Selective lesions of ITC cells reveal their role in extinction of conditioned fear.

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1b). Given this imbalance in receptor expression, we were confident that local injections of the μ-opioid receptor-targeted Dermorphin-SAP would be the optimal way of testing the impact of these cells on behavior, without compromising the integrity of the surrounding tissue.

To test whether the ITC cells play a role in extinction expression, rats were habituated and fear conditioned in one context, and then extinguished in a second context. One day after extinction, animals underwent surgery where they received bilateral infusions stereotaxically aimed at the ITC cells of either Dermorphin-SAP (3 pmol/μl, infusion rate .01 μl/min, total of 0.25 μl per hemisphere) or an equivalent amount of control--a non-targeted peptide conjugated to saporin, Blank-SAP (Fig 1a). This protocol did not result in any adverse effect on post-operative weight gain, posture or exploratory behavior.

After recovery, rats were tested for how well they remembered extinction training. During the extinction recall session, sham-lesioned animals displayed low freezing to presentations of the conditioned stimulus, consistent with having retained the extinction training. On the other hand, animals with ITC cell lesions had a dramatic increase in the amount of freezing to the previously extinguished conditioned stimulus, indicating that their recall of the previous session was substantially diminished (Fig 2a). Importantly, rats that received identical Dermorphin-SAP infusions in nuclei surrounding the ITC cells, recalled extinction training as well as controls, suggesting an ITC-specific effect.

Stereological cell counts revealed that properly placed tracks delivering Dermorphin-SAP decreased the number of ITC cells in lesioned animals by 34% as compared to sham-lesioned controls (Fig 2b). In contrast, cells in the adjacent central nucleus of the amygdala were unaffected by the lesion. In addition, an inverse correlation between the number of surviving ITC cells and freezing during extinction recall (r=-0.67) was observed, whereas there was no such correlation between the number of cells in the central nucleus and freezing during extinction recall (r=-0.13).

In this study, Dermorphin-SAP has served as an important tool to seek out a potentially useful target for clinical intervention. Dermorphin-SAP lesions have allowed us to safely and selectively eliminate a proportion of amygdala ITC cells, revealing the importance of these neurons in the expression of extinction. Given that extinction failure is a robust model for a number of anxiety disorders,[4] we can now explore ways of pharmacologically manipulating μ-opioid and other receptors expressed on the ITC cells to control amygdala output and facilitate extinction.

References:

1. Paré D, Quirk GJ, Ledoux JE. (2004) *J Neurophysiol* 92:1-9.
2. Quirk GJ, Likhtik E, Pelletier JG, Paré D. (2003) *J Neurosci* 23:8800-8807.
3. Millhouse OE (1986) *J Comp Neurol* 247:246-271.
4. Ressler KJ, Mayberg HS (2007) *Nat Neurosci* 10:1116-1124.

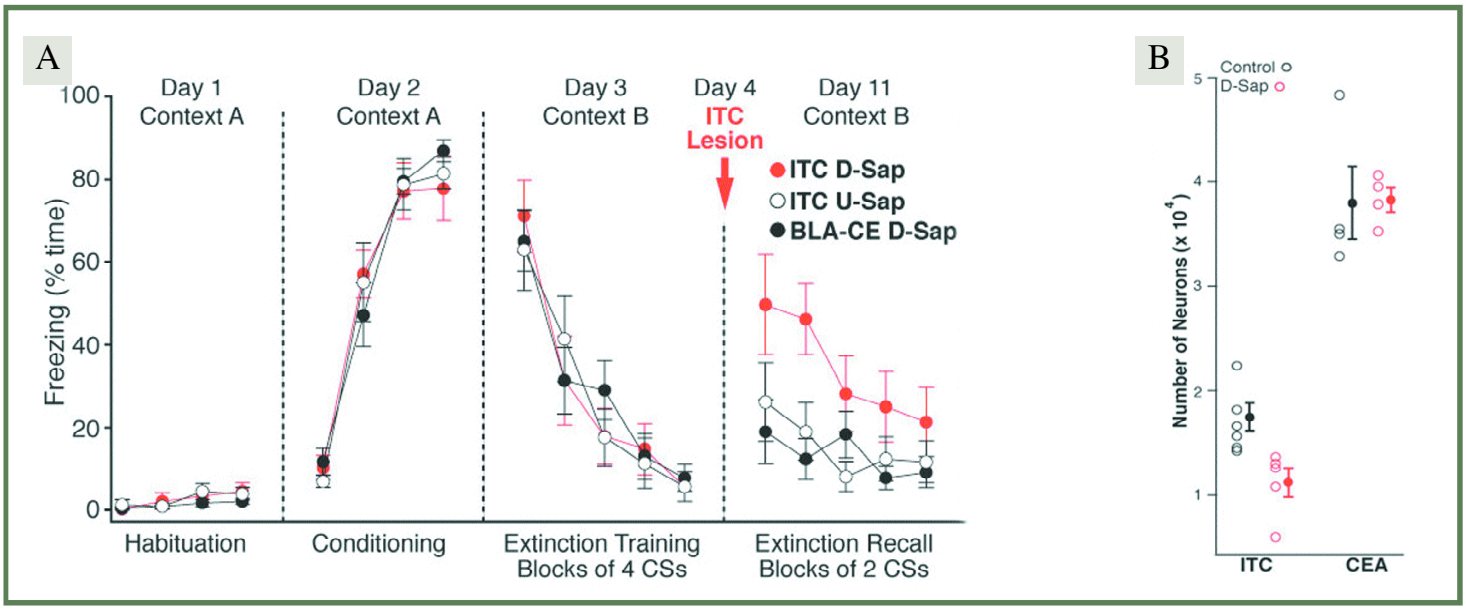

Figure 2. Infusions of Dermorphin-SAP decrease the number of ITC cells and lead to a deficit in extinction recall.
(A) Percent time freezing over experimental sessions in animals receiving Dermorphin-SAP injections in the ITC cells (red circles), in the BLA-CEA (black circles), or Blank-SAP injections in the ITC cells (white circles).
(B) Unbiased stereological estimates of cell numbers (mean ±sem, filled black circles) in the medial ITC cell clusters and central nucleus (CEA) in experimental (red circles) versus control (white circles) animals.