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Research report

Sign-tracking to an appetitive cue predicts incubation of conditioned fear in rats

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HIGHLIGHTS

- Fear to a tone extensively paired with shock "incubated", i.e. increased over time.
- Fear incubation only occurred in rats that preferentially approached reward cues.
- Rats that approached reward cues also had less prefrontal cortical BDNF.
- Prefrontal BDNF may protect against both addiction and pathological fear responses.

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ABSTRACT

Although post-traumatic stress disorder (PTSD) and addiction are very different disorders, both are characterized by hyperreactivity to trauma- or drug-related cues, respectively. We investigated whether an appetitive conditioning task, Pavlovian conditioned approach, which predicts vulnerability to reinstatement of cocaine-seeking, also predicts fear incubation, which may be a marker for vulnerability to PTSD. We classified rats based on whether they learned to approach and interact with a food predictive cue (sign-trackers), or, whether upon cue presentation they went to the location of impending food delivery (goal-trackers). Rats were then exposed to extensive Pavlovian tone-shock pairings, which causes the fear response to increase or "incubate" over time. We found that the fear incubation effect was only present in sign-trackers. The behavior of goal-trackers was more consistent with a normal fear response–it was most robust immediately after training and decayed slowly over time. Sign-trackers also had lower levels of brain-derived neurotrophic factor (BDNF) protein in the prefrontal cortex than goal-trackers. These results indicate that, while many factors likely contribute to the disproportionate co-occurrence of PTSD and substance abuse, one such factor may be a core psychological trait that biases some individuals to attribute excessive motivational significance to predictive cues, regardless of the emotional valence of those cues. High levels of BDNF in the prefrontal cortex may be protective against developing excessive emotional and motivational responses to salient cues.

Abbreviations: BDNF, brain-derived neurotrophic factor; CS, conditioned stimulus; ELISA, enzyme-linked immunosorbent assay; FR, fixed-ratio; GT, goal-tracker; IR, intermediate responder; ITI, intertrial interval; PCA, Pavlovian conditioned approach; PTSD, post-traumatic stress disorder; ST, sign-tracker; US, unconditioned stimulus; VI, variable interval; VR, variable-ratio.

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1. Introduction

Addiction is highly comorbid with post-traumatic stress disorder (PTSD). The overall prevalence of addiction in the United States is about 15% [1], while the prevalence of addiction among people with PTSD is up to 52% in the community and as high as 75% in treatment-seeking populations [2,3]. Similarly, overall prevalence of PTSD is about 7–10% [4], whereas, prevalence among patients with addiction has been reported as high as 42% [5]. Some possible explanations that have been proposed for the relationship between PTSD and addiction include self-medication of anxiety with drugs or alcohol [6,7], increased exposure to traumatic events due to activities involved in acquiring illegal substances [5,8,9], or addictive substances altering the brain's sensitivity to stress to make users more vulnerable to PTSD [10]. These possibilities are not mutually exclusive, and empirical support exists for each of them. However, another possibility is that common factors intrinsic to the individual can increase vulnerability to both disorders. For example, a number of twin studies have indicated significant overlap in genetic predisposition to PTSD and addiction [11–13].

There are many obvious phenomenological differences between addiction and PTSD, but there are also some striking similarities in the core psychological processes underlying both disorders. In particular, both disorders involve excessive motivational responses to cues associated with emotionally salient events. Excessive reactivity to trauma-related cues is especially well-documented in the case of PTSD and is described in no less than three of the diagnostic criteria for PTSD [14–16]. Similarly, addiction is characterized in part by excessive emotional and motivational responses to drug-related cues, i.e. cue-induced craving [17]. The extent to which drug-related cues induce such motivational responses in an individual is positively correlated with a number of clinically relevant variables, such as addiction severity, risk of relapse, and poor treatment outcomes [18,19]. Thus, a general tendency to attribute excessive motivational salience to conditioned cues, regardless of the emotional valence of those cues, would likely predispose an individual to developing both addiction and PTSD.

