Archival Report

Biological Psychiatry

Stress Enables Reinforcement-Elicited Serotonergic Consolidation of Fear Memory

Michael V. Baratta, Suhasa B. Kodandaramaiah, Patrick E. Monahan, Junmei Yao, Michael D. Weber, Pei-Ann Lin, Barbara Gisabella, Natalie Petrossian, Jose Amat, Kyungman Kim, Aimei Yang, Craig R. Forest, Edward S. Boyden, and Ki A. Goosens

ABSTRACT

BACKGROUND: Prior exposure to stress is a risk factor for developing posttraumatic stress disorder (PTSD) in response to trauma, yet the mechanisms by which this occurs are unclear. Using a rodent model of stress-based susceptibility to PTSD, we investigated the role of serotonin in this phenomenon.

METHODS: Adult mice were exposed to repeated immobilization stress or handling, and the role of serotonin in subsequent fear learning was assessed using pharmacologic manipulation and western blot detection of serotonin receptors, measurements of serotonin, high-speed optogenetic silencing, and behavior.

RESULTS: Both dorsal raphe serotonergic activity during aversive reinforcement and amygdala serotonin 2C receptor (5-HT2CR) activity during memory consolidation were necessary for stress enhancement of fear memory, but neither process affected fear memory in unstressed mice. Additionally, prior stress increased amygdala sensitivity to serotonin by promoting surface expression of 5-HT2CR without affecting tissue levels of serotonin in the amygdala. We also showed that the serotonin that drives stress enhancement of associative cued fear memory can arise from paired or unpaired footshock, an effect not predicted by theoretical models of associative learning.

CONCLUSIONS: Stress bolsters the consequences of aversive reinforcement, not by simply enhancing the neurobiological signals used to encode fear in unstressed animals, but rather by engaging distinct mechanistic pathways. These results reveal that predictions from classical associative learning models do not always hold for stressed animals and suggest that 5-HT2CR blockade may represent a promising therapeutic target for psychiatric disorders characterized by excessive fear responses such as that observed in PTSD.

Keywords: Amygdala, Fear, 5-HT2C receptor, Optogenetics, PTSD, Serotonin

http://dx.doi.org/10.1016/j.biopsych.2015.06.025

Stress exposure is a risk factor for the development of posttraumatic stress disorder (PTSD) in humans (1,2). Humans with PTSD often have strong memories for the traumatic experiences that underlie their disorder (3) but also exhibit heightened fear conditioning in laboratory settings (4,5). In preclinical studies, the relationship between stress exposure and subsequent trauma-related memory can be studied by exposing rodents to stressors and examining the impact on Pavlovian fear conditioning. In this model, fear conditioning itself does not lead to PTSD; only stress-exposed animals display the excessively strong fear memories that are also observed in humans with PTSD. The exaggerated fear response typically observed in stress-exposed animals (6) is often attributed to either strengthened encoding (7) or consolidation processes (8).

Serotonin plays a critical role in the regulation of emotion, and dysregulation of serotonergic systems is associated with stress-related affective disorders (9), including PTSD. Multiple lines of evidence suggest that excess serotonin is linked to altered threat processing. For instance, individuals that carry the short variant of the gene encoding the serotonin transporter (*SLC6A4*), which is thought to impair synaptic serotonin uptake, display increased amygdala reactivity to briefly presented (phasic) aversive stimuli (10). In rodent studies, during aversive learning, serotonin is released into projection regions of the dorsal raphe nucleus (DRN) via phasic firing changes in response to discrete stimuli (11–13). The extracellular serotonin levels in downstream DRN targets, like the basolateral amygdala (BLA), can remain elevated for at least an hour after learning is completed (14,15). Although serotonin acts through several receptor subtypes in the BLA, the serotonin 2C receptor (5-HT2CR) is of interest because these receptors are heavily expressed in BLA neurons that regulate anxiety (16) and 5-HT2CR agonists promote anxiety in humans (17). Furthermore, viral-mediated overexpression of 5-HT2CR in amygdala produces anxiogenic effects (18), while pharmacologic blockade of amygdala 5-HT2CR prevents stress-induced anxiety-like behaviors (19).

Here, we examine behavior in a rodent paradigm in which repeated exposure to stress produces a vulnerability to heightened fear learning (6) and demonstrate that this vulnerability emerges from a serotonergic fear memory consolidation

ISSN: 0006-3223

process that is not present in unstressed mice. This consolidation process requires serotonergic activity in the DRN during aversive reinforcement and 5-HT2CR signaling in the BLA, a major target structure of the DRN (20–24), after aversive learning. Interestingly, we also show that serotonin activation by either signaled or unsignaled footshocks is sufficient to enhance associative fear memory in stressed animals, an effect not predicted by classic theoretical models of associative learning. We show that stress enhances cell surface expression of 5-HT2CRs in the amygdala without affecting total serotonin levels during fear conditioning. Thus, aversive reinforcement is processed differently in the brain of a stress-exposed animal, and this profoundly impacts memory for later aversive experiences. These findings reveal fundamental mechanisms underlying the operation of a critical neural system in affective processing and provide new principles both for associative learning theory and the prevention of stress-related psychiatric disorders.

