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Research report

Adrenal-dependent diurnal modulation of conditioned fear extinction learning

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HIGHLIGHTS

- Conditioned fear extinction learning shows a robust time of day difference.
- This effect is dependent on the presence of adrenal hormones, likely glucocorticoids.
- This study is relevant to research aimed at optimizing therapy for anxiety related disorders.

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ABSTRACT

Post traumatic stress disorder (PTSD) is associated with altered conditioned fear extinction expression and impaired circadian function including dysregulation of glucocorticoid hormone secretion. We examined in adult male rats the relationship between conditioned fear extinction learning, circadian phase, and endogenous glucocorticoids (CORT). Rats maintained on a 12 h light:dark cycle were trained and tested across 3 separate daily sessions (conditioned fear acquisition and 2 extinction sessions) that were administered during either the rats' active or inactive circadian phase. In an initial experiment we found that rats at both circadian phases acquired and extinguished auditory cue conditioned fear to a similar degree in the first extinction session. However, rats trained and tested at zeitgeber time-16 (ZT16) (active phase) showed enhanced extinction memory expression during the second extinction session compared to rats trained and tested at ZT4 (inactive phase). In a follow-up experiment, adrenalectomized (ADX) or sham surgery rats were similarly trained and tested across 3 separate daily sessions at either ZT4 or ZT16. ADX had no effect on conditioned fear acquisition or conditioned fear memory. Sham ADX rats trained and tested at ZT16 exhibited better extinction learning across the two extinction sessions compared to all other groups of rats. These results indicate that conditioned fear extinction learning is modulated by time of day, and this diurnal modulation requires the presence of adrenal hormones. These results support an important role of CORT-dependent circadian processes in regulating conditioned fear extinction learning, which may be capitalized upon to optimize effective treatment of PTSD.

1. Introduction

The ability of organisms to form associations between innately dangerous events (unconditioned stimuli or US) and surrounding cues (conditioned stimulus or CS) serves an adaptive function – it permits organisms to better escape or avoid dangerous situations. Normally, conditioned fear responses diminish with repeated exposure to the CS in the absence of the US due to the acquisition of new competing conditioned fear extinction associations [1]. However, in many individuals with anxiety-related psychiatric disorders the expression of conditioned fear persists and becomes pathological [2,3]. Patients suffering from these maladies, as well as subjects in an animal model of post traumatic stress disorder (PTSD), show deficits in fear extinction learning [4,5]. Given the debilitating and persistent nature of PTSD, research leading to a better understanding of the underlying neural processes that regulate conditioned fear and extinction expression may lead to improved treatment strategies.

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Conditioned fear is an aversive learning process that is highly sensitive to environmental conditions such as context and degree of stress [6]. Time of day has also been found to modulate various aspects of conditioned fear expression and extinction in rodent and human studies [7–10]. Glucocorticoid hormones (CORT, cortisol in humans and corticosterone in rats) are a well-known transducer of the biological effects of environmental stressors [11] and time of day [12–14]. There has been some examination of CORT-dependent modulation of auditory cue and contextual conditioned fear acquisition, memory, and extinction but virtually no examination of CORT effects on auditory cue conditioned fear extinction learning (reviewed in Rodrigues et al. [15]). Furthermore, no prior studies have examined whether CORT regulates various aspects of conditioned fear and extinction expression in a diurnal fashion. Subregions of the amygdala are central to conditioned fear and extinction learning, and the medial prefrontal cortex (mPFC) and hippocampus contribute critical modulation of these processes [16–18]. Neuroplasticity in the mPFC and hippocampus is sensitive to circadian modulation [8,19], and a number of related cognitive functions exhibit diurnal performance patterns [20–22].

The goals of our study were to determine if there was a time of day difference in conditioned fear and/or extinction learning in rats (experiment 1), and to assess whether the presence of adrenal hormones was necessary for any observed time of day differences (experiment 2). Emotionally driven memories are strongly modulated by the presence and/or timing of acute CORT administration [23]. Therefore, diurnal variations in conditioned fear learning and/or extinction learning may be modulated by the presence or absence of endogenous CORT. For this study we assessed conditioned fear to a discrete auditory cue as well as extinction learning when it was presented in a context separate from the initial cue-shock pairing in order to assess the diurnal and adrenal-dependent effects on cue specific conditioned fear learning and extinction [24].

