



Repeated corticosterone enhances the acquisition and recall of trace fear conditioning



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ABSTRACT

Repeated exposure to high levels of stress hormones can enhance contextual and discrete fear conditioning in rats. A common belief is that this enhanced fear memory is largely mediated by the amygdala because both contextual and discrete fear conditioning are dependent on an intact amygdala. However, trace fear conditioning is thought to be amygdala independent, and therefore, it is not clear what impact stress would have on this form of fear learning. Here, we examined whether the stress hormone corticosterone (CORT) would enhance memory in a hippocampal-dependent trace fear conditioning test. Male Long-Evans rats received either 40 mg/kg of CORT or vehicle injections for 21 consecutive days. On day 22, rats received either 1, 2, or 5 tone-trace-shock pairings. On day 23, the rats were tested for behavior to the conditioned tone cues in a novel context. We found that CORT significantly increased the acquisition of trace conditioned fear. We also found that CORT significantly increased recall of trace conditioned cues, but only when a 2 trace-pairing protocol was used during training. These results suggest that CORT can enhance non-amygdala forms of fear learning and memory and that high levels of stress hormones modify the physiological substrates that mediate emotionally driven behavior in tasks that are less dependent on amygdala functioning.

1. Introduction

Chronic stress is known to produce aberrant brain plasticity and maladaptive behaviors. Some of the most prominent neuroplastic changes associated with repeated stress exposure occur in the hippocampus and amygdala as both structures are abundant in glucocorticoid receptors [1,2]. Within the hippocampus, chronic stress can lead to decreased dendritic branching, decreased mossy-fiber synapses, and decreased adult neurogenesis [3–5]. This aberrant plasticity has been associated with deficits in hippocampal dependent memory tasks [3,6]. However, within the amygdala, chronic stress seems to increase dendritic arborization and spine density [7,8], and learned fear associations that depend on the amygdala are similarly enhanced after either repeated restraint stress or repeated exposure to the stress hormone corticosterone (CORT) [9–11].

Fear conditioning assesses a rodent's ability to associate neutral cues with an aversive experience. Variations of the fear conditioning paradigm have been developed that require the activation of different brain structures to successfully learn the task [11,12]. For example, a typical delay fear conditioning protocol where the conditioned stimulus is followed immediately by or co-terminates with the unconditioned

stimulus is largely an amygdala-dependent task [11,13]. In contrast, trace fear conditioning, which separates the conditioned stimulus from the unconditioned stimulus by a temporal gap, relies on an intact hippocampus and is largely amygdala independent [12,14]. Repeated stress or CORT administration produces a robust increase in fear memory in a delay fear conditioning paradigm [10,15,16]. This is thought to occur due to an enhancement of amygdala functioning following chronic stress or glucocorticoid exposure [9]. Trace fear conditioning provides the opportunity to examine whether chronic stress would also enhance fear memories in a hippocampal-dependent task.

Surprisingly few experiments have addressed this question. One group reported that 2 weeks of immobilization stress decreases trace memory recall, with no effect on memory acquisition [17], but another group found that 3 weeks of chronic mild stress enhances trace memory recall [18]. However, immobilization stress can produce variable behavioral effects possibly as a result of habituation effects, and both immobilization stress and chronic mild stress may produce individual differences or have no effect on HPA axis responses [19–21]. We examined this issue here using a repeated CORT paradigm that reliably increases depression-like behavior and amygdala-dependent fear memory, and alters amygdalar and hippocampal plasticity [10,22–27].

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We examined the effects of repeated CORT administration on freezing behavior after three different trace fear conditioning training paradigms. Given previous findings in our lab, we expected to see an increase in trace fear memory in CORT treated animals [10]. Considering that we have also previously demonstrated that the effects of CORT on fear memory are dependent on the training paradigm [10], we also anticipated that these enhancements in fear will be dependent on the intensity of the trace fear conditioning protocol.

2. Materials and methods

2.1. Animals

Three separate experiments used 18 (experiment 1), 19 (experiment 2), and 18 (experiment 3) adult male Long-Evans rats (purchased from Charles River, Canada). Rats were housed individually in standard polypropylene cages maintained on a 12 h light/dark cycle (lights on at 07:00 h) in a room maintained at 21 °C. Rats had free access to water and Purina rat chow. All experimental procedures were conducted during the light phase under an animal care protocol approved by the University of Saskatchewan's Animal Research Ethics Board.

2.2. Repeated CORT injections

Rats were handled daily for 7–14 days prior to the injections. Rats were assigned to one of two groups based on body weight (i.e., so both groups had approximately equal average weight from the start). One group received 40 mg/kg CORT injections ($n = 9$ experiment 1; $n = 9$ experiment 2; $n = 10$ experiment 3) and the second group received vehicle injections ($n = 9$ experiment one; $n = 9$ experiment two; $n = 9$ experiment 3). Injections were administered subcutaneously once per day in a volume of 1 ml/kg for 21 consecutive days. CORT (Steraloids, Newport, RI) was suspended in 0.9% (w/v) physiological saline with 2% (v/v) polyoxyethylene glycol sorbitan monooleate (Tween-80; VWR, West Chester, PA). The 40 mg/kg dose of CORT was chosen based on previous research showing that this dose reliably increases depression-like behavior on a variety of measures [23,26,28,29].

