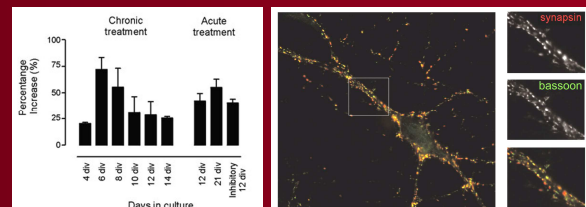
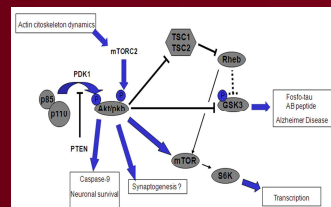


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Dendritic spines are the postsynaptic sites for the majority of excitatory synapses in mammalian nervous system; they have been regarded as the possible locus of long-term synaptic plasticity associated with the storage of memories in the brain. Previous observations of our group indicate that overactivation of PI3K (Phosphoinositide 3-kinase), increases synapse density in primary cultures of rat hippocampal neurons. Here, we show evidence that PI3K is also able to modulate spinogenesis both in cultured and in vivo pyramidal neurons. In hippocampal neurons in culture, PI3K overactivation produces an increase of 22 %, while in vivo this augment is about a 30%. Changes in spine density in vivo correlates with the formation of a higher number of small and short-neck spines. These changes in spine density are associated with enhanced cognitive behavior in treated animals in a fear conditioning assay. Furthermore, we are characterizing the mechanisms by which PI3K controls spine formation and morphology changes.

1.- PI3K signaling pathway and the synaptogenic effect



PTD4-PI3KAc, a transduction peptide was employed to stimulate PI3K activity.

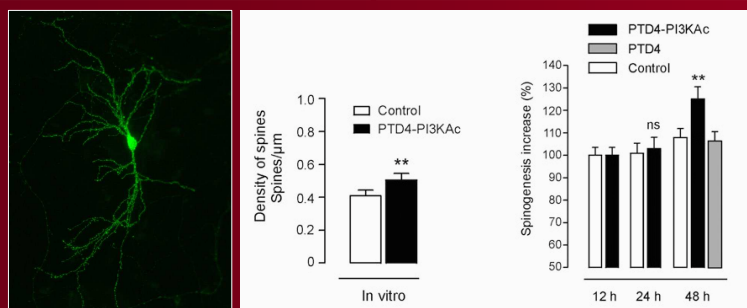
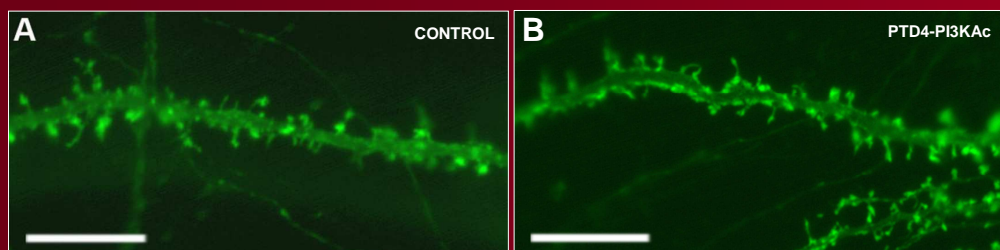
Effects of PTD4-PI3KAc in cultures of rat hippocampus. Colocalization of presynaptic markers: Synapsin + Bassoon.

