Pain Hurts the Emotional Brain: Impairment of mPFC Neurons to Signal Memory of Extinction in Rats with Neuropathic Pain

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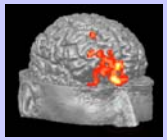
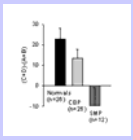
INTRODUCTION

We have shown that chronic pain patients exhibit behavioral and brain changes beyond pain itself, including impaired performance on emotional decision-making tasks, dorsolateral prefrontal cortex atrophy and medial prefrontal cortex (mPFC) hyperactivity.





We have also documented an increase in cortical expression of IL-1beta in rats with neuropathic pain (Apkarian et al 2006) as well as an impairment in extinction of fear conditioned responses (see companion poster). Here we investigate the mechanisms of such behavioral deficits by testing the hypothesis that chronic neuropathic pain in rats will result in impaired mPFC neuronal activity and impairment of memory of extinction to the fear conditioned responses. We follow up on Milad & Quirk (2002) observations who showed that mPFC neurons signal the memory of fear extinction. We use adult male rats with spared nerve injury (SNI) in contrast to sham controls, and submit them to standard fear conditioning testing while recording neuronal activity in mPFC.

METHODS

Electrode Implantation and SNI Surgery

Male Sprague-Dawley rats (225-250 grams) were anesthetized using isofluorane 5%, and 30% N_2O and 70% O_2 and then implanted with eight channel microwire electrodes (50mm diameter, NB Labs, Denton TX), in mPFC: 2.9 mm anterior, 1.0mm lateral, and 4.1mm ventral to Bregma. Rats were allowed to recover for two weeks then recorded for baseline neuronal activity. Half the animals then underwent SNI (ligation of tibial and peroneal branches of the sciatic, Decosterd and Woolf, 2000). Two weeks later the rats were fear conditioned (as in Milad & Quirk, 2002) while recording from mPFC. Neuronal activity was recorded using multiple channel amplifiers, the amplified signal from each electrode was digitized (20kHz) and saved on a PC for off line spike sorting (Plexon Inc, Dallas TX).

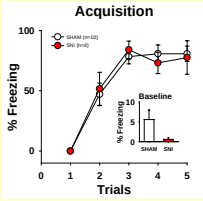
Fear Conditioning

Rats were pre-exposed to the conditioning cage for 20 min on the day prior to conditioning. Next day, the rats were first adapted for 60 s and presented with a 30 s tone. Two minutes later they received 5 pairings of a 30 sec tone that co-terminated with a 1 sec shock (0.8 mA) delivered to the stainless steel bar floor, each pairing separated by 2 min. The animals were then returned to their home cage. Extinction trials were conducted 1 and 24 hours later in which after a 2 min adaptation period the rats were presented with 10 x 30 sec tone separated by 2 min. Electrophysiological recording were done only during extinction.

Data Analysis

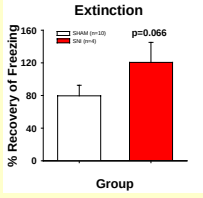
Rats were video taped with a camera mounted on the side of the conditioning cage. The amount of freezing during conditioning was analyzed by the Freeze View software (Actimetrics, Willmette IL) and normalized to baseline values.

Fear Conditioning



A plot of freezing behavior across trials. Inset: baseline freezing is low in both groups.

Sham and SNI rats exhibit good freezing behavior during acquisition, indicating both groups learn that tone predicts the shock.



%Recovery of Freezing =

($Freeze_{1\ hour}$ tone 1 & 2) / ($Freeze_{24\ hours}$ tone 1 & 2).

Defined as ratio of freezing for 1st two tones at 1 hour vs. 24 hours post-conditioning.

SNI rats exhibit a trend for poor recovery of freezing compared to SHAM rats.

This demonstrates poor memory of the previous day's extinction trial in SNI rats.

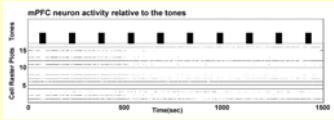
Brain Activity During Fear Extinction

Sham Rats

Good recovery of freezing

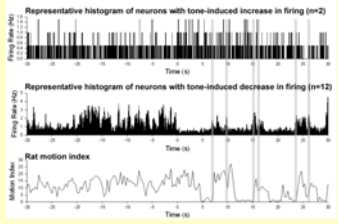
Seen in 2 rats and 30 cells

Behavior for the rat with best freezing recovery (23%) is illustrated:



Firing activity for all 16 mPFC cells isolated in this animal during extinction (10 consecutive tones) at 24 hours post-conditioning.

Across the population of cells, firing rate is reduced with repeat exposure to tone alone.

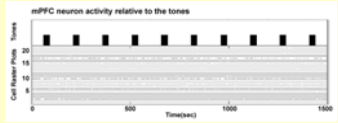


Of the 16 cells, 2 showed increase activity and 12 decreased activity with repeated tone presentation. Activity for the two groups are shown as an average across 30 seconds prior and 30 seconds during tone presentation, averaged for two consecutive tones. Top & middle panels: Average firing rate for each group of cells. Bottom panel: motion index. Gray bars line up the panels, where neural activity seems to predict increased motion. Onset of tone at 0 seconds, sustained for 30 seconds.

Poor recovery of freezing

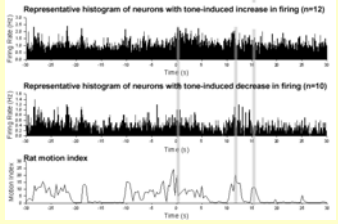
Seen in 8 rats and 68 cells

Behavior for a rat with poor freezing recovery (95%) is illustrated:



Firing activity for all 22 mPFC cells isolated in this animal during extinction (10 consecutive tones) at 24 hours post-conditioning.

Across the population of cells, firing rate does not change significantly.



Of the 22 cells, 12 showed increase activity and 10 decreased activity with repeated tone presentation (details same as in left panel).

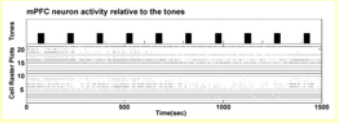
Gray bars line up the panels, where neural activity does not seem to predict motion.

SNI Rats

Poor recovery of freezing

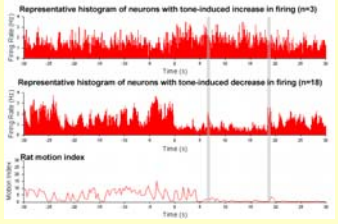
Seen in 4 rats and 35 cells

Behavior for a rat with poor freezing recovery (122%) is illustrated:



Firing activity for all 21 mPFC cells isolated in this animal during extinction (10 consecutive tones) at 24 hours post-conditioning.

Across the population of cells, firing rate is reduced with repeat exposure to tone alone.



Of the 23 cells, 3 showed increase activity and 18 decreased activity with repeated tone presentation (details same as in left most panel).

Gray bars line up the panels, where neural activity does not seem to predict motion.

SUMMARY

Sham rats that show good memory of previous extinction trials have neurons in mPFC with increased activity that directly precedes decrease in freezing during the tone, whereas rats that show poor memory do not have mPFC neurons were the activity directly precedes decrease in freezing.

In contrast, all of the SNI rats show a poor memory of previous extinction trials and increases in firing rate rarely precede decreases in freezing during the tone on the second day of extinction .

These results demonstrate the cortical impact of long term neuropathic pain beyond the classical pain perceptual brain network, emphasizing the need to further study these aspects of chronic pain.