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Research report

Short-term, high-dose administration of corticosterone by injection facilitates trace eyeblink conditioning in young male rats

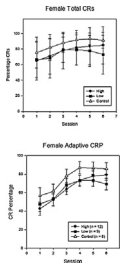
Christine L. Wentworth-Eidsaune, Michael B. Hennessy, Dragana I. Claflin*

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

HIGHLIGHTS

- Oscillating corticosterone elevations facilitate trace eyeblink conditioning.
- Corticosterone's effects appear to be specific to male rats.
- Direction of corticosterone effect on conditioning varies with delivery method.

GRAPHICAL ABSTRACT



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ABSTRACT

Glucocorticoids released as part of the physiological response to stress are known to affect cognitive function, presumably via effects on the hippocampus. Trace classical eyeblink conditioning is an associative learning task which depends on the hippocampus and has been used to examine the development of learning processes in young mammals. Previously, we demonstrated deficits in trace eyeblink conditioning associated with postnatal administration of the glucocorticoid corticosterone by creating a sustained elevation with methods such as subcutaneous timed-release pellets and osmotic mini-pumps which were active over several days. In the present study, we examined the effects of an oscillating pattern of corticosterone elevation on subsequent trace eyeblink conditioning. Twice daily corticosterone injections (high, low, or vehicle) were administered over a 3-day period, starting at postnatal day 15. Then, on postnatal day 28, animals underwent trace classical eyeblink conditioning to examine the possible influence of earlier corticosterone elevations on the development of learning and memory. Eyeblink conditioning was affected by corticosterone treatments, but only for males, and only very early in acquisition; Males receiving the high dose of corticosterone exhibited facilitation of learning relative to controls. These data demonstrate that oscillating corticosterone elevations produce opposite effects on this associative learning task than do sustained elevations.

1. Introduction

Glucocorticoids play an important role in cognitive functions. Most often, fluctuations in glucocorticoid levels are associated with learning and memory deficits (for reviews, see [1,27,29]). However, glucocorticoids can also enhance learning [44]. The effects are typically described as having an inverted-U shaped relationship such that very high and very low levels of glucocorticoids impair learning, whereas moderate levels tend to facilitate learning and memory processes [45,65]. Glucocorticoid effects also depend

Abbreviations: MR, mineralocorticoid receptor; SR, startle response; GR, glucocorticoid receptor; SEM, standard error of the mean; LTP, long term potentiation; HPA, hypothalamic-pituitary-adrenal axis; PND, postnatal day; SHRP, stress hyporesponsive period; EMG, electromyography; CPR, conditioned response percentage; CRA, conditioned response amplitude; CL, response onset latency; CML, latency to maximum peak.

* Corresponding author: Fax: +1 937 775 3347.
E-mail addresses: christine.eidsaune@gmail.com (C.L. Wentworth-Eidsaune), michael.hennessy@wright.edu (M.B. Hennessy), dragana.claflin@wright.edu (D.I. Claflin).

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on a number of other variables including task complexity, contextual and temporal factors, and the duration of the glucocorticoid exposure (reviewed in [14,16,30,41,45].

The brain region most associated with learning and memory is the hippocampus. A large number of glucocorticoid receptors are found in the hippocampus, making it particularly sensitive to changes in glucocorticoid levels produced by both endogenous and exogenous sources [4,48,58]. It is suggested that the differential effect of glucocorticoids on hippocampal function is mediated by the ratio of occupied glucocorticoid receptor types, Type 1 (mineralocorticoid; MR) and Type II (glucocorticoid; GR). Activation of MRs, such as occurs under resting conditions, appears to facilitate hippocampal function, enhancing LTP and improving spatial memory in the *Y*-maze. Memory consolidation is impaired in the absence of glucocorticoids following adrenalectomy or administration of antagonists. In contrast, activation of GRs, such as occurs during exposure to stressors, appears to impair hippocampal excitability associated with learning plasticity, suppressing LTP and impairing performance on spatial memory tasks (see reviews: [10,14,32,33,43]. Prolonged glucocorticoid elevations have been found to have neurotoxic effects on the hippocampus in adult humans and in other animals, including rats (for review, see [26,28,50]. However, short-term glucocorticoid elevations have been shown to support neural plasticity and normal learning, specifically post-training memory consolidation [24,50].

The neurotoxic effects of glucocorticoids produce devastating effects on brain development during the perinatal period. Neonatal glucocorticoid administration inhibits differentiation and growth of neurons and glia in widespread areas, but particularly those undergoing extensive postnatal differentiation, including the hippocampus [4,15,17,18,53,55]. A number of behavioral outcomes, including cognitive impairment, have been observed across several species. Glucocorticoid treatments for neonatal respiratory distress and asthma in children have been associated with deficits in verbal memory [2,3], but may also preferentially impair other cognitive tasks known to be mediated by the hippocampus [42,64].