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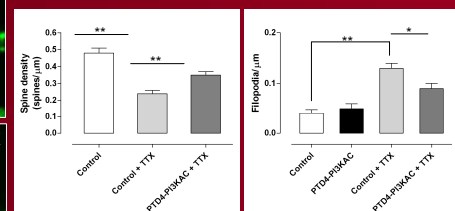
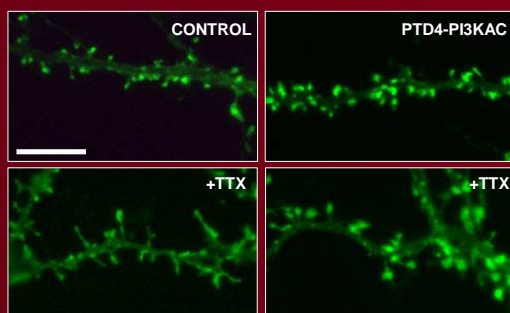
2.- PI3K overactivation in cultures: spinogenic effect

Cell cultures of neonate rat hippocampus transfected with a eGFP-βActin vector.

PI3K overactivation produces a significant increment (22 %) in spines density after 48 hours.

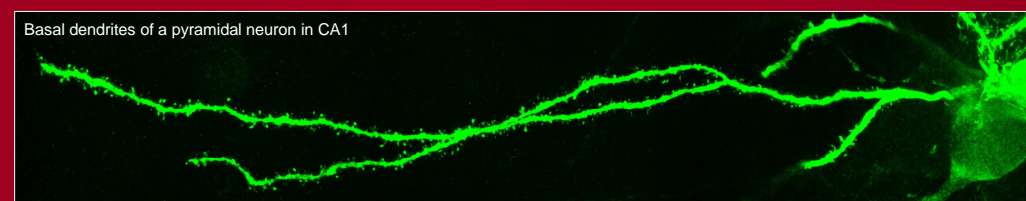


3.- Spinogenesis is partially independent of neuronal activity



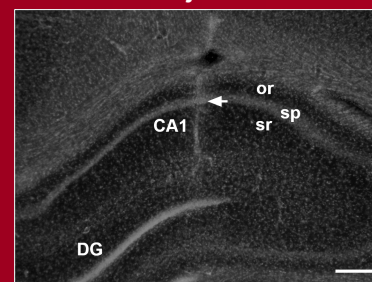
4.- PI3K overactivation in vivo: spinogenic increase

Lucifer Yellow microinjection allows filling of individual neurons.

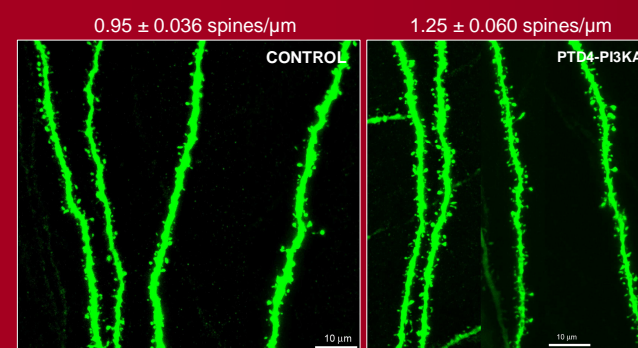


Spine density changes as a function of the distance to the cell soma, reaching a plateau at 45 μm.

