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RESEARCH HIGHLIGHT

An innovative approach to examining the role of neurotransmitters in fear circuitry

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Anxiety-related disorders affect millions of people but lack effective treatments—requiring scientists to explore new and innovative approaches. A comprehensive understanding of the pathophysiology of anxiety-related disorders and related conditions necessitates greater foundational knowledge of the underlying neurobiological processes. Serotonin (5HT) has long been implicated in regulating emotional mood. Selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors are commonly prescribed medications for individuals diagnosed with anxiety disorders, and are generally thought to increase 5HT and norepinephrine present in the synapse. A better understanding of the precise synapses that these drugs impact is integral to advancing treatments for anxiety-related disorders.

Fear, a common symptom of anxiety-related disorders, involves neurotransmission in the limbic system, with the amygdala serving as a key nexus governing fear-related behaviors. Signaling from the central amygdala (CeA) to the ventrolateral periaqueductal gray is known to modulate defensive behavior in mice [1]. However, the role that 5HT plays in this signaling pathway is not fully understood. In their recent *Neuropsychopharmacology* article, Hon et al. examine the role that 5HT plays on a novel circuit between the ventral periaqueductal gray (vPAG) and the CeA, and its role in fear learning [2].

Hon et al. aimed to map the PAG projection to the CeA in greater depth than previously explored. To investigate this circuit the authors utilized optogenetic stimulation of $vPAG^{VGAT}$ neurons and channelrhodopsin-assisted circuit mapping. This allowed the authors to outline clear regions of influence between the CeA and the vPAG. In doing so, the authors determined the monosynaptic, $GABA_A$-mediated nature of this system. Previous work conducted by the authors shows that 5HT is released in amygdala subregions during fear learning [3]. Using a novel genetically encoded membrane-bound 5HT sensor, iSeroSnFr developed by Unger et al., the authors were able to visualize 5HT release in the CeA [4]. By coupling this biosensor with fiber photometry, the authors found a previously uncharacterized in vivo 5HT signal. The iSeroSnFr-expressing mice showed a decrease in extracellular 5HT during shock delivery (as well as a rebound response above baseline), and an increase in freezing behavior relative to naive control mice. This initial aim explored by Hon et al. outlined a unique and translational approach toward understanding the mechanism of neurotransmitter signaling between these two regions.

To determine the role that 5HT plays in the $vPAG^{VGAT}$-CeA circuit, the authors again employed optogenetics to optically evoke inhibitory post-synaptic currents (oIPSCs) in the CeA in an ex vivo preparation. The authors showed that bath application of 5HT increased the amplitude of the initial oIPSC and decreased the paired pulse ratio relative to baseline. This effect persisted in the presence of tetrodotoxin and 4-aminopyridine, indicating a direct impact of 5HT on GABA release at $vPAG^{VGAT}$-CeA synapses. The authors were able to highlight the unique role of 5HT on presynaptic $5HT_{2C}$ receptors using specified receptor antagonists. Previously published work by the authors shows that $vPAG^{VGAT}$ neuron inhibition during fear learning will subsequently impair fear expression [3]. The previous findings fell in line with this new work performed by Hon et al. to modulate the $vPAG^{VGAT}$-CeA circuit during fear learning. Mice that underwent fear learning showed a smaller response relative to baseline than naive mice when treated with 5HT in bath application optogenetic experiments. The authors' results suggest that smaller responses to 5HT in fear mice relative to naive mice imply neuroplasticity in the $vPAG^{VGAT}$-CeA circuit.

The authors then further explored these circuit dynamics using fiber photometry. A clear increase in activity relative to basal activity was noted in both the vPAG and CeA during fear learning sessions. This increase was not present in the response of naive mice to tone as no significant difference was noted in either region. The above experiments outlined a clear mechanism for the roles of the vPAG and CeA regions in fear learning. The importance of this connection also lies in the ability of this circuit to modulate specific fear responses. The authors further examined the signaling between the $vPAG^{VGAT}$ and CeA using machine learning to track, classify, and align behavior with fiber photometry signals. In doing so, the authors were able to tease apart individual frame-by-frame behavioral shifts and their corresponding signals. This led to the identification of an inverse relationship between the $vPAG^{VGAT}$ region and freezing behavior. A similar trend was found in the CeA where a decrease in z-scored fiber data corresponded with an increase in freezing behavior. Interestingly, both effects were present regardless of fear learning condition. It is important to note that this work does not examine sex differences in these regions and necessitates further exploration into the underlying mechanisms of this fear signaling circuit between sexes. Further investigation should use similar techniques to examine other behavior phenotypes.

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Although important to the understanding of fear circuitry, the true translational impact of this work lies beyond the mechanistic findings noted by Hon et al. The application of both machine learning and genetically encoded biosensors showcases a unique approach to understanding circuitry foundational to behavior. Unger et al. previously showed the efficacy of this unique neurotransmitter-specific biosensor and were able to highlight its power using fiber photometry. In addition to biosensors, open-source programs for data analysis have grown in scope and efficacy in recent years. Machine learning algorithms and behavioral analysis packages provide a powerful tool for examining animal behavior outside of commercial standards. SimBA is an open-source package that allows for frame-by-frame analysis of rodent behavior and provides greater flexibility than commercial software [5]. Hon et al. utilized SimBA, coupled with fiber photometry, to quantify specific episodes of freezing and mobility during fear learning sessions, and this application of cutting-edge techniques allowed for a more thorough characterization of the 5HT system. Overall, the work by Hon et al. provides convincing evidence for the endogenous role of 5HT in fear learning, and highlights the unique and exciting role of emerging biosensors and technologies.

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AUTHOR CONTRIBUTIONS

KRG wrote the initial draft. NAC edited the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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