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Research report

Modest elevation of corticosterone in preweanling rats impairs subsequent trace eyeblink conditioning during the juvenile period

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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.

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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 μl/h 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 μg/dl versus 6.02 μg/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.

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 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 Refs. [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

Abbreviations: 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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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 Days 2–14, the pup undergoes the so-called stress hyporesponsiveness 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 h 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 h later, at 1000 h 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 1000 h, osmotic mini-pumps (Alzet, Model 1003D) were preloaded, under sterile conditions, according to manufacturer instructions, with 100 μl of either 50 mg/ml corticosterone (Sigma, No. C2505) dissolved in polyethlylene 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 h prior to implantation. The pumps were designed to release 1 μl/h 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 CO_2 exposure until unconscious (<1.5 min) 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 1 h 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, 2 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 (1 mm 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-3Tl Medwire) of a custom-made differential electromyographic (EMG) recording electrode