2. Methods

2.1. Animals

For experiments 1 ($n = 6$, $N = 12$) and 2 ($n = 6$, $N = 24$) male Sprague Dawley rats (250–280 g; Harlan Laboratories, Indianapolis, IN) were housed two per cage (polycarbonate tubs, 47 cm × 23 cm × 20 cm) and given food (Teklad Rodent Diet 8640; Harlan) and water *ad libitum*. For experiment 2, rats were given food and either tap water (Sham rats) or 0.9% saline (adrenalectomized, ADX rats) *ad libitum*. Rats were housed in two separate rooms within a suite of rooms that included the behavioral testing rooms. Each housing room had independent control of its light/dark cycle, and all rats were maintained on a 12 h light/dark cycle but with the phase adjusted so that behavioral testing occurred between 0900 and 1100 h. All behavioral tests took place at either zeitgeber time (ZT; hours after light phase onset in rats housing room) 4 ± 60 min or ZT16 ± 60 min. Rats were given 2 weeks to adjust to their respective light/dark phase prior to behavioral testing. For experiment 2, the surgical procedure occurred in the middle of the 2 week adjustment period, allowing for 1 week of surgical recovery before the onset of behavioral testing. Experimental procedures were conducted in accordance with the ethical treatment of animals and were approved by the University of Colorado Institutional Animal Care and Use Committee.

2.2. Adrenalectomy surgery

For experiment 2 rats received either bilateral adrenalectomy (ADX) or Sham-ADX. During surgery rats were anesthetized with halothane. Bilateral incisions were made through the dorsal lateral skin and peritoneal wall near the kidney, and adrenal glands of ADX rats were isolated with forceps and excised. Sham rats went through the same procedure, but adrenal glands were left in place.

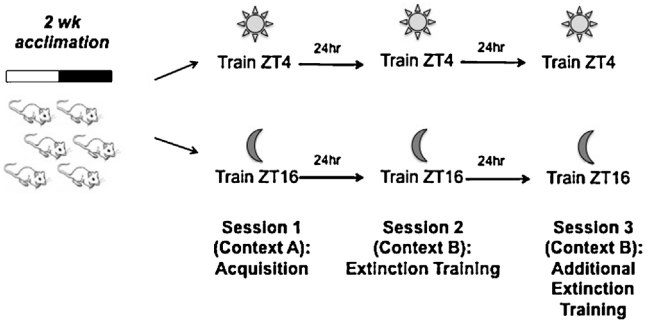

Fig. 1. Experimental timeline. Rats were trained for auditory cue conditioned fear at either ZT4 or ZT16 and tested for extinction learning at the same ZT. For experiment 2 rats received either adrenalectomy or sham surgery 1 wk into acclimation.

2.3. Fear conditioning and testing apparatus

Conditioned fear acquisition took place in rectangular chambers (25.4 × 25.4 cm × 30.4 cm) comprised of 3 stainless steel walls and a Plexiglas front with a shock grid floor comprised of stainless steel rods placed 2 mm apart (context A). Shock grid floors were connected to a current generator (Precision Regulated Animal Shocker, Whitehall, PA) used to provide a 2 s 0.8 mA foot shock. Audio speakers (Coulbourn Instruments, Whitehall, PA) were located on the top of the shuttle boxes and designed to provide a 30 s 1 kHz 70 dB tone. Extinction training took place in clear Plexiglas boxes (28.6 cm × 18.5 cm × 22 cm) with wire mesh floors (context B). Extinction boxes were contained in sound attenuating icebox coolers containing the same type of audio speakers used in context A. Context B boxes, but not context A boxes, were cleaned with 70% ethanol and allowed to dry before and after each session. Context B boxes were scented with peppermint oil (Now Foods, Bloomingdale, IL) during the extinction sessions.

2.4. Behavioral testing

Development of conditioned fear or conditioned fear extinction can be acquired within a single session, and memory of each can be directly assessed in a follow-up session. Each of these training and testing phases of conditioned fear expression can be effectively accomplished within a relatively short session (<40 min), which allows for scheduling of these sessions at the same time each day for each experimental subject. Thus, we trained and tested rats over the course of 3 separate sessions that took place either during the rats' inactive (lights-on) or active (lights-off) circadian phase. For both experiments rats were trained and tested at either ZT4 ± 60 min or ZT16 ± 60 min (Fig. 1). At ZT4 house lights remained on for testing. At ZT16 training and testing were performed under red light conditions. Session 1 (conditioned fear acquisition): rats were placed in context A for a 5 min pre-exposure period after which they were trained to fear a 30 s 1 kHz 70 dB tone conditioned stimulus (CS) that co-terminated with a single 2 s 0.8 mA foot shock unconditioned stimulus (US; one CS–US pairing). Rats remained in the testing chambers for 1 min post-shock. Session 2 (conditioned fear recall and extinction training): 24 h after session 1 rats were pre-exposed to context B for 3 min followed by fifteen 30 s 1 kHz 70 dB tones unaccompanied by a foot shock (CS alone, random inter-trial interval 90–120 s). Freezing during the first 3 trials (trial block 1) was used as a measure of conditioned fear recall. Session 3 (additional extinction training): 24 h after session 2 rats were pre-exposed to