Stereotaxic Injections

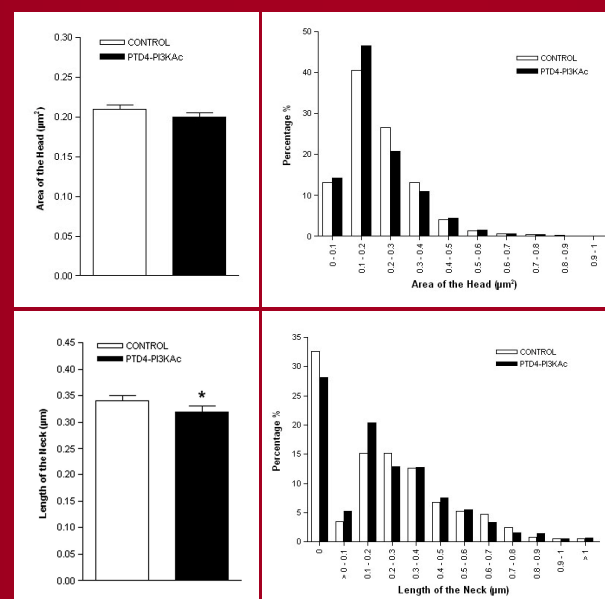


anteroposterior: -2.8 mm
 lateral: -1.5 mm
 dorsoventral: -2.8 mm



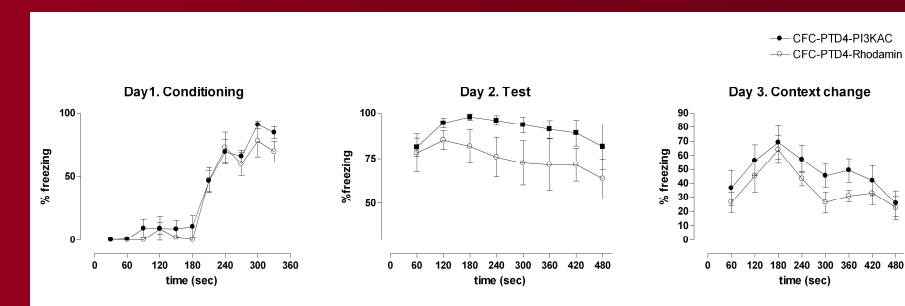
Student t-Test (p < 0.005)

5.- What kind of spines PI3K overactivation forms?

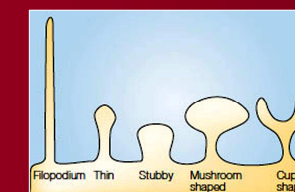


6.- PI3K activation leads to changes in behavior

Dorsal Hippocampal injection of PTD4-PI3KAc in adult rats.



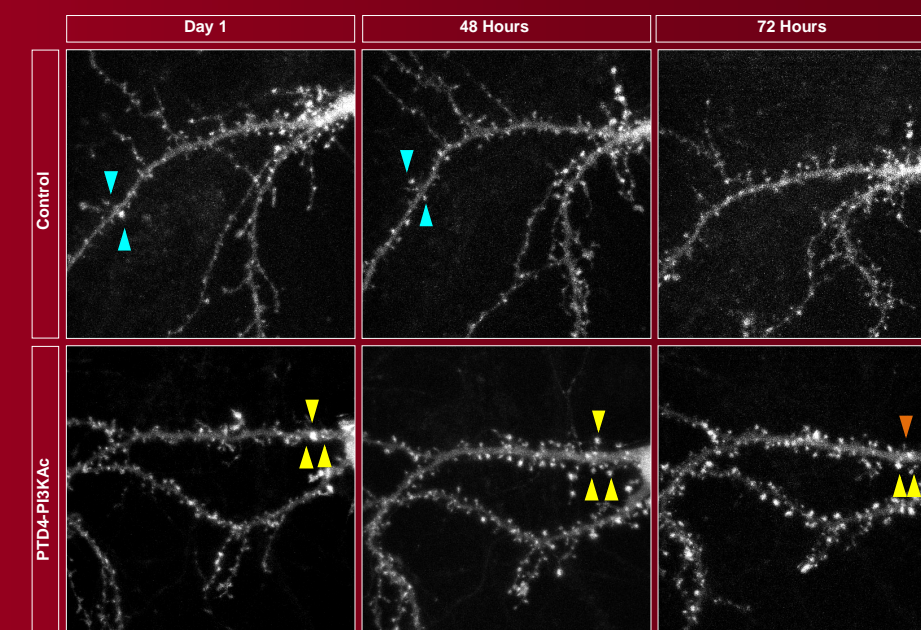
Two-way ANOVA repeated measures: p = 0.0482



Study of spines morphology is currently in progress.

7.- Time-lapse experiments confirms the spinogenic effects

Spines are highly dynamic: size and shape continually shift on a time scale of seconds to minutes.



▲ Persistent spines
 ▲ New spines
 ▲ New spines that disappear with time