Calcium waves alter dendritic spine morphology in basolateral amygdala projection neurons

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Connections between excitatory neurons are generally made on small, specialized compartments called spines. Spines are connected to the dendrite by a thin spine neck that restricts diffusion between the spine head and the dendrite. Rises in spine calcium are a critical trigger for synaptic plasticity and this arrangement enables calcium signals evoked by high frequency stimulation (HFS) of synaptic inputs to become concentrated within individual spines (Zucker, 1999). In projection neurons in the basolateral amygdala (BLA), HFS also activates metabotropic receptors and leads to a focal rise in dendritic calcium that propagates as a wave along the dendrite (Power & Sah, 2007). Here we investigated whether synaptic connections are altered by the passing dendritic calcium wave.

Brain slices were prepared from Wistar rats (21-35 d) anesthetized with a lethal dose of isoflurane, and killed by decapitation. Whole-cell patch-clamp recordings and two-photon fluorescence images were made from BLA projection neurons loaded with the calcium indicator Fluo5F and the calcium insensitive dye Alexa 594 to measure spine morphology. Calcium waves evoked by HFS (100 Hz 1s) differentially invaded spines as they propagated along the dendrite, preferentially invading those with short necks. Repeated bouts of HFS (5 times at 1 min intervals) resulted in a reduction in the spine head volume 10 minutes after the first wave was evoked (87 ± 6 % of baseline; n = 9; $P = 0.073$) in spines with short necks (< 0.75 μm). The volume of long-necked spines (> 0.75 μm), which are shielded from dendritic waves, was unchanged (104 ± 6 % baseline; n = 9; $P = 0.49$; short *versus* long $P = 0.067$). Spines located on dendrites where no calcium waves were evoked were unchanged (102 ± 2 % of baseline; n = 52) following HFS. Changes in synaptic strength are accompanied by concomitant changes in the volume of the spine head with a decreases in synaptic strength being accompanied by a decrease in spine volume (Zhou *et al.*, 2004). As calcium waves preferentially invade spines with short necks these results suggest that calcium wave invasion depresses the strength of synaptic connections. Calcium waves and IP3 receptors have been implicated in heterosynaptic long-term depression of unstimulated inputs following the induction of long-term potentiation (Nagase *et al.*, 2003; Royer & Pare, 2003). Our results provide an explanation for these results.

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