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TITLE: Fear memory engram is the mind-killer

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ABSTRACT

Stegemann et al have unraveled a long-lasting role for fear engrams in future pain perception. Prefrontal pain and fear representations become entangled following a fearful event. As subsequent painful experiences also reactivate the fear network, silencing the fear engram alone can alleviate both tonic pain and hypersensitivity in chronic pain.

MAIN TEXT

In Frank Herbert's novel "Dune", Paul Atreides has to endure a tremendous amount of pain, without flinching, by putting his hand in a mysterious device presented to him by the Bene Gesserit's reverend mother, as a test of his presence of mind. Paul (or Timothée Chalamet, for the more cinematographically-inclined scientists) manages to do so by reciting the 'litany of fear': "I must not fear. Fear is the mind-killer [...]", trying to feel no pain by suppressing his fear of the pain itself. As he was writing those lines, Herbert probably didn't realize how true the litany would eventually ring to neuroscientists.

Indeed, pain and fear are inextricably linked. Fear can both powerfully suppress and enhance pain sensation, in different scenarios: sudden fear can temporarily suppress the perception of pain, such as when facing a threat; conversely, previously experienced fear episodes can chronically enhance pain, e.g., in the form of hyperalgesia in chronic pain patients¹. While

fear-mediated acute analgesia is well-documented and its mechanism explained², we know very little about the neuronal mechanisms governing the opposite interaction.

In their paper, Stegemann et al.³ showed that cells active in the mouse medial prefrontal cortex (PFC) during recall of long-term fear memory (LTFM), which form the fear memory 'engram', are involved in subsequent responses to painful events. They demonstrated that, in chronic pain models, the PFC fear-memory network extends to PFC pain-responsive cells and even to PFC touch-responsive cells. Moreover, optogenetic silencing of the LTFM engram cells alleviated hypersensitivity in mouse chronic pain models, providing hope for possible future PFC engram-targeting therapeutic strategies to curb chronic pain in human patients.

In this work, the target was the 'engram', that is, the ensemble of cells representing the physical substrate of a memory trace. These cells are activated when an event occurs and undergo the morphological changes necessary to recruit them again when we remember the same event⁴. This study focused on the ensemble of cells reactivated at a remote time point in the PFC. As a region known for its implication in both pain⁵ and long-term fear memory⁶, the authors reasoned that the PFC may represent the hub connecting the two. Are pain and fear encoded in overlapping circuits within this region and is it realistic to study one without considering its influence on the other?

Here³, the authors wished to specifically tag subsets of cells that are active at a particular time (e.g. the engram cells, and pain-responsive cells) using a well-established system called Tet-Off⁷. Briefly, the expression of a reporter protein is under the control of both cell activity (regulated by the promoter of the c-Fos protein, a marker of neuronal activity) and the absence of doxycycline (Dox), which is administered to the animals through their food. Hence, the reporter is only expressed in cells that are active during an experimenter-determined time window. To first identify the ensemble of neurons that are active during both long-term fear memory recall and a subsequent tonic pain event (i.e., a hind paw injection of capsaicin⁸), the LTFM engram was Tet-off-tagged by opening the tagging window during fear recall and using

mCherry as a reporter protein. At the same time, cells expressing c-Fos during tonic pain were GFP-immunostained. Fluorescent cell counting revealed a strong, significant overlap between those two populations. Electrophysiological recording of the same PFC cells across the two behaviors confirmed the existence of this overlap.

Proving the existence of this overlap is, in and of itself, interesting. But, perhaps more importantly, it immediately becomes the prime suspect for LTFM-mediated potentiation of future pain. To test it, Stegemann et al.³ changed the reporter protein so that the tagged engram expressed either an inhibitory or excitatory opsin. This ensemble of cells could then be manipulated during a subsequent tonic painful stimulation (Fig1c). As predicted, but still surprisingly, optogenetically silencing the LTFM engram reduced pain-related behaviors, effectively creating an “optogenetic Litany Against Fear”. Conversely, activating the LTFM engram increased such behaviors. These outcomes were specific to the engram since no effect was detected when a similar-sized but random population of cells was targeted and manipulated.

