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MODEST ELEVATION OF CORTICOSTERONE IN PREWEANLING RATS IMPAIRS SUBSEQUENT TRACE EYEBLINK CONDITIONING DURING THE JUVENILE PERIOD

Dragana I. Claflin, Leslie R. Greenfield, and Michael B. Hennessy

Department of Psychology 335 Fawcett Hall Wright State University 3640 Colonel Glenn Hwy Dayton, OH 45435 USA

Abstract

The hippocampus is known to be especially sensitive to the deleterious effects of glucocorticoids. Previously, we administered exogenous corticosterone, the major stress-related glucocorticoid in rats, to young developing rats using subcutaneous pellets which produced high pharmacological levels of circulating corticosterone as well as a sex-specific learning deficit for males on a hippocampus-mediated associative learning task, trace eyeblink conditioning [1]. The present study evaluated the effects of corticosterone administered at a physiologically-relevant level by a more consistent release method, osmotic mini-pumps. Pumps were implanted subcutaneously in 15-day-old rats to deliver either corticosterone or the vehicle control (PEG) at a rate of 1 μ L/hour over 3 days. On Day 28, learning was assessed using trace eyeblink conditioning. The results of the present experiment revealed that a small elevation in corticosterone (11.77 μ g/dl vs 6.02 μ g/dl for controls) within the normal physiological range impaired learning as determined by a significantly lower percentage and amplitude of total conditioned responses (CRs) and lower amplitude of adaptive responses relative to the control group. There were no significant differences in response timing, although the corticosterone group tended to produce CRs which began and peaked a little later than controls. These findings indicate that even modest elevations of corticosterone for several days can produce later impairments on this hippocampally mediated learning task.

Keywords

trace conditioning; eyeblink; corticosterone; hippocampus; development; learning

1. Introduction

The physiological response to stress involves an attempt to sustain homeostasis through activation of the hypothalamic-pituitary-adrenal (HPA) axis and the release of glucocorticoids [2-4]. Changes in glucocorticoid levels can have both positive and negative effects on cognitive performance following an inverted-U function. Pathologically low or

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Corresponding Author: D. I. Claflin dragana.claflin@wright.edu Department of Psychology 335 Fawcett Hall Wright State University 3640 Colonel Glenn Hwy Dayton, OH 45435 USA Tel: 001 937 775-2391 Fax: 001 937 775-3347. Co-Author E-mails: michael.hennessy@wright.edu leslie.greenfield@us.army.mil mildragana.claflin@wright.edu.

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very high levels of glucocorticoids produce cognitive deficits whereas moderate levels can enhance cognitive processes, depending on a number of variables including task complexity, contextual and temporal factors, and the intensity and duration of the stress exposure [reviewed in 5-7]. Both stress-induced and pharmacologically induced glucocorticoid elevations have been associated with memory impairments [8]. Moreover, the use of glucocorticoid medications has been shown to produce cognitive deficits in declarative memory throughout the lifespan [9-13]. For this reason it is important to better understand the behavioral effects and neurobiological mechanisms underlying glucocorticoid effects on learning and memory.

Chronic elevations of glucocorticoids can have severe effects on the hippocampus, a structure heavily involved in declarative learning and memory. Increased glucocorticoid levels have been shown to cause a decrease in the volume of hippocampus [14], reduce long-term potentiation in the hippocampus--a proposed mechanism for memory formation-- [15-16], and produce cognitive deficits on hippocampus-dependent tasks [1, 17- 20]. In contrast, *acute* administration of glucocorticoids decreases blood flow in the medial temporal lobe, increases activation of the hippocampus, and enhances long-term potentiation [21-23]. Indeed, acute increases in glucocorticoid levels appear to be necessary for normal memory consolidation [24- 26].

