

Perspective

Dopamine-dependent synaptic plasticity in an amygdala inhibitory circuit controls fear memory expression

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Of the numerous events that occur in daily life, we readily remember salient information, but do not retain most less-salient events for a prolonged period. Although some of the episodes contain putatively emotional aspects, the information with lower saliency is rarely stored in neural circuits via an unknown mechanism. We provided substantial evidence indicating that synaptic plasticity in the dorsal ITC of amygdala allows for selective storage of salient emotional experiences, while it deters less-salient experience from entering long-term memory. After activation of D4R or weak fear conditioning, STDP stimulation induces LTD in the LA-ITC synapses. This form of LTD is dependent upon presynaptic D4R, and is likely to result from enhancement of GABA release. Both optogenetic abrogation of LTD and ablation of D4R at the dorsal ITC *in vivo* lead to heightened and over-generalized fear responses. Finally, we demonstrated that LTD was impaired at the dorsal ITC of PTSD model mice, which suggests that maladaptation of GABAergic signaling and the resultant LTD impairment contribute to the endophenotypes of PTSD. [BMB Reports 2016; 49(1): 1-2]

The amygdala is one of the most widely studied brain regions due to its critical roles in the acquisition and expression of emotional memory. Signal transfer within and between the amygdala nuclei is causally modulated not only by the cellular features of individual amygdala neurons, but also by dynamic regulation of the comprising neural circuits. Prior anatomical

studies revealed that ITCs are divided into several clusters, each of which has distinct connectivity and thereby plays potentially different physiological roles in fear memory. Among those clusters, the dorsal ITC receives excitatory inputs from the lateral amygdala (LA) and projects its GABAergic inputs into neighboring regions, such as the lateral compartment of the central amygdala (CeA) or the ventral ITC, which project to the common outputs: the medial compartment of the CeA. Besides the excitatory inputs from other brain areas, the dorsal ITC also receives abundant DAergic inputs from the midbrain. Additionally, it was reported that a subset of DAergic neurons are robustly activated by aversive stimuli and the excitability of ITC neurons are affected by activation of DA receptors. Accordingly, we reasonably hypothesized that DA plays a regulatory role in fear memory by modulating ITC cells and their synaptic plasticity.

To recapitulate two types of fear memory possessing distinct saliency in mice, we paired strong (0.8 mA) or weak (0.4 mA) electric shock with the same auditory cue. While strongly fear-conditioned mice exhibited apparent freezing behavior even 3 days after fear conditioning, weakly fear-conditioned mice showed an only moderate level of freezing after 24 hours but failed to consistently recall thereafter. Using these behavioral paradigms, we sought to explore whether dorsal ITC synapses are altered differentially by each learning paradigm. Interestingly, after weak fear conditioning but not after strong fear conditioning, spike-timing-dependent plasticity (STDP) stimulation could induce LTD in the LA-ITC pathway. LTD induction appears to be regulated by the saliency of fear-related stimuli, which suggests that the amount of DA released by a distinct level of saliency in fear conditioning may determine or control synaptic plasticity in the LA-ITC pathway.

To provide mechanistic insights into STDP-induced LTD, we pharmacologically modulated each subtype of DA receptor. Rather than any other subtypes, D4R was necessary and sufficient for triggering LTD in the LA-ITC synapses, which was corroborated by data from D4R knockout mice. Importantly, this form of LTD does not result from a decrease in excitatory transmission itself. Rather, disynaptic IPSC and miniature IPSC data indicate that D4R-induced LTD is driven by an increase in GABAergic transmission from neighboring ITC cells. The coefficient of variation, simultaneous monitoring of monosynaptic and disynaptic postsynaptic currents and selective blockade of

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Abbreviations: ITC, intercalated cell mass; D4R, dopamine receptor subtype 4; DA, dopamine; LTD, long-term depression; PTSD, post-traumatic stress disorder; STDP, spike-timing dependent plasticity

