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RESEARCH HIGHLIGHT

Pathway Matters: Prefrontal Control of Negative Emotions *via* Distinct Downstream Regions

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The infralimbic cortex (IL) is a major subregion of the medial prefrontal cortex (mPFC) that is essential for many advanced brain functions including emotions. Mounting studies have unraveled a critical role of the IL in the processing of negative emotions such as fear and anxiety. However, these studies also generate controversial data regarding the precise functions of the IL in the regulation of either fear or anxiety. On one hand, pharmacological inactivation of the IL attenuates anxiety-like behaviors and consistently, pharmacological activation of the IL produces anxiety-like behaviors [1]. On the other hand, there are opposing reports that electrical stimulation of the IL generates fear-releasing effects: reduction in conditioned fear responses and facilitation of fear extinction [2]. These contradictory reports raise the possibility that subgroups of IL neurons differentially regulate negative emotions *via* distinct downstream regions. The specific projections and their causal roles in emotional regulation are of clinical importance, and yet are to be determined.

Now, a study published in *The Journal of Clinical Investigation* [3] set out to address this important issue and investigated distinct IL projections regulating negative emotions. The authors first examined the function of the IL in regulating anxiety-like behaviors in mice with two well-established behavioral assays: the elevated plus maze and the open field test (OFT). Optogenetic activation of IL glutamatergic neurons robustly induced an anxiogenic effect. Optogenetic inactivation of IL glutamatergic neurons induced an anxiolytic effect. These results indicate that the IL is indeed able to bidirectionally regulate anxiety-like behaviors. Given that a previous study reported that activation of the entire ventromedial (vm) PFC [including the IL and the dorsal peduncular cortex (DP)] has no effect on anxiety, the authors hypothesized that the function of the DP in anxiety regulation might be opposite to that of the IL. In this case, when the entire vmPFC was activated, the anxiogenic effect of IL activation would be counterbalanced by the anxiolytic effect of DP activation. Consistent with this hypothesis, the authors found that specific activation of the DP has an anxiolytic effect.

By using anterograde tracing, the authors further demonstrated that IL neurons have dense axon terminals in the lateral septum (LS), central nucleus of the amygdala (CeA), basolateral amygdala, and bed nucleus of the stria terminalis, all of which play important roles in regulating anxiety-like behaviors [4]. This structural association suggests that the IL can regulate anxiety-like behaviors *via* its projections to these downstream regions. Interestingly, however, only activation of IL axons either in the LS or the CeA had an effect on anxiety-like behaviors. Specifically, activation of the IL-LS pathway had an anxiogenic effect. Such effect is in line with previous studies showing that activation of IL or LS Crfr2^{+} neurons increases anxiety [5]. Conversely, inhibition of the IL-LS pathway reduced anxiety-like behaviors. In striking contrast to the IL-LS pathway, activation of the IL-CeA pathway had an anxiolytic effect, and inhibition of this pathway increased anxiety-like behaviors. Together, these projection-specific manipulation experiments provide

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convincing evidence that both the IL-LS and IL-CeA pathways are closely involved in anxiety regulation. More importantly, their regulatory effects on anxiety-like behaviors are opposite (Fig. 1a).

The above behavioral effects of activation of IL-LS and IL-CeA pathways were blocked by the pre-application of glutamate receptor antagonists (NBQX and AP5) in the LS and CeA, suggesting that the excitatory transmission from IL to LS and CeA are important for anxiety regulation. Consistent with this, using *ex vivo* electrophysiological recordings, the authors demonstrated that IL neurons form monosynaptic and functional excitatory synapses with LS and CeA neurons. Furthermore, IL neurons projecting to the LS and CeA are distinct populations with almost no overlap. Counts revealed more LS-projecting than CeA-projecting neurons in the IL.

The anxiolysis produced by activation of the IL-CeA pathway raised the possibility that activation of this pathway could reverse stress-induced high anxiety. To test this hypothesis, the authors induced anxiety-like behaviors in mice with the chronic restraint stress paradigm. Specifically, mice were restrained for 1 h each day for 3 consecutive days and the restrained animals developed an elevated anxiety phenotype. In agreement with their hypothesis, the stress-induced anxiety phenotype was successfully rescued by activation of the IL-CeA pathway. These results suggest that activation of the IL-CeA pathway alleviates stress-induced anxiety, therefore this pathway could serve as a treatment target for anxiety disorders.

Next, the authors turned to dissect the role of the IL in fear regulation with a classical fear conditioning assay in mice. Consistent with previous studies [2], they found that activation of the IL suppresses fear expression and facilitates fear extinction, while inhibition of the IL impaired the formation of extinction memory. Having established the critical role of the IL in regulating fear extinction, the authors then explored the neural circuit mechanisms underlying this regulation. By using optogenetic manipulations, they showed that activation of the IL-LS pathway impaired fear extinction, whereas activation of the IL-CeA pathway facilitated fear extinction. On the contrary, inhibition of these pathways had opposite consequences on fear extinction. Together, these results indicate that fear-like behaviors are also under tight control of the IL *via* its distinct subcortical targets. Both the IL-LS and IL-CeA pathways participate in fear extinction, but they act in opposite directions (Fig. 1b).