The authors also targeted cells upon initial presentation of TMT, an odor present in urine and feces of foxes, which are natural mouse predators⁹, hence tagging an innate-fear engram. In this case, optogenetic activation yielded the opposite effect (i.e., suppression of pain) compared to activating a previous remote fear memory. This apparently contrasting result may hide a more complex mechanism, where if an animal experiencing tonic pain is artificially re-exposed to a threat, the result is temporal analgesia to the pain. This experiment also confirmed that the effects of engram manipulation on pain-related behaviors were specific to the PFC LTFM engram. Furthermore, the authors tried replacing the tonic pain session with a pain-unrelated white-noise avoidance task or water reward-learning. In both cases, fear engram inactivation failed to modify mouse behavior, demonstrating that the LTFM engram specifically affects behaviors that are directly linked to pain.

Up until this point, the authors were looking at the influence of fear conditioning on acute tonic exposure to a painful stimulus. However, in chronic pain, clinical studies have shown that patients experience extreme, pathological sensitivity to non-painful stimuli. Knowing that fear memory can exacerbate this hyperalgesia¹⁰, one could wonder if fear engram manipulation will change subsequent chronic pain sensitivity.

To answer this question, the authors used both a neuropathic and an inflammatory chronic pain model, the former induced by unilateral peripheral spared nerve injury (SNI) and the latter by unilateral paw inflammation with Complete Freund's Adjuvant (CFA)¹¹. In both models, hypersensitivity was achieved in the contralateral, unaffected paw, as expected. Fear conditioning the mice prior to chronic pain induction significantly heightened hypersensitivity — specifically, hyperalgesia in a thermal withdrawal test in the CFA model, and allodynia in a tactile stimulation test in the SNI model — once chronic pain set in. Similarly to the tonic pain results, fear-responsive cells and thermal or tactile-responsive cells significantly overlapped in mice with chronic pain compared to controls.

Interestingly, the overlap between fear and tactile representations was not confined to the PFC micro-network but expanded across the entire brain. In fact, the authors³ used Tet-Off-tagging during tactile stimulation to study projections of the tactile-responsive cells, and compared it with the fear-responsive cells. In naive animals, the two populations connected to very distinct networks (Fig1a). However, after both fear conditioning and chronic-pain induction (SNI), the tactile cells' connectivity strongly shifted to match that of the LTFM network, including hub regions for fear (Fig1b) such as amygdala, agranular insular cortex, mediodorsal thalamus, and anterior cingulate cortex¹². This suggests a mechanism for fear-mediated heightened nociception in chronic pain: the fear network extends itself to include touch-sensitive cells of the PFC.

Considering how touch cells and their connectivity get incorporated into the fear network after chronic pain and fear conditioning, could one imagine reducing hypersensitivity by targeting the PFC LTFM engram? Indeed, when the authors Tet-Off-tagged the LTFM engram cells and optogenetically inactivated them in mice with SNI- or CFA-induced chronic pain, nociceptive hypersensitivity was significantly reduced both shortly (days) and long (weeks) after chronic pain onset (Fig1c).

An important drawback is that optogenetic inactivation only transiently reduced pain hypersensitivity: at the later timepoint, an additional optogenetic inactivation was necessary to reduce the regained hyperalgesia. This result leaves us wondering whether the fear engram can be definitively 'extinguished', in other words, whether its representation can be reverted to separate, untangled representations of fear and pain to achieve a lasting treatment for allodynia and hyperalgesia in chronic pain.

In their experiments, Stegemann et al.³ consistently tagged the fear engram during memory recall and shortly thereafter presented the pain experience (either tonically or chronically). However, two different events have a higher chance of recruiting overlapping ensemble activity if they happen closer in time^{13,14}. This raises another question: would a broader time window between fear memory and pain experiences lead to similar results?

Pain can be transiently silenced at critical moments in our lives when necessary. The mechanisms involved are rapidly induced and short-lived. We also know that fear memory can lead to a life-long enhancement of nociception. This opposite effect being long-lasting, it seems reasonable to assume it could be explained by long-term mechanisms such as plasticity or memory. Indeed, Stegemann et al.³ demonstrate here that an LTFM engram in the PFC is involved in long-term pain heightening following a fearful event. They report evidence that fear-responding cells are not only primed to partially become pain-responding cells in the future, but that, in chronic pain models, fear conditioning results in whole-brain connectivity changes for touch-responding cells: these become entangled in the brain-wide

fear network. Furthermore, inactivating the LTFM engram during tactile stimulation after induction of chronic pain greatly reduces hypersensitivity. Hence, the PFC, where fear and pain representations can merge, exchange, and connect, could prove a target of choice for novel therapeutic approaches, where precisely interfering with the mechanisms of fear memory (and not, as one could expect, to pain directly) could prove effective for reducing pain in chronically afflicted patients.