A long history of research in laboratory rats has demonstrated devastating effects on neural and cognitive development of glucocorticoids administered during gestation or in the first 2 weeks after birth [e.g., 20], but few studies have explored lasting effects of administration during the later preweaning period. However, dramatic changes occur in the HPA system during the first 2 weeks of life in the rat. Shortly after birth, circulating levels of corticosterone (the primary glucocorticoid in the rat) are high and responsiveness to some stressors is impaired [27- 28]. From about Day 2-14, the pup undergoes the so-called “stress hypo-responsiveness period”(SHRP) when both resting and stress-induced elevations are markedly reduced [27, 29]. A primary function of the SHRP appears to be to protect rapidly developing brain structures from the catabolic effects of glucocorticoids [30]. It is only from about Day 15 forward that the HPA axis of pups is capable of responding to stressors in a mature fashion. However, brain development, including the hippocampus, continues after the SHRP, raising the question of how stress-induced elevations of corticosterone at this time might affect the developing hippocampus and later cognitive development. Previously, we reported that implanting subcutaneous corticosterone pellets on Day 15 impaired eyeblink conditioning beginning on Day 28 in male, but not female, rats. This effect was specific for “trace” eyeblink conditioning, which is highly dependent on the hippocampus [31-35] and not for “delay” conditioning, which is primarily mediated by the cerebellum and other brainstem structures [31-32, 34]. The corticosterone pellet was designed to produce a low, constant release of hormone over a 21-day period, but plasma assays showed otherwise. There was a large supraphysiological increase in circulating corticosterone levels (to ~ 80 µg/dl) for about three days after implantation and a return to normal levels by the time testing occurred. Thus, a pronounced elevation of circulating corticosterone concentrations over several days, occurring just after the SHRP, disrupted a hippocampus-mediated learning task up to 10 days later. However, because the magnitude of the corticosterone elevations produced was well into the pharmacological range, the implications of the findings for corticosterone elevations of the level associated with stress remains unclear. In the present study, we administered corticosterone using an alternative, more reliable method that yielded the low level (within a normal physiological range) and constant rate of delivery we had originally expected. Furthermore, since the ~3-day period of elevation in the previous study was sufficient to produce lasting effects on behavior, we chose to use an osmotic mini-pump that was designed to deliver corticosterone at a low and constant rate over a 3-day period only. The purpose of this study then was to determine the influence of a

modest elevation of circulating corticosterone on a hippocampal-mediated learning task (trace eyeblink conditioning) 10 days after the treatment.

2. Material and methods

2.1 Subjects, procedures, and design

Timed-pregnant Long-Evans female rats were received from Charles River Laboratories (Raleigh, NC) around embryonic day 15. On PND 4-5, born litters were culled to 10 pups, 5 female and 5 male whenever possible. Animal housing and procedures were approved by the Laboratory Animal Care and Use Committee of Wright State University in Dayton, Ohio. A 12:12 hr light:dark cycle was maintained throughout the study, with lights on at 0700. *Ad libitum* access to food and water was provided. Pups continued to be housed with their dams until weaning on PND 21. At that time they were separated into groups of same-sex littermates until the beginning of behavioral procedures on PND 26, when they were placed into individual housing for the remainder of the study.

On PND 15, pups were randomly assigned to one of two drug treatment groups (CORT or Control), balanced for sex. No more than one male and one female from each litter were assigned to a particular experimental condition. All animals received a preloaded osmotic mini-pump, implanted under the skin at the back of the neck (described below). Approximately 24 hours later, at 1000h on PND 16, a blood sample was obtained from the heart and frozen for later assay of plasma corticosterone level. Animals recovered quickly and on PND 26 underwent a second surgery to implant electrodes for behavioral testing. On PND 28-29, rats received 6 sessions of trace eyeblink conditioning (3 sessions/day) and were euthanized at the end of the study. The health of the rats was monitored throughout the study both by visual examination for appearance and normal grooming patterns and by weight, which was measured every other day starting on PND 15.

The overall design of the study included 2 treatment groups, 2 sexes, and 6 conditioning sessions. The final data set included 24 Long-Evans rat pups taken from 9 litters. The CORT group consisted of 11 pups (5 males, 6 females) and the Control group consisted of 13 pups (8 males, 5 females).