2.3. Fear conditioning

Rats were trained and tested in two identical fear-conditioning chambers (25.5 cm × 32 cm × 25.5 cm) connected to a computer that controlled both the shock and tone presentations and video recorded all rat behavior (Med Associates, St. Albans, VT). The front door and ceiling of the chambers were made of clear Plexiglas, the back wall was made of white plastic and the side walls were made of stainless steel. The floor of each chamber comprised 19 stainless steel rods spaced apart by 1 cm. Each rod was wired to a shock generator and scrambler that delivered foot-shocks as unconditioned stimuli. The ceiling of the chamber held a speaker that presented individual tones as the conditioned stimuli. A stainless steel pan covered by two sheets of paper towel collected urine and fecal matter under the grid floor. The entire chamber was inserted into a white sound-attenuating cubicle (73 cm × 64 cm × 42 cm) that contained a video camera on the inside door. A fluorescent lamp on the ceiling of the cubicle lit each chamber.

2.3.1. Training experiment 1 (high intensity protocol)

Injections were completed on day 21 of the experiment. On day 22 (training day), rats were placed individually into one of the fear conditioning chambers and given 180 s to acclimatize to the surroundings. After acclimatization, rats received 5 tone-trace-shock pairings with each tone having a volume of 90 dB, frequency of 2 kHz, and a rise time of 50 ms. Each tone-trace-shock pairing comprised the tone played for 16 s, followed by a 30 s trace and ending with a 2 s 0.5 mA foot-shock. An inter-trial interval of 210 s was used in between pairings. Rats remained in the chamber for 210 s after the final tone-shock-pairing. The

chambers were cleaned with 0.4% glacial acetic acid in between each training session. Training was conducted between 09:00 and 13:00 h. The experimental protocols were adapted from previously reported procedures [30,31].

2.3.2. Training experiment 2 (medium intensity protocol)

In this experiment, rats received 2 tone-trace-shock pairings. The tone-trace-shock pairings had identical parameters to those used for experiment 1. All other experimental procedures were identical to those used for experiment 1.

2.3.3. Training experiment 3 (low intensity protocol)

In this experiment, rats received 1 tone-trace-shock pairing. The tone-trace-shock pairing had identical parameters to those used for experiments 1 and 2. All other experimental procedures were identical to those used for experiment 1 and 2.

2.3.4. Testing

Retrieval of the tone-trace-shock association occurred 24 h after the completion of training. Testing took place in the same two chambers described above, but contextual details within the chambers and the testing room were altered in several different ways. First, black horizontal strips were fitted to the outside of the chambers changing both the appearance of the ceiling and the lighting of the chamber. Second, a white plastic plate was placed over the grid rods to change tactile cues and make the floor completely flat. Third, a plastic insert containing two blue vertical stripes and one red vertical stripe was inserted into the chamber giving the chamber a semicircle shape around the side panels and rear of the chamber. Fourth, cardboard shapes of various colours were placed on the inner walls of the sound attenuating cubicle to alter visual cues and a white noise generator created background noise (65 dB) in the cubicle. Fifth, blue curtains were draped from the ceiling changing both the shape and colour of the room. Lighting in the conditioning room was also changed by draping a blue curtain over the fluorescent ceiling light dimming the room. Sixth, olfactory cues were changed by scenting the room with vanilla coconut pet deodorizer. As well, the chambers were cleaned with 75% ethanol instead of 0.4% glacial acetic acid between trials.

During testing, rats were placed into the opposite chamber from the one they were trained in. Rats were given 180 s to acclimatize to the new contextual surroundings before receiving 5 tones, each lasting 16 s (the same volume, frequency, and rise time used for training) with an ITI of 210 s. Rats remained in the chamber for 210 s after the 5th tone before being returned to the housing room. All testing took place between 09:00 h and 14:00 h. Testing procedures were identical for experiments 1, 2, and 3.

Behavior in the conditioning chambers was recorded by a video camera attached to the inside door of the sound attenuating cubicles. Freezing during testing was quantified by software (i.e., Video Freeze, v 1.16.0.0, Med Associates) using a motion threshold. Freezing was defined as immobility with the exception of movement required for respiration. The percentage of time spent freezing was based on a minimum freeze duration of 1 s.

2.4. Body weight

All rats were weighed daily for later analyses.

2.5. Statistical analyses

Statistical significance for all comparisons was set at $p \leq 0.05$. Training day data were analyzed using two-way mixed factor analysis of variance (ANOVAs) for experiments 1, and 2 (Treatment as the between measures factor and Tone, Trace, or Post-shock Interval as the repeated measures factors). For experiment 3, training day data were analyzed using separate *t*-tests. Testing day data were analyzed using

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