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RESEARCH HIGHLIGHT

Heightened fear in the absence of the kainate receptor auxiliary subunit NETO2: implications for PTSD

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About 4% of individuals who experience a traumatic event in their lifetime develop post-traumatic stress disorder (PTSD) [1], characterized by the heightened fear and the presence of intrusive memories of past traumatic events. Based on the conditioning theory of PTSD, various cues or conditioned stimuli (CS) present at the time of the traumatic event (unconditioned stimulus; US) become associated with the emotional response caused by the traumatic event. This can lead to enhanced pathological fear responses even in the absence of real threat. Two possibilities may underlie why PTSD develops in some individuals while sparing others.

1. Individuals who are more prone to acquire conditioned responses to the traumatic US, i.e., those with a higher fear conditionability may be more likely to develop PTSD.

2. The failure to extinguish conditioned fear may contribute to the development of PTSD. In either case, patients who suffer from PTSD show excessive conditioned fear and a failure to learn safety cues in the absence of danger.

The classical Pavlovian fear conditioning paradigm is commonly used in rodents to model PTSD. In this paradigm, animals are trained to associate a neutral stimulus (CS) such as a context or an auditory cue (tone) with an aversive event (US) usually presented in the form of a mild electrical shock [2]. Assessment of fear learning is performed by re-exposing the animals to the context or to the cue while measuring the duration of the time they spend freezing. That is, the absence of any movement except respiration. Studies using this model have demonstrated that conditioned fear learning and expression are mediated by the activation and connectivity of a network of brain regions that include the amygdala, the medial prefrontal cortex, and the hippocampus. Understanding the biological components and mechanisms that mediate fear learning and extinction within this circuitry may offer novel pharmacological targets for the treatment of diseases characterized by enhanced fear expression and extinction such as PTSD.

In their recent article in *Neuropsychopharmacology*, Mennesson et al. [3] describe the involvement of the auxiliary subunits of kainate receptors (KARs), NETO1, and NETO2 in contextual and cued fear responses, respectively. KARs are a type of ionotropic glutamate receptors that mediate excitatory and inhibitory neurotransmission, enhance cell excitability, and control neurotransmitter release [4]. The KAR interacting proteins NETO1 and 2 regulate channel kinetics, modulate opening probability of KARs and regulate KAR-mediated synaptic transmission [5]. Therefore it is likely that a disruption of the activity of NETO subunits will have a detrimental effect on the function of KARs affecting the efficacy of KAR-regulated synaptic networks. How this translates to behavior has not previously been described. This is where the work by Mennesson et al. comes into play. Considering the previous reports showing an association between KAR subunit expression levels and several neuropsychiatric diseases, as well as alterations in anxiety-like behaviors observed in animal models lacking specific KAR subunits, the authors hypothesized that NETO proteins may be involved in the regulation of emotional behaviors. They performed a comprehensive behavioral analysis using mice which are null mutants of *Neto1* (*Neto1*$^{-/-}$) and *Neto2* (*Neto2*$^{-/-}$) genes and their wild-type littermates as controls. The authors found no difference between the innate anxiety-like behaviors of *Neto1*$^{-/-}$ and *Neto2*$^{-/-}$ mice and controls assessed in four different behavioral paradigms including the elevated plus maze, elevated zero maze, light/dark box, and open field tests. However, in all of these tests, female *Neto2*$^{-/-}$ mice had reduced activity, which was absent when monitored in their homecage suggesting that the absence of NETO2 may interfere with their adaptation to a novel environment. Despite this alteration in activity within a novel setting, which may indicate a heightened stress response, there were no differences in the stress-related behaviors or physiological parameters including stress-induced hyperthermia, corticosterone levels, and depression-like behaviors between *Neto2*$^{-/-}$ mice and wild-type controls.

To determine the role of NETO1 and NETO2 proteins in fear memory, the authors performed contextual and cued fear conditioning in *Neto1*$^{-/-}$ and *Neto2*$^{-/-}$ mice. The absence of NETO1 specifically in the females was associated with enhanced contextual fear memory. On the other hand, *Neto2*$^{-/-}$ mice showed significantly higher freezing levels during the both fear acquisition and retrieval indicative of hyper-conditionability. Additionally, the mutant mice exhibited higher freezing during the extinction phase of cued fear conditioning suggesting impaired fear extinction. Importantly, the changes in fear response of *Neto2*$^{-/-}$ mice were not due to increased sensitivity to pain or sensory stimuli assessed by the hot plate test and acoustic startle response measurements.

To further dissect the importance of NETO2 for the conditioned fear response, the authors investigated its pattern of expression in the brain regions critical for fear memory. They identified that *Neto2* was expressed in both excitatory and inhibitory neurons within the amygdala, medial prefrontal cortex, and hippocampus.

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The absence of NETO2 was also associated with reduced synaptic abundance of the major subunits of KARs including Gluk2/3 and Gluk5 in fear-associated brain regions. These findings suggested that in addition to its modulatory role for KARs, NETO2 is required for maintaining the expression levels of the major KAR subunits within the synapses of the fear circuitry, which may be important for the acquisition, expression, and extinction of fear memories.

Taken together, Mennesson et al. provide compelling evidence that supports NETO2-induced modulation of KARs as an important mechanism in the maintenance of normal fear response in mice. The absence of NETO2 leading to a decrease in the major KAR subunits at the syn`apses of the brain's fear centers results in the emergence of PTSD-like phenotypes in mice. These findings also raise important questions for future studies. It will be imperative to determine how the KAR-mediated synaptic transmission is affected in the absence of its auxiliary subunits. It will be interesting to know which part of the fear circuitry is especially vulnerable to the loss of these proteins. Investigation of functional changes in KAR and NETO proteins in humans suffering from trauma-related disorders may further shed light on the underlying mechanisms and lead to novel pharmacological approaches for the treatment of PTSD.

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The author declares no competing interests.

ADDITIONAL INFORMATION

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