2.2. Surgeries

2.2.1 Osmotic minipump implantation—On PND 14, at 1000h, osmotic mini-pumps (Alzet, Model 1003D) were preloaded, under sterile conditions, according to manufacturer instructions, with 100 μ L of either 50mg/mL corticosterone (Sigma, #C2505) dissolved in polyethylene glycol (PEG, viscosity 400, sterilized by syringe-driven filter) or vehicle alone. Because of the difficulty of getting corticosterone to stay in solution, the mixture was made a day ahead and maintained on a heated stirring plate and shaken on a vortexer just before being loaded into the pumps. The pumps were then placed in sterile saline in a warm water bath to be primed, as recommended by the manufacturer, for at least 24 hours prior to implantation. The pumps were designed to release 1 μ L/hour over 3 days. Also on PND 14, the back of each pup's neck was shaved in preparation for surgery the next day. On PND 15, animals were weighed and the surgical area was disinfected using an alternating Betadine and ethanol (70%) scrub procedure. Animals were anesthetized by CO₂ exposure until unconscious (<1.5min) and unresponsive to toe-pinch. A small incision was made across the nape of the neck and the sterile minipump was inserted with sterile forceps into a subcutaneous pocket; the flow modulator cap was directed rostrally. The incision was closed with sterile staples and antibiotic ointment was applied to the wound. A dose of Buprenorphine (.025 mg/kg) was administered for postoperative pain management. Animals recovered in a clean cage on a heating pad for about an hour until they were alert and

responsive, at which time they were returned to their dam. Postoperative monitoring of the surgical site continued at least every other day throughout the duration of the study.

2.2.2. Electrode implantation for eyeblink conditioning—On PND 26, two days before eyeblink conditioning, stimulating and recording electrodes were implanted according to procedures described previously [1, 36]. A subcutaneous bipolar stimulating electrode terminated in a v- shape (1mm exposed tips; Plastics One, Roanoke, VA) and was placed caudal to the left eye for delivery of a mild periorbital shock that would elicit an eyeblink reflex. Two fine Teflon-coated wires (316-SS-3TI Medwire) of a custom-made differential electromyographic (EMG) recording electrode were threaded through the left upper eyelid to monitor muscle activity (*obicularis oculi*) and record eyeblink behavior.

Prior to surgery, each animal was anesthetized with an intraperitoneal injection of ketamine and xylazine (75:5 mg/kg). If the animal showed signs of awakening during the surgery, an additional half dose of the cocktail was administered. Ophthalmic ointment was applied, as needed, to lubricate the eyes. The head was shaved and a midline incision exposed the surface of the skull. A few drops of the topical analgesic Bupivacaine were applied. Two triangular sterile stainless steel hooks were secured to the skull to anchor the electrodes and their connectors in place when embedded in dental acrylic, which also served to seal the wound. Postoperative discomfort was managed with Buprenorphine (.05mg/kg, s.c.). Animals were placed in a clean recovery chamber on a heating pad and monitored during recovery from the anesthesia. Animals were returned to individual cages for the duration of the study.

2.3 Blood sampling and corticosterone assay

To determine the circulating corticosterone levels for each animal one day after mini- pump implantation, blood samples (~0.2 ml) were collected between 1000 h and 1100 h on PND16. Animals were taken from the home cage individually and the time to collection was less than 4 minutes in order to minimize activation of the HPA system in response to the blood collection procedures [37]. Due to the small size of preweanling rats, blood sample collection directly from the heart proved to be more efficient for obtaining a sufficient amount of blood for analysis than was collection from either the tail or ear vein. Animals were anesthetized with CO₂ (< 1.5min) until unresponsive to toe pinch. Blood samples were collected using a heparinized syringe and centrifuged (4° C, 3000 rpm for 20 minutes) to separate plasma, which was frozen until assay at a later date. The radioimmunoassay was conducted with a standard kit (¹²⁵I Rat Corticosterone; Siemens). For this study, the intra-assay coefficient of variation was calculated to be 7.6% and the inter-assay coefficient was 12.9%.

2.4 Trace Eyeblink Conditioning

Trace conditioning consists of a tone conditioned stimulus (CS) that initially produces little or no response, and an unconditioned stimulus (US) which is a mild shock to the side of the face that produces an eyeblink. The tone CS and the shock US are separated by a 500-ms stimulus free period. During this stimulus free period, the animal must maintain a memory trace of the tone in order to form the association with the subsequent US (see Figure 1). Learning takes place after several CS-US pairings and the animal begins to produce an eyeblink in response to the tone but preceding and anticipating the shock. This is known as a conditioned response (CR).

