Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Differential Involvement of ASIC1a in the Basolateral Amygdala in Fear Memory and Unconditioned Fear Responses

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Review of Coryell et al. (http://www.jneurosci.org/cgi/content/full/28/51/13738)

Emotion is critical to provide an adequate response to the environment. Hyperemotionality induces strongly invalidating psychiatric diseases such as posttraumatic stress disorders or phobias. Investigating how the brain controls and regulates fear expression is crucial to better understand these troubles.

Fear conditioning is the main paradigm used in clinical and preclinical research to investigate the mechanisms underlying fear learning and fear memory. Classical or pavlovian fear conditioning results from the association of a conditioned stimulus (CS), initially neutral, and a noxious unconditioned stimulus (US), usually an aversive footshock (fear training). This association drives the development of defensive conditioned responses following a reexposure to the CS (fear memory).

Surgical, pharmacological, and electrophysiological studies have highlighted neuronal pathways underlying associative learning, especially the limbic system (hippocampus, amygdala) and related cerebral structures (prefrontal, perirhinal, and entorhinal cortices). Among these sites, the amygdala is notably involved in encoding and storing the "CS–US" association. The basolateral amygdala complex (BLA), composed of the lateral, the basolateral, and the basomedial nuclei, is of particular interest since BLA inactivation using neurotoxic lesions impairs the occurrence of cue or contextual conditioned fear behavior (Maren, 2001). CS–US association in the BLA relies on NMDA receptor-dependent long-term potentiation (LTP) mechanisms and the activation of the PKA-CaMKII downstream signaling cascade (Kim and Jung, 2006). However other mechanisms might be involved. For example, intra-amygdala infusion of the $GABA_A$ receptor agonist muscimol in rats or disruption of functional metabotropic glutamate receptors, mGluR1 and mGluR3, in mice impair pavlovian fear conditioning.

ASIC1a, an acid-sensitive ion channel widely expressed in the central and peripheral nervous system, has a key function in mechanosensation and nociception. Interestingly, a role in fear learning and memory is also emerging. Recently, ASIC1a has been shown to be essential to innate fear but also to fear conditioning behaviors (Coryell et al., 2007). Considering the high ASIC1a expression level in the BLA, Coryell et al. (2008) hypothesized that (1) ASIC1a could be involved in neuronal mechanisms associated with fear learning and that, consequently, (2) disrupted fear memory in mice lacking functional ASIC1a (ASIC1a−/−) could be rescued by a specific viral administration of the ASIC1a gene in the BLA (Coryell et al., 2008).

To determine the role of ASIC1a in fear memory, the authors applied context fear conditioning to mice deficient for the ASIC1a gene. Context fear conditioning consisted in the association of a conditioning chamber with five footshock series (1 s, 0.75 mA). The total time during which an animal exhibited freezing behavior was used as a measure of context fear conditioning.

To assess whether ASIC1a in BLA is indeed involved in fear training, the expression level of the protein cFos was evaluated following context fear conditioning. ASIC1a−/− mice showed decreased c-fos expression compared with wild-type (WT) mice, suggesting that the deficit in ASIC1a decreased neuronal activity in BLA [Coryell et al. (2008), their Fig. 1].

To test more directly whether fear conditioning requires ASIC1a activation in the BLA, the authors investigated the impact of rescuing ASIC1a expression specifically in the BLA of ASIC1a−/− mice. Adeno-associated virus (AAV)-mediated gene transfer was used to restore ASIC1a expression bilaterally in the BLA. WT and ASIC1a−/− mice treated with AAV-ASIC1a virus or control, a vector contain-

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ing the green fluorescent protein (AAV-GFP), were trained and tested to the context–footshock conditioning.

As previously shown (Wemmie et al., 2003), ASIC1a−/− mice froze less often than WT mice during the training phase. Moreover, rescuing ASIC1a expression in the BLA was not sufficient to restore the WT level of freezing [Coryell et al. (2008), their Fig. 2*E*]. This suggests that the role of ASIC1a in fear training involves cerebral structures other than BLA.

