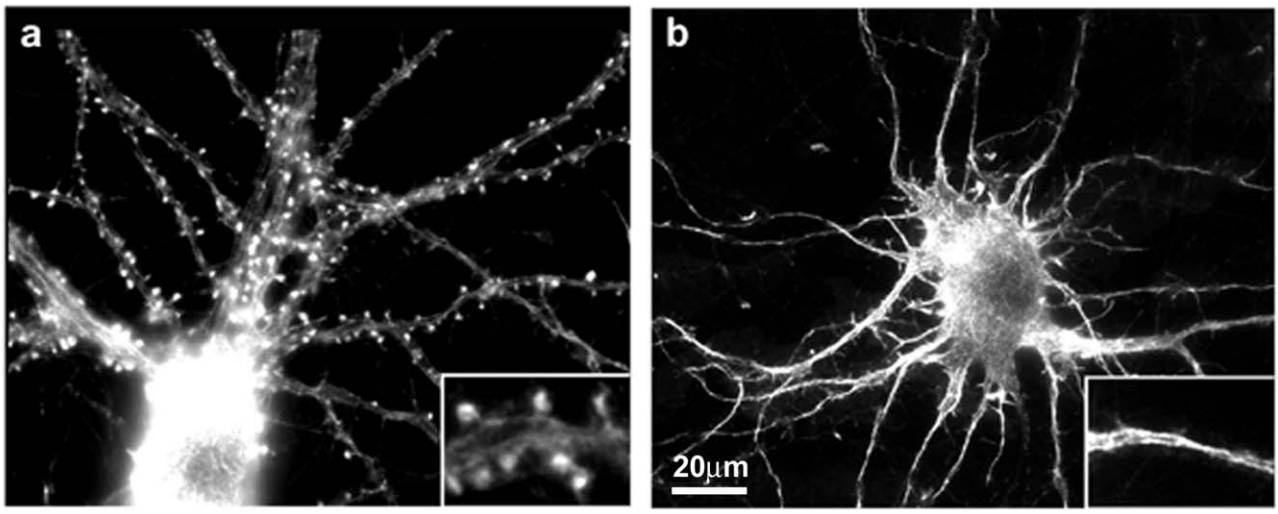

Figure 1. Sub-lethal exposure to NMDA results in spine collapse. Cultured hippocampal neurons either un-treated (a) or exposed to 50μm NMDA (b) stained with fluorescent phalloidin. Insets are 3x enlargements. Notice the lack of dendritic spines in the treated neurons.

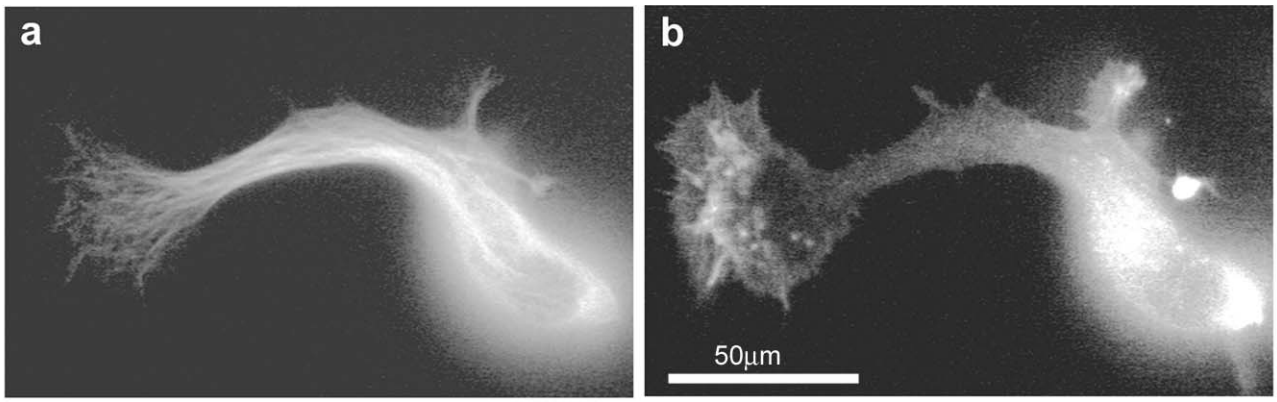

Figure 2. MAP2c expression involved in neurite formation. Cultured Neuro2a cells were transfected with CFP-MAP2c (a) and YFP-actin (b). MAP2c induced lamellipodia to condense around microtubules, producing a neurite shaft with an actin-rich growth cone at the tip.

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