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THE EFFECT OF CHRONIC CORTICOSTERONE ON FEAR LEARNING AND MEMORY DEPENDS ON DOSE AND THE TESTING PROTOCOL

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Abstract—Chronic exposure to the stress hormone corticosterone (CORT) is known to alter plasticity within hippocampal and amygdalar circuits that mediate fear learning and memory. The purpose of this experiment was to clarify the effects of chronic CORT on Pavlovian fear conditioning, which is dependent on intact hippocampal and amygdalar activity. In particular, we assessed whether the effect of chronic CORT on fear learning and memory is influenced by two factors—the dose of CORT and the order in which rats are tested for freezing to context versus tone cues. Male Long–Evans rats received low-dose CORT (5 mg/kg), high-dose CORT (40 mg/kg), or vehicle injections once daily for 21 days. On day 22, the rats were trained in a fear-conditioning paradigm. On days 23 and 24, the rats were tested for the retrieval of fear memories to context and tone cues in a counterbalanced way—half the rats received context testing on day 23 and then tone testing on day 24 and half the rats received tone testing on day 23 followed by context testing on day 24. Our results revealed dose-dependent effects of CORT on memory retrieval: Rats injected with high-dose CORT froze significantly more than control rats to both context and tone cues regardless of what testing day these cues were presented. However, rats injected with low-dose CORT froze significantly more than control rats to tone cues only. We also found an order effect in that the effects of CORT on freezing were greater on the second day of testing, regardless of whether that testing was to context or tones cues. This order effect may be due to a lack of extinction in the CORT rats. Overall, these results suggest a relationship between stress intensity and testing conditions that should be taken into account when assessing the effect of stress on fear memories.

Key words: stress, amygdala, fear conditioning, hippocampus, behavior, rat.

INTRODUCTION

The relationship between chronic stress, brain plasticity, and cognition is complex. It is well established that chronic exposure to the stress hormone corticosterone (CORT) can alter plasticity within brain regions involved in cognition. The hippocampus and amygdala are particularly susceptible to CORT-mediated changes as both structures contain a large number of glucocorticoid receptors (Morimoto et al., 1996; Feldman and Weidenfeld, 1999). Given the robust effect of CORT on hippocampal and amygdalar structure, it is of interest to understand how CORT affects the learning and memory of events that rely on the integrity of these two brain structures. One way to study this is through the use of Pavlovian fear conditioning, which assesses a rodent's ability to associate neutral cues such as a tone or context (conditioned stimuli (CS)) with an aversive experience such as a mild footshock (unconditioned stimuli (US)). The CS predicts an aversive outcome and comes to elicit a conditioned response (CR), such as defecation, piloerection, or freezing behavior. The conditioning of fear responses to contextual and tone cues is processed by both overlapping and dissociable pathways. That is, hippocampal lesions can interrupt conditioning of fear responses to contextual cues, whereas amygdala lesions can interrupt conditioning of fear responses to both contextual and tone cues (Phillips and LeDoux, 1992). As CORT facilitates synaptic plasticity within the amygdala, one would expect to see enhanced learning and retrieval of fear memories in rats subjected to chronic stress or CORT exposure. Indeed, previous research has shown that chronic stress or CORT administration can facilitate the acquisition of tone-shock fear associations (Bisaz and Sandi, 2010; Farrell et al., 2010; Monsey et al., 2014). However, the specific effect of these forms of stress on the learning and memory of dissociable cues such as tone and context are less clear. Few studies have explored the effect of chronic stress on context and tone conditioning within the same rats and the studies that have examined both types of cues have revealed variable results. For example, 21 days of restraint stress enhanced freezing to both tone and contextual cues when both types of cues were presented in the same session (Conrad et al., 1999), but it did not enhance freezing to context or tone cues when testing to context cues was conducted before testing to

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Abbreviations: AU, activity units; ANOVA, analysis of variance; CS, conditioned stimuli; CORT, corticosterone; ITI, inter-trial interval; US, unconditioned stimuli.

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tone cues or when tone cues were presented in a novel context (Conrad et al., 2001). Additionally, rats exposed to 400 mg/ml of CORT in their drinking water for 21 days showed enhanced freezing to contextual cues, but not tone cues, when testing to context cues occurred before testing to tone cues (Conrad et al., 2004). These results suggest that the order of testing (i.e., context first versus tone first) might be an important factor to consider in conducting these types of experiments.

