Inhibition of the PI3 Kinase Cascade in Corticolimbic Structures: Possible Target to Erase Fear Memories

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Introduction

Repeated presentations of a conditioned stimulus without the unconditioned aversive stimulus result in extinction of conditioned fear. Impairments in extinction are linked to maladaptive behavior and anxiety disorders.

The basolateral amygdala (BLA) and the infralimbic subregion (IL) of the medial prefrontal cortex are known to play pivotal roles in the acquisition and extinction of conditioned fear. The BLA is extensively implicated in the acquisition and consolidation of fear conditioning memory as well as in the acquisition of extinction memory[1]. The IL is implicated in the consolidation of extinction[2].

The specific mechanism by which these two brain areas mediates the extinction of fear memories is still to be established.

The phosphoinositide 3-kinase (PI3K) cascade is present in all the cells of the body and has been found vital in cell proliferation and the prevention of apoptosis. In the brain, PI3K is implicated in synaptic plasticity. In particular, this cascade is implicated in the formation of fear memory in the amygdala[3].

In this study we aimed to investigate the role of PI3K cascade in the IL and the BLA in acquisition of contextual fear memory and extinction.

Methods

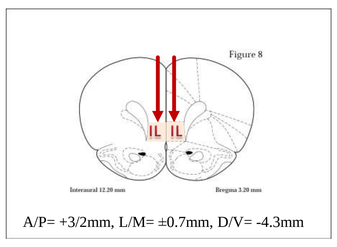
Male Sprague-Dawley rats (200-300gr) were used. They were bilaterally implanted with stainless steel guide cannulae into the BLA or the IL (see microinjection sites).

Contextual fear conditioning: The rat was placed in a conditioning chamber and received 3 foot shocks (0.5mA for 0.5 sec) with 2 min inter-trial interval.

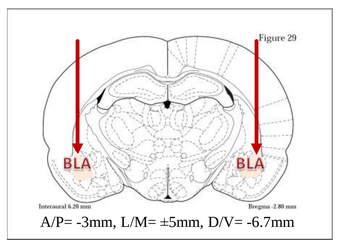
Extinction: Training took place over the next 3 days: The subject was placed in the context of the conditioning chamber for 10 min, and the duration of freezing was measured. The behavioral results are presented as percentage of time the animal spent freezing

Pharmacology: PI3K inhibitor LY294002 was dissolved in DMSO and diluted with ACSF (Artificial Cerebral-Spinal Fluid) (12.5 μM/rat). The control group (vehicle; VH) received DMSO with ACSF only.