METHODS AND MATERIALS

Subjects

Adult male C57BL/6 mice (Taconic, Germantown, New York) or transgenic mice (25) were used in all experiments. All procedures were approved by the Committee on Animal Care at the Massachusetts Institute of Technology and the Animal Care and Use Review Office at the U.S. Army Medical Research and Materiel Command.

Virus

Adeno-associated virus vectors were serotyped with adeno-associated virus 2/8 capsids and packaged by the Vector Core at The University of North Carolina at Chapel Hill. The final viral concentration was approximately 1.0 to 2.0 $\times$ 10^{11} infectious particles per milliliter.

Surgical Procedures

For some experiments, mice received cannulae implants, optical fiber implants, or virus infusions, as described in the Supplement.

In Vivo Recording. Single-unit recordings were conducted in anesthetized SERT-Cre mice weeks after stereotactic delivery of virus to the DRN. Cell-attached recordings, which enabled well-isolated single-unit recordings, were obtained using a standard blind in vivo patching technique (26). See the Supplement for details.

Drugs. The selective 5-HT2CR antagonist 6-chloro-2,3-dihydro-5-methyl-N-[6-[(2-methyl-3-pyridinyl) oxy]-3-pyridinyl]-1H-indole-1-carboxyamide dihydrochloride (SB242084; Tocris Bioscience, Minneapolis, Minnesota) was dissolved in .9% sterile saline.

Immobilization Stress. Mice were transferred to an experimental room and placed in ventilated plastic Decapicone bags (Braintree Scientific, Braintree, Massachusetts) for 1 hour on each of 2 consecutive days. While fear conditioning is also a type of stress exposure, here we use the term stress to exclusively refer to immobilization stress.

See the Supplement for additional procedures and assays.

RESULTS

Repeated Stress Enhances the Consolidation of Fear Memories Established Under Degraded Contingency

Stress exposure can enhance learned fear memories (6,27,28), modeling the way in which a history of stress exposure can predispose humans to disorders of fear or anxiety (1,29). Here, we exposed mice to either 2 days of immobilization stress (stress; 1 hour/day) or handling (no stress), followed by auditory fear conditioning (Figure 1). Unlike previous studies that examined the relationship between stress and subsequent auditory fear memory (6,27), we used an auditory fear conditioning protocol in which two of four tone and footshock presentations were explicitly unpaired (50% pairing), thereby reducing the tone-footshock contingency. Such a paradigm may be more sensitive to the effects of stress than a conventional protocol where the pairing is 100% (30). Conditional fear to the tone was assessed in a novel environment either 2 hours (short-term memory) or 24 hours (long-term memory) after fear conditioning (Figure 1A).

Prior stress did not impact the amount of conditional freezing to the tone during fear acquisition (Supplemental Figure S1A) or the short-term memory test (stress: $F_{1,19}$ = .020; stress $\times$ tone interaction: $F_{1,19}$ = .384, ps = ns, n = 10–11/group; Figure 1A, left) but did enhance tone-elicited freezing in mice tested 24 hours later (stress: $F_{1,18}$ = 1.64, p = ns; stress $\times$ tone interaction: $F_{1,18}$ = 11.790, $p < .01$; Fisher's protected least significant difference [PLSD] comparing no stress = 37.22 $\pm$ 9.22% and stress = 62.78 $\pm$ 6.26%, $p < .05$, n = 10/group; Figure 1A, right). All groups exhibited comparable, low levels of freezing during the 3-minute baseline period of the auditory fear test (Fisher's PLSD comparing no stress with stress, ps > .230; Figure 1A, left and right), indicating no generalization between the conditioning and testing contexts. Stress did not enhance fear memory via changes in pain processing, general motor activity, or memory retrieval (Supplemental Figures S1B–D and S2). Enhanced fear memory was also observed only after repeated stress (Supplemental Figure S3). The findings that repeated stress enhances long-term but not short-term fear memory when given before fear conditioning suggests that immobilization stress enhances fear responses by strengthening fear memory consolidation.

Serotonergic Fear Memory Consolidation Is Selectively Enabled by Stress

Because our stress paradigm enhanced fear memory consolidation and serotonin is also implicated in the consolidation of memories (31–34), we determined whether stress-related enhancement of long-term fear memory consolidation is mediated by serotonin signaling in the BLA. Mice were implanted with bilateral cannulae in the BLA before stress or handling. Intra-BLA administration of the highly selective 5-HT2CR antagonist SB242084 (.4 μg/.4 μL) (24) immediately following fear conditioning completely blocked stress-induced