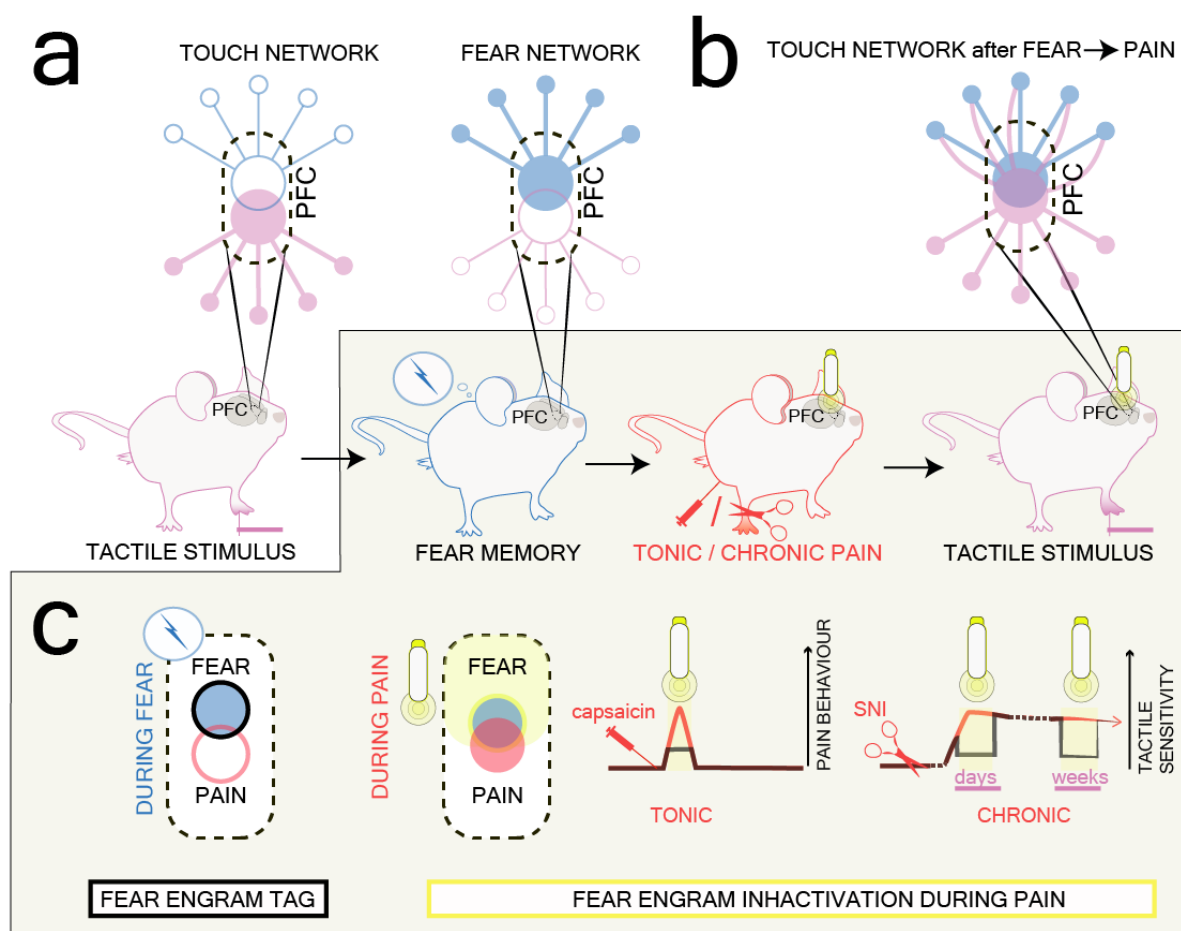


Fig.1 | Silencing the PFC fear memory engram suppresses pathological hypersensitivity in a chronic pain model. **a**, Tactile and fear-responding cells in the PFC have distinct brain network connectivities. **b**, However, if a pain-related fear experience precedes pain, the same tactile stimulus will activate touch-responding cells that are strongly connected with the fear network. **c**, If the fear engram is tagged during long-term fear memory recall, and is subsequently optogenetically silenced during a tonic painful experience such as capsaicin injection, this leads to alleviation of pain behaviors. Similarly,

artificial inactivation of the fear engram alleviates allodynia during contralateral-paw tactile stimulation in mouse models of chronic pain, such as SNI.

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