Microinjection Sites



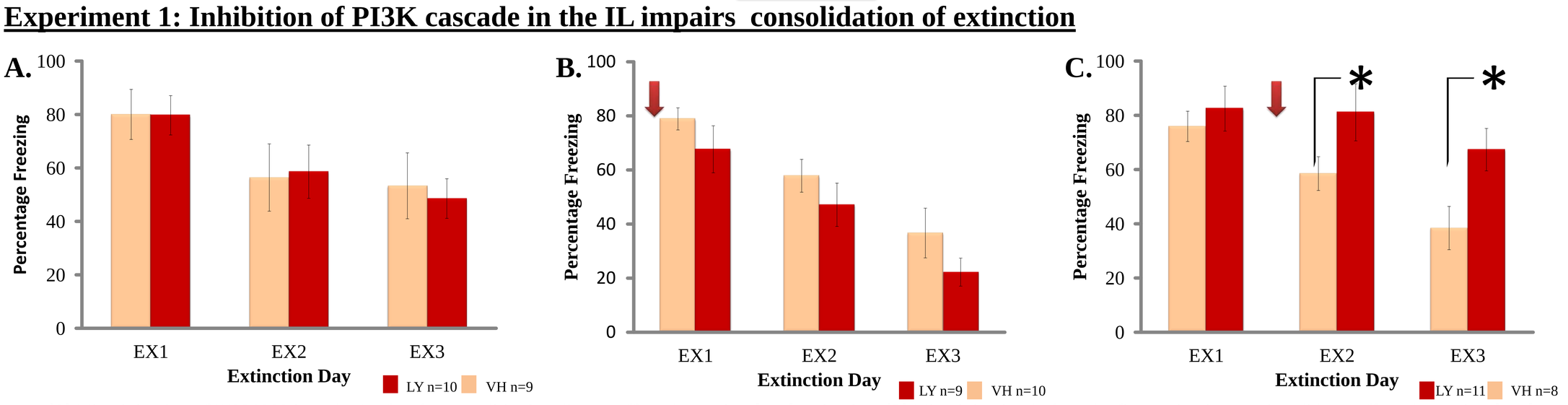
A/P= +3/2mm, L/M= ±0.7mm, D/V= -4.3mm



A/P= -3mm, L/M= ±5mm, D/V= -6.7mm

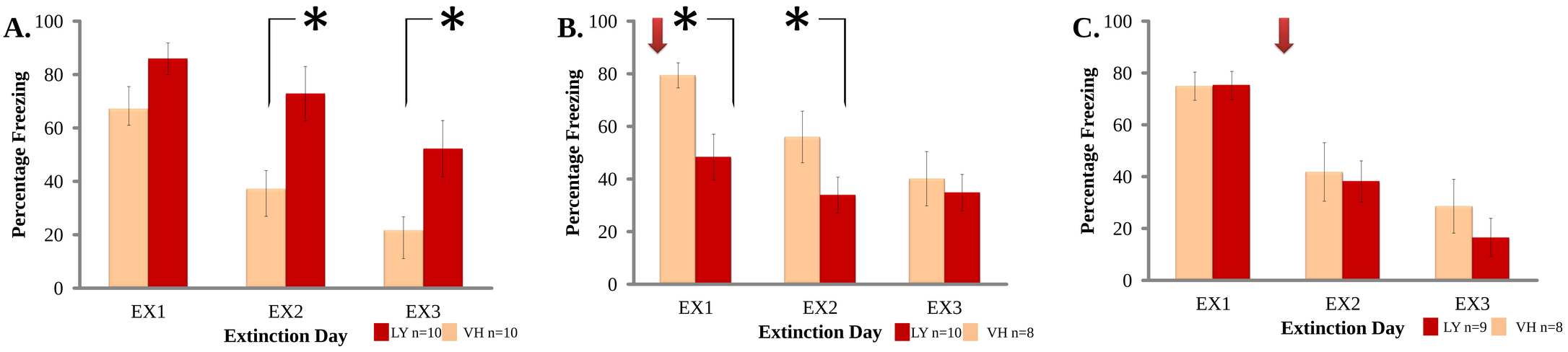
Results

Experiment 1: Inhibition of PI3K cascade in the IL impairs consolidation of extinction



(A) Microinjection of LY294002 into the IL after fear conditioning did not effect the retrieval and extinction. **(B)** Microinjection before the first extinction session did not effect the retrieval and extinction.**(C)** Microinjection of LY294002 15 min into the IL after the first extinction session resulted in enhanced fear memory and impairment of extinction over the subsequent extinction days($p<0.05$).

Experiment 2: Inhibition of PI3K cascade in the BLA has a differential effect on fear formation and extinction depending on the time of microinjection



(A) Microinjection of LY294002 into the BLA 15 min after the fear conditioning resulted in enhanced fear memory and impairment of extinction over the subsequent extinction days ($p<0.05$).**(B)** Microinjection into the BLA before the first extinction session reduced freezing, an effect that also persisted 24h later ($p<0.05$).**(C)** When applied after the first extinction session the treatment did not effect the normal course of extinction.

Discussion

Using PI3K inhibition, our results support the assumption of differential roles of the IL and BLA during fear and extinction.

Microinjection of the inhibitor into the IL had no effect on consolidation of fear conditioning memory, while in the BLA it caused a significant increase in freezing levels. However, when fear memory was retrieved 24h after conditioning, PI3K inhibition in the BLA suppressed freezing. This is in line with the assumption that the IL is not involved in the consolidation and retrieval of fear conditioning memory. This dissociation was further shown by targeting the consolidation phase of extinction; while inhibition of PI3K cascade in the IL was associated with impairment in extinction consolidation which resulted in an increase in freezing levels, inhibition at the same time point in the BLA resulted in intact extinction.

These results point on differences in the temporal parameters of the effects of PI3K cascade inhibition in the IL or BLA and suggest differential involvement of these structures in long-term fear and extinction memory. Furthermore, the current results point on the interesting possibility that the microinjection of PI3K inhibitor into the BLA during retrieval of fear could be used to erase fear associations. These findings may be relevant to the treatment of human pathologies.

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References:

[1] LeDoux J. (2007) The amygdala. Curr Biol 20:R868-74.

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[3] Lin CH, Yeh SH, Lin CH, Lu KT, Leu TH, et al. (2001) A role for the PI-3 kinase signaling pathway in fear conditioning and synaptic plasticity in the amygdala. Neuron 5:841-51