2.4.1 Procedures and apparatus—Trace eyeblink conditioning consisted of repeated presentations of a 380-ms tone conditioned stimulus (CS; 2.8 kHz, 90 dB) and a 100-ms periorbital shock unconditioned stimulus (US; 1.5 mA), distinct and separated by a stimulus-

free period of 500 ms. This produced a CS-US interstimulus interval of 880ms. Animals were presented with 6 conditioning sessions over a 2 day period (3 sessions/day). Each session consisted of 100 trials, 90 paired CS-US trials and 10 CS-alone test trials, with an average intertrial interval of 30 s (20- 40 s range).

During conditioning, animals were allowed to move freely within a Plexiglas test chamber (28x24x30cm) with a stainless steel grid floor contained within a sound attenuating chamber (Med Associates, Inc., St. Albans, Vermont). Each chamber consisted of a fan (background noise level 65-70 dB), dim light (15W), and two speakers (2-12kHz range) which were used for presenting the tone CS. The shock US was produced by a constant-current, 60Hz square wave stimulator (World Precision Instruments, Sarasota, FL). The electrodes secured to the animals' skull were connected to wire leads that passed through an opening in the chamber ceiling to a commutator suspended above the chamber, allowing maximum mobility. A custom- built Eyeblink Conditioning System (JSA Designs, Raleigh) controlled stimulus presentations and recorded EMG activity from the eyelid. EMG signals were sampled in 3.5- ms bins during the 1400-ms trial epoch. The raw signal was amplified (5K), rectified, and integrated for quantitative analysis. The threshold for registering an EMG response was set 0.4 arbitrary units above the average baseline amplitude during the pre-CS period (280 ms prior to CS onset).

2.4.2 Measures

2.4.2.1 Learning measures: Learning was characterized using several different measures related to the conditioned/learned responses (CRs), i.e., responses that began after the tone but occurred before the shock. These include the total percentage of CRs (Total CRs), the percentage of well- timed CRs (Adaptive CRs), CR amplitude, and CR onset and peak latency relative to the tone CS. CRs were represented by muscle responses that exceeded the threshold during the allotted time window. Total CRs were recorded during the 800 ms prior to the shock US, leaving an initial 80ms window for measuring reflexive startle responses to the tone (see *Control measures* below). Adaptive CRs represent a subset of the total CRs which anticipated the shock within just 200ms before the shock was presented. Percentages of both total and adaptive CRs were calculated using the paired CS-US trials that made up 90% of training sessions. Along with CR amplitude (CRA) these reflect the frequency and strength of anticipatory responding, respectively. Learning is represented by an increase in the percentage and amplitude of CRs across conditioning sessions. Because the purest measure of the CR is acquired during CS-alone trials, in the absence of a US, average CR amplitude, onset latency and peak latency were calculated using CS-alone trials. CR onset latency (CL) and latency to maximum peak of eye closure (CML) further characterize the conditioned or learned response. Changes in CR latency over conditioning sessions therefore reflect adaptation of response timing to anticipate the expected US.

2.4.2.2 Control measures: Differences in sensitivity to the CS or US can influence acquisition of eyeblink conditioning. The startle response (SR) is used to measure sensitivity to the tone, and the unconditioned response (UR) is the standard measure of sensitivity to the US. Possible SRs were measured during the first 80 ms after the CS presentation, indicating a reflexive/orienting response to the sound. An increase in SR frequency that parallels increases in CRs over training would suggest that the changes in CRs may not actually reflect learning but rather some form of sensitization to the CS, perhaps caused by fear-potentiation of the acoustic startle. The UR was measured at the end of the US (shock stimulus interferes with recording during the US), indicating a reflexive response to the shock. The percentage of SRs and maximum amplitude of URs during paired CS-US trials were analyzed as measures of stimulus effectiveness and subject sensitivity, respectively.

2.5 Data Analysis

2.5.1 Analysis of corticosterone levels and body weight—Plasma corticosterone concentrations were analyzed using an independent samples t-test to compare circulating levels of that hormone in the CORT and Control Groups 24 hours after implantation of the mini-pumps, on PND 16. At the beginning of conditioning on PND 28, body weights were also compared using a t-test between the CORT and Control Groups as a gross assessment of potential health effects resulting from corticosterone treatment that might be a factor for consideration if behavioral differences were observed.