The CeA consists of the CeL and CeM, of which the CeM receives inhibitory control from the CeL and is the output nucleus of the CeA [6]. Following fear conditioning, CeL_{on} cells ($PKC\delta^{-}$) are excited by the conditioned stimulus and inhibit CeL_{off} cells ($PKC\delta^{+}$) that project to the CeM. This disinhibitory circuit enhances CeM output and hence drives the expression of fear (freezing) [6]. Since IL neurons project to both CeL and CeM, it is interesting to determine which specific target(s) mediate the behavioral effects following activation of the IL-CeA pathway. The authors demonstrated that activation of IL neurons projecting to the CeM decreases anxiety-like behaviors and facilitates fear extinction, while activation of IL neurons projecting to the CeL only increased center exploration in the OFT and had no effect on fear extinction. These results suggest that the fear-releasing behavioral effects of activating the IL-CeA pathway are mainly mediated by IL projections to the CeM.

The study by Chen *et al.* [3] delineates the detailed neuronal circuit mechanisms by which the IL regulates negative emotions. The authors, for the first time, provide compelling evidence that divergent pathways of the IL exert antagonistic effects on anxiety and fear. Specifically, the IL-LS pathway has anxiogenic effects and promotes fear responses, whereas the IL-CeA pathway has anxiolytic effects and reduces fear responses. These findings not only advance our understanding of the prefrontal circuits underlying emotion regulation but also inspire future

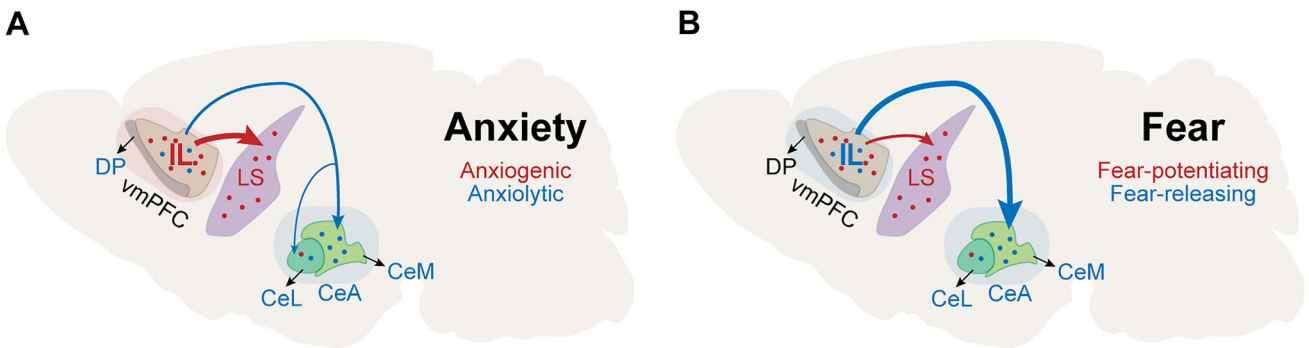

Fig. 1 Distinct IL projections have opposite effects in modulating anxiety **a** and fear **b**. DP, dorsal peduncular cortex; IL, infralimbic cortex; vmPFC, ventromedial prefrontal cortex; LS, lateral septum; CeL, lateral subdivision of the central amygdala; CeM, medial subdivision of the central amygdala; CeA, central nucleus of the amygdala.

scientific quests. For instance, how are these distinct pathways recruited in the intact brain under physiological conditions? As for long-range input, prefrontal anxiety-related neuronal activity is strongly modulated by theta activity in the ventral hippocampus (vHPC) [7]. At the local circuit level, vasoactive intestinal polypeptide (VIP) interneurons in the mPFC are activated by vHPC input and disinhibit cortical pyramidal neurons [8]. This disinhibitory microcircuit mediates the transmission of anxiety-related information from the vHPC to the PFC and promotes anxiety-like behaviors. Besides, recent studies have reported that prefrontal somatostatin (SST) interneurons disinhibit pyramidal cells *via* their inhibitory interaction with PV interneurons, and thereby modulate prefrontal output to orchestrate fear responses [9, 10]. Therefore, it would be interesting to determine whether these long-range and local circuits are differentially involved in driving IL neurons projecting to the LS and CeA. In addition, the current study suggests that a proper emotional state is determined by a balance of the IL-LS and IL-CeA pathways. Disruption of this balance could lead to anxiety-related dysfunctions commonly reported in neuropsychiatric disorders. Therefore, how IL neurons projecting to the LS and CeA and their corresponding projections are impaired in anxiety-related disorders is another important question to be resolved. Answers to these questions could help develop novel pharmacological treatments for anxiety-related disorders.

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Conflict of interest The author declares no competing interest.

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