The purpose of this experiment was to clarify the effect of chronic CORT exposure on fear learning and memory by examining two important factors: the order of cue presentation during fear memory testing and the dose of CORT administered to the rats. Previous work has shown that chronic CORT given over a 21-day period dose dependently increases depression-like behavior (Johnson et al., 2006; Marks et al., 2009; Sterner and Kalynchuk, 2010), with large and robust increases seen in rats given a high dose of 40 mg/kg and few increases seen in rats given low doses of 5 mg/kg or 10 mg/kg. Therefore, we examined the effect of 21 days of either low-dose (5 mg/kg) or high-dose CORT (40 mg/kg) on the acquisition and retrieval of fear memories. We hypothesized that CORT would dose dependently enhance the retrieval of fear memories and that the magnitude of CORT's effects on the retrieval of fear memories would depend on the order in which rats are tested for freezing to context versus tone cues.

EXPERIMENTAL PROCEDURES

Animals

We used 42 adult male Long–Evans rats (purchased from Charles River Canada, Montreal, Quebec, Canada) in this experiment. The rats weighed between 215 and 250 g at the time of arrival from the breeder. They were individually housed in standard polypropylene cages in a housing room maintained on a 12-h-light/dark cycle (lights on at 07:00 am) at a temperature of 21 °C. Rats had free access to Purina rat chow and water. All experimental procedures were carried out during the light phase and were conducted according to an animal care protocol approved by the University of Saskatchewan Committee on Animal Care and Supply.

Repeated CORT injections

Rats were handled and injected in a procedures room separate from the housing room. Handling occurred daily for 7–14 days prior to the onset of any injections in order to familiarize the rats with the vivarium and researchers. At the end of this handling phase, each rat was assigned to one of three experimental conditions based on body weight (i.e., so that all groups had approximately equivalent average weight). The three experimental conditions were: a 5 mg/kg CORT group (CORT 5 group, $n = 14$), a 40 mg/kg CORT group (CORT 40 group, $n = 14$), and a vehicle control group (vehicle group, $n = 14$). All injections were administered subcutaneously at a volume of 1 ml/kg once per day for 21 consecutive days between 09:00 h and 11:00 h. CORT (Steraloids Inc., Newport, RI, USA) was suspended in 0.9% (w/v) physiological saline with 2% (v/v) polyoxyethylene glycol sorbitan monooleate (Tween-80; VWR International, Mississauga, ON, Canada). The 5 mg/kg dose of CORT was chosen to mimic physiological levels of endogenous CORT under conditions of moderate stress (Stein-Behrens et al., 1994). The high dose of 40 mg/kg was chosen as it reliably increases depression-like behavior in the forced swim test and dysregulates the hypothalamic–pituitary–adrenal axis in response to a novel stressor (Kalynchuk et al., 2004; Gregus et al., 2005; Brummelte et al., 2006; Johnson et al., 2006).

Experimental design

The design of this experiment is shown in Fig. 1. We used two different testing protocols to examine the effect of the order of testing to contextual versus tone cues in rats treated with a low or high dose of CORT. After 21 days of low CORT, high CORT, or vehicle injections, rats were assigned to one of two protocols. In protocol 1, rats were conditioned to contextual and tone cues on day 22 (training day), and then they were tested with contextual cues on day 23 (contextual testing) and tone cues on day 24 (tone testing). In protocol 2, the testing order was reversed: Rats received the same conditioning to contextual and tone cues on day 22 (training day), but they were tested with tone cues on day 23 and with contextual cues on day 24. This experimental design allowed us to assess two main factors: the effect of CORT dose on the acquisition and retrieval of fear memories and the effect of order of cue presentation on the magnitude of any CORT-induced changes observed during fear memory retrieval.

Fear conditioning

Apparatus. The rats were trained and tested in two identical video fear-conditioning chambers (25.5 cm × 32 cm × 25.5 cm) that were attached to a personal computer that controlled all shocks and tone presentations and recorded all rat behavior (Med Associates, St. Albans, VT, USA). The four sidewalls of each chamber were made of aluminum. The front door and ceiling were made of clear Plexiglas, and the back wall was made of white plastic. The floor of each chamber comprised 19 stainless steel rods spaced 1 cm apart; each rod was wired to a shock generator and scrambler that was used to deliver footshocks as unconditioned stimuli (US). The ceiling held a small speaker that allowed for the presentation of individual tones that acted as CS. A stainless steel pan covered by two sheets of paper towel was inserted beneath the grid floor for the collection of urine and fecal matter. The entire chamber was placed inside a white sound-attenuating cubicle (73 cm × 64 cm × 42 cm). Each chamber was lit by a fluorescent lamp.

Training. On day 22 of the experiment (i.e., the training day), rats were placed individually inside one of