2.5.2 Analysis of learning measures—Data were analyzed using separate repeated-measures ANOVAs for different measures of learning (Total CRs, Adaptive CRs, CRA, CL and CML) with a 2 (Treatment) x 2 (Sex) x 6 (Session) design. Where assumptions of sphericity were violated, Huynh-Feldt corrected degrees of freedom and *F*-values are reported. Post-hoc Tukey tests were performed for significant main effects on between-groups variables. Significant interactions were further analyzed using simple means analysis with Bonferroni's adjustment, where applicable. All analyses were conducted with SPSS statistical software.

2.5.3 Analysis of control measures—For control measures of sensory processing, analyses of SR percentages were conducted using the same repeated-measures design described above, but UR amplitude was analyzed only for the first day of conditioning. The SR percentage and amplitude of URs was compared across treatment groups and sex to make certain the conditioning stimuli produced similar reactions in all groups and could not be a contributing factor to learning-related changes that might be observed.

3. Results

3.1 Corticosterone levels and body weight

An independent samples t-test compared circulating corticosterone levels for corticosterone-treated animals versus vehicle controls. Corticosterone-treated animals exhibited significantly higher ($M = 11.77 \mu\text{g/dl}$, $SEM = 2.24$) circulating corticosterone levels on PND16, 24 hours after the mini-pump implant, relative to control animals [$M = 6.17 \mu\text{g/dl}$, $SEM = 1.39$; $t(22) = 2.16$, $p = .044$]. The elevated levels obtained here (see Figure 2) are best considered in the low-moderate stress range [e.g., 38-39]. Furthermore, corticosterone did not have a statistically significant effect on body weight as measured at the time of behavioral testing PND28 [CORT: $M = 72.17\text{g}$, $SEM = 2.76$; Control: $M = 79.45\text{g}$, $SEM = 2.66$; $t(22) = 1.89$, $p = .0722$].

3.2 Trace eyeblink conditioning

3.2.1 Learning measures

3.2.1.1 Total CRs: A 2 (Treatment) x 2 (Sex) x 6 (Session) repeated-measures ANOVA for the percentage of Total CRs yielded a main effect for treatment, $F(1,20) = 5.79$, $p = .026$. This effect reflects impaired acquisition of the eyeblink CR for corticosterone-treated animals relative to controls, as demonstrated by lower CR percentages across sessions (see Figure 3, left panel). A main effect for session was marginally significant, $F(3.15, 62.93) = 4.42$ ($p = .06$), indicating that across all the subjects, there was a slight increase in CR responding over the course of training. There were no significant interactions for this measure.

3.2.1.2 Adaptive CRs: A similar analysis of well-timed CRs yielded no main effects or interactions for treatment or sex, but there was a strong main effect for session, $F(3.15,$

63.05) = 11.14, $p < .001$, with the CR percentage increasing over sessions (see Figure 3, right panel).

3.2.1.3 CR Amplitude: Separate analyses of CR amplitude were conducted for both Total and Adaptive CR periods using the same repeated-measures design and yielded similar results. However, analyses of CR amplitude were based on measures taken during CS-alone test trials. Consistent with the Total CR percentage data, a main effect of treatment was observed for Total CR amplitudes (see Figure 4, left panel), but in addition there was a significant effect of treatment on Adaptive CR amplitudes (see Figure 4, right panel, respectively, $F(1, 20) = 9.44$, $p = .006$; $F(1, 20) = 5.09$, $p = .035$). Response amplitudes were significantly lower in the CORT groups relative to vehicle controls throughout training. The significant finding here for Adaptive CR amplitude may suggest that CR amplitude is more sensitive than percentage to group differences during the shorter time window associated with the adaptive CR period. Furthermore, a main effect of session was observed for the amplitude of both Total and Adaptive CRs, respectively, $F(5, 100) = 2.44$, $p = .039$; $F(5, 100) = 5.26$, $p < .001$.

3.2.1.4 CR Onset and peak latency: Repeated-measures ANOVA for the onset and peak latency revealed no significant main effect of treatment, but the significant main effect of session was repeated [onset: $F(5, 100) = 3.04$, $p = .014$; peak: $F(5, 100) = 10.65$, $p < .001$]. For both treatment groups, a general increase in onset and peak latencies was observed across sessions (Figure 5), but is most evident for latency of the CR peak. CRs peaked between CS and US onset and were initially closely associated with CS offset but moved closer to US onset with training (see Figure 5, right panel).