Pavlovian conditioned approach (PCA) behavior has been used to assess the propensity of individual animals to attribute motivational salience to a reward cue [20,21]. In this procedure, a discrete cue, i.e. a conditioned stimulus (CS), predictive of a food reward, is separated spatially from the location of reward delivery. All animals learn the predictive value of the CS, but a subset of animals (sign-trackers; STs) are especially prone to attribute motivational value to the CS, as evidenced by approach and physical interaction with it. Other animals (goal-trackers; GTs) learn to approach the location of reward delivery upon CS presentation but rarely approach the CS. STs also attribute more motivational salience to drug-paired cues and are more susceptible to drug- and cue-induced reinstatement of drug-seeking behavior than GTs [22–25]. In addition to differences in conditioned appetitive behaviors, STs show more fear toward a CS paired with footshock than GTs [26]. This may indicate that STs would be more likely to develop abnormal fear responses in procedures that model PTSD-like behavior.

In typical fear-conditioning paradigms, in which a tone (CS) is paired with footshock, a fear response to the CS develops quickly, after as little as one tone-shock pairing, and then either remains stable or slowly decays over time [27–30]. However, if the tone-shock pairing is repeated extensively, the fear response increases or "incubates" over time and becomes maximal ~30 days after conditioning [31], similar to the delayed-onset pattern of symptom development often seen in PTSD patients [32]. Interestingly, fear incubation shows considerable individual variability, with some animals showing a large incubation effect while others show no incubation at all [33]. Here, we sought to test whether individual variation in the attribution of motivational value to reward cues, as measured by PCA behavior, would predict incubation of conditioned fear.

To address possible neurobiological differences that could account for individual differences in behavior, we also measured expression of brain-derived neurotrophic factor (BDNF). This molecule was chosen because the BDNF *Val66Met* polymorphism has been implicated in the development of both addiction [34–39] and PTSD [40,41]. Heterozygote BDNF knockout mice self-administer more alcohol than wild-type mice [42,43], and exhibit impaired extinction of conditioned fear [44]. In addition to these effects of global differences in BDNF expression, several pre-clinical studies have shown effects of BDNF manipulation on both drug-seeking behavior and conditioned fear, with BDNF either increasing or decreasing conditioned motivational behavior in a highly region- and circuit-specific manner [45,46]. We therefore tested STs and GTs for differences in BDNF expression in multiple brain regions within the emotional circuitry relevant to both PTSD and addiction [47].

2. Material and methods

All procedures were approved by the University Committee on the Use and Care of Animals.

2.1. Subjects

Ninety-four male Sprague–Dawley rats weighing 275–300 g were obtained from Harlan and Charles River for use in these studies. Subjects were counterbalanced for supplier throughout all phases of the experiment. The rats were housed individually with ad libitum access to water throughout the study. As detailed below, food was also provided ad libitum until training on the PCA task was complete. Subsequent to PCA training, rats were food-restricted to maintain 85% of free-feeding weight, and food restriction continued until brains were harvested at the end of the experiment. The vivarium was kept on a 12:12-h light:dark schedule with temperature maintained at 70–73 °F and humidity at 65–70%. All experimental procedures were performed during the light portion of the cycle.

2.2. Pavlovian conditioned approach

Training on the PCA task took place in Med Associates behavioral testing chambers (24.1 × 20.5 cm floor area, 29.2 cm high; Med Associates, St. Albans, VT). Each chamber had its own sound-attenuating enclosure and a ventilating fan that provided masking noise. Red room lights were used throughout each session, and a red house light in each chamber was illuminated during testing, as well. A recessed food cup was located in the center of one wall of each testing chamber, into which 45-mg banana-flavored pellets could be delivered from a pellet dispenser using a programmable schedule. To the left or right of the food cup, according to a counterbalanced design, was a retractable stainless-steel lever. Whenever the lever was extended into the chamber, an LED mounted inside the lever mechanism illuminated the slot through which the lever protruded. A tray with corn-cob bedding was placed beneath the stainless-steel grid floor.

For two days prior to training, rats received ~15 banana pellets in their home cages to familiarize them with this food. On each of the next two days, rats underwent a pretraining session consisting of 25 pellets delivered non-contingently into the food cup on a variable interval (VI) 30-s schedule, i.e. one food pellet was delivered on average every 30 s, but the actual interval between pellets varied randomly between 1 and 60 s. The lever remained retracted throughout the pretraining sessions. Two rats did not consume all 25 pellets by the end of a second pretraining session and were eliminated from the study. PCA training sessions then commenced