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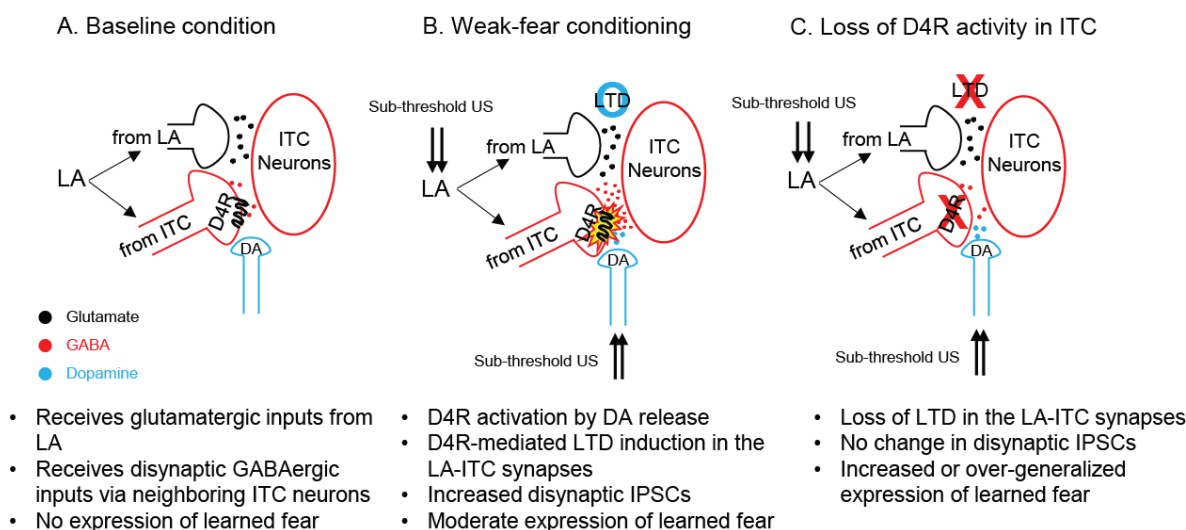


Fig. 1. A schematic description of D4R activity during less-salient experience. (A) In the baseline condition, dorsal ITC neurons receive monosynaptic excitatory inputs from LA, and GABAergic inputs from neighboring dorsal ITC neurons. As the other ITC neurons also receive monosynaptic inputs from the LA, this pathway forms a feed-forward inhibitory circuit with disynaptic IPSCs. (B) During weak fear conditioning, DA is released from the midbrain to the dorsal ITC neurons and activates D4Rs on axonal terminals of the dorsal ITC neurons. Activation of D4R could lead to induction of LTD, leaving glutamatergic transmission unaffected. (C) D4R activity is required to induce LTD in the LA-ITC synapses. In case of pharmacological or genetic D4R depletion, GABA release within the dorsal ITC area would not be increased. The deficit in LTD is likely to result in excessive and generalized fear behavior even toward less-salient experience.

G-protein signaling in neighboring ITC neurons, indicated that the activity of presynaptic D4R plays a critical role in LTD induction in the dorsal ITC (Fig. 1). Importantly, increases in disynaptic IPSCs and miniature IPSCs were also observed after weak fear conditioning, which is consistent with D4R-dependent increases in inhibitory inputs upon exposure to less-salient experience.

Subsequent behavioral experiments revealed the functional roles of D4R in the dorsal ITC. We micro-infused D4R antagonist into the dorsal ITC or transduced a newly developed, cell-type-specific AAV vector expressing shRNA against D4R before weak fear conditioning. Both manipulations for D4R activity led to increases in fear responses in weakly fear-conditioned mice. Furthermore, we optogenetically reversed LTD with applying theta-burst stimulation (TBS) *in vivo*, and then assessed fear recall behavior. After TBS-induced reversal of LTD, ChR2-expressing mice showed a significantly higher freezing level than EYFP-expressing control mice. Taken together, our results indicated that D4R activity and/or D4R-mediated LTD in the dorsal ITC is necessary for preventing less-salient experience from eliciting fear memory.

Finally, we took advantage of an animal model that exhibited PTSD-like memory impairment. As demonstrated previously, CORT-injected mice showed generalized freezing behavior: unusual freezing responses upon exposure to the contexts or the auditory cues that were never paired with noxious stimuli. Importantly, weak fear conditioning failed to make LTD available and to increase disynaptic IPSCs in the LA-ITC pathway of

PTSD mice. Furthermore, D4R-depleted mice displayed a significantly higher level of freezing behavior in the context that was not paired with electric shocks, compared to control mice. Therefore, our behavioral assessments of PTSD-like symptoms point to the existence of common cellular mechanisms of PTSD-causing and D4R-triggered signaling pathways.

In conclusion, our findings provide a novel viewpoint of the physiological and functional roles of the amygdala inhibitory circuit in expression of fear memory. In contrast to the classical view, it became clear that some signaling pathways, including the downstream cascade of D4R, could be activated and play certain roles after exposure to less-salient stimuli and the subsequent processing. Furthermore, our report is important because it is the first delineation of both the physiological role of synaptic plasticity at the LA-ITC synapses and the functional relevance of feed-forward inhibitory connections in the dorsal ITC. Taken together, D4R-induced synaptic plasticity in the dorsal ITC is one of the major neural components that control storage and recollection of fear memory, and deficits in this cellular or molecular pathway could contribute to the endophenotypes of PTSD such as excessive and/or over-generalized fear responses.

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