3.2.2 Control measures

3.2.2.1 SR Percentage: Repeated measures ANOVA for SR percentage revealed no main effects for treatment, sex, or session. Although a significant interaction was found for Treatment x Sex x Session, $F(4.08, 81.49) = 3.36$, $p = .013$, *post hoc* tests revealed no significant differences between treatments for either sex during any session, suggesting a spurious finding that may have been due to control females exhibiting an uncharacteristically low SR percentage during Session 5. Together, these data suggest that differences in sensitivity to the CS did not contribute to the differences observed in learning.

3.2.2.2 UR Amplitude: ANOVA for UR amplitude during Session 1 of conditioning revealed no significant differences for treatment, sex, or session. No interactions were found, suggesting that US sensitivity was not a contributing factor to any differences reported for learning measures.

4. Discussion

In the present study, corticosterone administered immediately following the SHRP, on PND 15-17, impaired hippocampus-mediated trace eyeblink conditioning 10 days after treatment, on Days 28 and 29. Corticosterone-treated rats produced significantly fewer CRs than vehicle-treated controls. CR amplitudes were significantly lower for the CORT group when measured during the adaptive period as well as overall. There were no significant differences between treatment groups with regards to measures of response timing. Further, there were no sex differences in the effects corticosterone produced in this study in which osmotic mini-pumps were used for drug delivery. Importantly, the corticosterone elevations produced in this study were moderate and within the range of normal stress values. Moreover, treated rats showed no loss of body weight, appeared to be in good health and exhibited normal grooming patterns.

In our previous study, impairments in trace eyeblink conditioning were observed after an administration procedure that produced supraphysiological levels of plasma corticosterone and significantly reduced the weight gain of the pups [1]. The current results demonstrate that corticosterone-induced impairment of this form of learning does not require such extreme conditions. Rather, an approximate 3-day period of exposure to corticosterone in the normal stress range was sufficient to reduce future learning performance. Because results in the previous experiment were limited to males, the most unexpected result of the present study was that females showed a deficit as well. One explanation is that there may have been insufficient power in the present study to detect reliable sex differences, although the sample sizes were only slightly smaller than in our 2005 paper. Why a less-extreme, but not a more-extreme, level of exposure to corticosterone should produce a learning deficit in females cannot easily be explained, but raises the possibility that the two exposure protocols affected performance through distinct mechanisms.

Given the high presence of glucocorticoid receptors in the hippocampus and the importance of this structure for trace eyeblink conditioning, it is very likely that the corticosterone effects observed here are mediated by the hippocampus. Extreme glucocorticoid levels have been shown to cause overexcitation of hippocampal neurons leading to cell death [40] and changes in hippocampal structure and function. In fact, studies of human patients with hypercortisolemia have shown impairment of trace eyeblink conditioning [41]. Glucocorticoid receptor sub-types, Type 1 (mineralocorticoid; MR) and Type 2 (glucocorticoid; GR), are found within the CA1, CA3, and dentate gyrus regions of the hippocampus [30,40]. Type 1 receptors are activated by normal daily fluctuations in glucocorticoids levels [42], whereas Type 2 are activated in response to elevated or chronic stress levels [43-44]. This distinction in receptor function may, therefore, support the idea that there are two distinct mechanisms for corticosterone's effects on trace conditioning, as demonstrated by our behavioral data.

Of course the hippocampus alone does not mediate trace conditioning. The cerebellum, other brain stem areas and prefrontal cortex are also significant players [45-49]. However, it is less likely that corticosterone's negative effects on learning are mediated by these other brain regions. Our previous study included delay eyeblink conditioning, a version of associative learning dependent on cerebellum and brainstem but not hippocampus, and found it to be unaffected by high levels of exogenously administered corticosterone. However, neither does our CORT group resemble trace conditioning in animals with lesions of hippocampus. The mild elevations of plasma corticosterone produced here specifically affected CR frequency and did not have the same impact on CR latency that is typically observed following hippocampal lesions.