The day after training, mice were returned to the conditioning chamber to test context-evoked freezing. Untreated ASIC1a−/− mice froze less than WT mice during testing. Surprisingly, the conditioned freezing in AAV-ASIC1a-treated mutant mice was the same as that of WT [Coryell et al. (2008), their Fig. 2*F*], indicating that ASIC1a expressed in the BLA plays a crucial role in context-dependent conditioned fear responses. This result raises the question of how AAV-ASIC1a-treated mice could display an appropriate fear-conditioned response while they still present fear training deficits? This would imply that fear memory and fear training rely on different ASIC1a-dependent memory mechanisms.

Results of the ASIC1a rescue experiments suggest that ASIC1a activity in the BLA plays a role in fear conditioning, but raises the perplexing question of why the conditioned response (CR) was larger than the unconditioned response (UR). Given that ASIC1a has been shown to be involved in nociception (Mazzuca et al., 2007) and to contribute to pain-elicited currents in the spinal cord (Wu et al., 2004), ASIC1a−/− mice are likely to present nociceptive sensory deficits. This could involve a weakened perception of pain which could consequently impair the CS–US pairing.

It thus appears essential to further assess ASIC1a−/− mice nociceptive sensitivity using acute pain models, for example the hot-plate test. Indeed, a somatic painful stimulation such as intraplantar injection of formalin induces increased c-fos mRNA expression in the BLA (Nakagawa et al., 2003). Consequently, the decreased c-fos expression observed in ASIC1a−/− mice BLA following footshock–context conditioning could arise from a reduced response to pain. In contrast, a similar pain threshold of ASIC1a−/− and WT mice would suggest that freezing deficits during the training phase result from modified emotional responses to pain or modified ASIC1a-dependent fear training mechanisms. Since BLA treatment with ASIC1a-AAV did not rescue freezing deficits, these mechanisms are likely to involve ASIC1a activation in other cerebral regions such as the medial prefrontal or the entorhinal and perirhinal cortices which are known to play a key role in context fear memory. Viral transduction of ASIC1a-AAV in these structures before contextual fear learning could provide answers to this issue.

In another experience, Coryell et al. (2008) attempted to determine whether all unconditioned responses were impaired in ASIC1a−/− mice. The nonpainful US (predator odor) produced the same deficit in freezing as the painful one [Coryell et al. (2008), their Fig. 2*G*]. This result supports the hypothesis that the strength of the CS–RS is not directly tied to the strength of the US–UR. Nevertheless, to avoid the confusing influence of pain perception on memory processes, the authors could have used a nonpainful aversive conditioned stimulus (e.g., predator odor). However, any US that evokes a fear response would also produce anxiety. A recent study demonstrated that ASIC1a antagonists have anxiolytic effects (Dwyer et al., 2009). Consequently, less freezing during training in ASIC1a−/− mice could be interpreted as a loss of ASIC1a anxiolytic properties. Anxiety levels in mice could be evaluated using an elevated-zero maze, which is relevant due to the prominent role of amygdala in emotional behavior.

The mismatch between ASIC1a−/− mice freezing during testing and the expression of long-lasting fear memory during training remains to be elucidated. As the test was performed 24 h following the training, plasticity mechanisms such as consolidation could have occurred during this period and contributed to the formation of a stable memory. To test this hypothesis, the time interval between training and testing could be reduced (to 30 min) or one could consider using ASIC1a antagonists following the training phase. Thus, preventing the consolidation would blunt the freezing ASIC1a-AAV rescue observed during testing.

In summary, Coryell et al. (2008) provide evidences that ASIC1a is not solely involved in context-dependent fear behavior and that the site of ASIC1a action is likely to be the BLA. Strikingly, these results report that expressing ASIC1a in the BLA of ASIC1a−/− mice rescues fear memory but not the freezing deficit observed during training. This study raises several questions for future investigations. A better understanding of how "CS–US" association is established and determining the contribution of different brain structures like BLA to fear memory would help the development of new behavioral or pharmacological strategies to treat pathological fears.

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