In studies of glucocorticoid administration three commonly used routes of administration are injection, commercially-available, timed-release, surgically-implanted biodegradable pellets, and precision-release osmotic minipumps. Glucocorticoids administered by these different methods can produce differences in temporal pattern as well as in blood levels. Pellets and minipumps are intended to produce prolonged constant elevations, whereas injections produce relatively short-lived increases and decreases in blood levels. But even methods intended to produce constant levels might not always do so. Hermann et al. [20] observed little consistency of circulating concentrations with different drug delivery methods, in terms of both plasma level and duration of elevation. Specifically, they reported that: (1) corticosterone pellets produced high levels at 24 h that dropped dramatically to zero at 7 days instead of the intended 21 days. (2) osmotic mini-pumps failed to consistently alter plasma levels, and (3) injections only elevated corticosterone levels for about 4 h. When applied to studies of learning, these results raise the possibility that performance may be affected not only by the intended dose level, but also by the inconsistency and variability in hormone administration that might follow the use of pellets or osmotic pumps. Further, the relatively short-lived increase and then decrease in circulating levels one sees following injection may also be important, in as much as Liston et al. [25] recently reported that oscillating levels of corticosterone, such as occur during the natural circadian cycle facilitate the formation of learning-induced synapses at the peak of the cycle, whereas a prolonged constant elevation disrupted these same processes.

In a previous study we used corticosterone pellets to assess the effects of elevated hormone levels on hippocampal-mediated associative learning during the preweaning period. The neural substrates of trace classical eyeblink conditioning have been well established and are known to be hippocampus-mediated [23,36,59]. We found that the pellets, implanted subcutaneously on postnatal day 15, produced an initial marked elevation in circulating corticosterone reaching a pharmacological level of 80 μg/dl on Day 3 post-implant which returned to control levels by Day 6. Associative learning, as measured by an increase in conditioned responding using trace eyeblink conditioning procedures was assessed on postnatal day 28. Learning was impaired, but only in males, suggesting that the effects of corticosterone on learning lasted beyond the drug's effective period and that sex differences in vulnerability to corticosterone were present at this stage in development [11]. In order to determine the effects of corticosterone within a more relevant physiological range, we then implanted subcutaneous minipumps designed for a slow and constant rate of release over 3 days. The result was corticosterone levels in the moderate physiological range of 12 μg/dl at 24 h. Once again learning was impaired, but in this case males and females showed comparable deficits [10]

The present study examined whether corticosterone elevations at the same approximate levels would produce the same pattern of deficits if corticosterone was administered by injection. Rats were injected twice daily with doses producing peak plasma levels that approximated those of our earlier studies with pellets and minipumps. Injections continued over 3 days to mimic the length of exposure in the previous studies. Effects on trace eyeblink conditioning were then assessed 10 days later.

2. Material and methods

2.1. Subjects

Timed-pregnant female Long-Evans rats were received from Charles River Laboratories (Raleigh, NC) around E15. On PND 4 or 5, litters were culled to 10 pups, 5 male and 5 female whenever possible. Animals were housed with dams until weaning in a colony room accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). Animals were maintained on a 12:12 h light:dark cycle with lights on at 0700. Ad libitum access to food and water was provided. Animals were randomly assigned to one of three dose groups (high, low and control) and groups were roughly balanced for sex.

Starting on PND 15, two subcutaneous injections were given daily for 3 days, one at 0900 and one at 1700 h. Animal health and weight were monitored every other day throughout the experiment. Pups were housed with dams until weaning on PND 21, at which time they were housed with same-sex littermates until surgery on PND 26. On PND 26, pups underwent a surgery to implant electrodes used during eyeblink conditioning on PND 28–29. After surgery, and during behavioural training, animals were housed individually.

The final data are derived from 57 Long-Evans rat pups, 29 female and 28 male sampled from 15 litters. No more than 1 female and 1 male from the same litter were assigned to a particular condition to control for litter effects. The high dose group consisted of 20 pups (12 f, 8 m), the low dose group consisted of 20 pups (9 f, 11 m) and the control group consisted of 17 pups (8 f, 9 m).

2.2. Procedures

2.2.1. Injections

Corticosterone (*Sigma* C2505) was dissolved in sesame oil (*Sigma* S-3547), vortexed and placed in a warm water bath 24 h prior to injections and maintained at 37 °C until injections were completed. In order to establish doses that produced blood levels