Typically, hippocampal lesions result in CRs that occur very early in the trial and early during tone presentation [50-51] whereas the animals in our study continued to respond during the latter part of the tone period and into the interstimulus interval. Therefore, the effects of corticosterone in this study appear to be consistent with a milder insult. Further studies of hippocampal glucocorticoid receptor involvement may reveal a mechanism for graded or variable effects on behavior.

An alternative to the differential receptor sub-type recruitment in response to low- and high-corticosterone levels, is the possibility is that corticosterone disrupts fundamental neural development through processes such as neurogenesis. In adult rats, acute stress, chronic unpredictable stress and exogenous corticosterone administration all produce changes such as suppression of synaptic plasticity as well as a decrease in neurogenesis in hippocampal regions [52-54]. Certainly, in the adult, neurogenesis and the presence of immature neurons appear to be important for stress adaptation and regulation [55]. Mice exposed to a mild

environmental stressor following suppression of neurogenesis, produced a potentiated corticosterone response suggesting negative feedback disruption to the HPA axis in the absence of new neurons [56].

Because the rat species is born immature, a great deal of neural development takes place postnatally making them extremely sensitive to glucocorticoid exposure during neonatal development [57]. Species such as the guinea pig and human that are born more mature experience significant brain and neuroendocrine development in utero, resulting in greater vulnerability to glucocorticoid exposure and stress during the prenatal period. Furthermore, studies in the rat and guinea pig have revealed that moderate glucocorticoid exposure during a period of significant brain growth results in modified corticosteroid receptor expression in the adult brain [58-59]. The present study is consistent with these and similar studies indicating that even short duration and modest glucocorticoid exposure at a time of continued brain growth and neuroendocrine development produces effects that last beyond the time of the exposure. In humans, development of the hippocampus begins prenatally and continues into the postnatal period [60-61]. Pre- and early postnatal stress, as well as childhood exposure to corticosteroid-based asthma medications, have been associated with later learning deficits [9, 62, 63]. Therefore, our results suggest that further studies of hippocampal-dependent learning tasks that can be performed in both humans and other animals—such as trace eyeblink conditioning—may reveal important information about the associations between stress, glucocorticoids, the hippocampus, and developmental learning disorders.

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Abbreviations (Non-standard; In order of appearance): PEG: Polyethelyne Glycol

HPA	Hypothalamic-Pituitary-Adrenal Axis
SHRP	Stress Hyporesponsive Period
PND	Postnatal Day
EMG	Electromyography
CRA	Conditioned Response Amplitude
CL	Response Onset Latency
CML	Latency to Maximum Peak
SR	Startle Response
SEM	Standard Error of the Mean

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Highlights

- !! Limited exposure to CORT impairs eyeblink conditioning 10 days later in young rats
- !! Impairment of trace conditioning suggests CORT effect mediated by hippocampus
- !! Developmental vulnerability to glucocorticoids extends beyond hyporesponsive period

Figure 1.

A schematic diagram of the timing of stimulus presentations for trace eyeblink conditioning. A 380ms tone precedes a 500ms silent period ending with a 100ms periorbital shock. This creates an 880ms interstimulus interval between tone onset and shock onset during which conditioned responses occur once the CS-US association is learned.

Figure 2.
Mean ($\pm$ SD) plasma CORT concentrations at 24 hours after mini-pump implantation.

Figure 3.

The average percentage of Total and Adaptive CRs ($\pm$ SEM) are presented here for each treatment group across 6 sessions of trace eyeblink conditioning. CORT (open symbols) significantly impaired learning as measured by Total but not Adaptive CRs as compared to vehicle controls (filled symbols).

Figure 4.

The average amplitude (in arbitrary EMG units) of Total and Adaptive CRs ($\pm$ SEM) are presented here for each treatment group across 6 sessions of trace eyeblink conditioning. CORT (open symbols) significantly impaired learning as measured by both Total and Adaptive CRs as compared to vehicle controls (filled symbols).

Figure 5.

No significant differences were observed between treatment groups for the onset and peak latency of conditioned responses which shifted closer to US onset as training progressed. However, there was a non-significant trend separating the CORT group (open symbols) after Session 2 and shifting CRs closer to US onset (later in the interstimulus interval) than occurred for controls (filled symbols).