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Research report

Influence of environmental enrichment on hypothalamic-pituitary-adrenal (HPA) responses to single-dose nicotine, continuous nicotine by osmotic mini-pumps, and nicotine withdrawal by mecamylamine in male and female rats

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HIGHLIGHTS

- Environmental enrichment (EE) reduced baseline ACTH and CORT in males and females.
- EE lowered HPA axis responses to SAL, nicotine (NIC), and mecamylamine in most groups.
- Effects of EE were sexually diergic: females were more sensitive to EE than males.
- EE may alter coping during NIC habituation and withdrawal.
- EE may be a useful approach for stress reduction in animal models of NIC addiction.

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ABSTRACT

In the present study, we determined the effects of environmental enrichment (EE; Kong Toys® and Nestlets®) on sexually diergic HPA axis responses to single-dose nicotine (NIC), single-dose NIC following continuous NIC administration for two weeks, and NIC withdrawal by single-dose mecamylamine (MEC) in male and female rats. Blood sampling occurred before and after MEC and NIC administrations for the determination of adrenocorticotropic hormone (ACTH) and corticosterone (CORT).

Supporting and extending our previous findings, EE appeared to produce anxiolytic effects by reducing hormone responses: Male and female rats housed with EE had lower baseline ACTH and significantly lower HPA axis responses to the mild stress of saline (SAL) injection than did those housed without EE. The sexually diergic responses to single dose NIC, continuous NIC, and MEC-induced NIC withdrawal were reduced by EE in many male and female groups. ACTH responses to continuous NIC and MEC-induced NIC withdrawal were blunted to a greater extent in female EE groups than in male EE groups, suggesting that females are more sensitive to the anxiolytic effects of EE. Because EE lowered stress-responsive hormones of the HPA axis in most groups, EE may be a useful intervention for stress reduction in animal models of NIC addiction. As well, the effectiveness of EE in animal studies of NIC withdrawal may enlighten human studies addressing coping styles and tobacco cessation in men and women.

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1. Introduction

The potentially stressful nature of an organism's environment is often overlooked in research on laboratory animals. Factors including caging, social disruption, restraint, transport, noise, routine cleanings, and lighting can have adverse physiological and behavioral consequences [1–7] and can significantly alter neuroendocrine and autonomic responses [8,9]. For example, individually housed rats have increased stress hormone levels compared to group-housed rats [10–13]. Environmental enrichment (EE) can improve the quality of life of the caged animal, distracting the animal from an otherwise monotonous environment [6]. Stress reduction can be achieved by enriching an animal's environment with devices that promote an animal's normal instinctive tendencies, thereby enhancing the animal's homeostatic physiology [6,14–20].

The hypothalamic-pituitary-adrenal (HPA) axis is an important modulator of homeostasis and the stress response. HPA axis activity

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is reflected peripherally by plasma concentrations of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) released from the anterior pituitary and adrenal cortex, respectively. ACTH release is stimulated by corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) secreted from the paraventricular nuclei (PVN) of the hypothalamus [21–23]. HPA axis activity is modulated by muscarinic and nicotinic cholinergic receptors in brain areas such as the PVN and supraoptic nuclei of the hypothalamus, the hippocampus, and the brainstem [24–26], as well as by cholinergic receptors within the axis itself [27,28].

Nicotine (NIC) administration increases HPA axis activity in both humans [29–32] and rodents [33–38] through stimulation of presynaptic nicotinic cholinergic receptors, which activate noradrenergic projections to the PVN [35,39,40]. Because few studies have reported sex differences in HPA axis responses to NIC [41–43], our studies have focused on sexual diergism (functional sex differences) in HPA axis responses to cholinergic stimulation [26,37,38,44–47].

We previously reported that NIC activates the HPA axis in a sexually diergic manner, stimulating ACTH and CORT in female rats to a significantly greater degree than in males [37,38,46]. As well, NIC habituation to continuous NIC administered for two weeks by osmotic mini-pumps, and withdrawal by a single dose of 5 mg/kg of mecamylamine (MEC), influence stress responses in a sexually diergic manner, also stimulating ACTH and CORT in female rats to a significantly greater degree than in males [48]. In these studies, chronic jugular-vein cannulation for serial blood sampling necessitated that animals be housed individually, potentially leading to elevated stress; alleviating this potential stress to achieve low and stable baseline HPA activity therefore was imperative. As well, stress can play an important role in predisposing to drug addiction [49–52] and in maintaining addiction and triggering relapse [50,52], and thereby could possibly alter sexually diergic responses to NIC habituation and withdrawal. For these reasons, we determined the effects of stress reduction by EE on sexually diergic HPA axis responses to single-dose NIC, single-dose NIC immediately following continuous NIC administration for two weeks, and NIC withdrawal by single-dose MEC in male and female laboratory rats. It was hypothesized that EE would reduce the stress of individual housing and facilitate NIC withdrawal in both males and females.

2. Materials and methods

2.1. Animals

Eight-week old, jugular vein-cannulated (JVC), male and female Sprague-Dawley rats weighing 200–225 g (Taconic Farms, Inc., Germantown, NY, USA) were housed singly in a well-ventilated, temperature- and humidity-controlled environment (22–25 °C, 50–75% humidity) under a standard 12-h light/dark cycle (lights on at 0700 h). Laboratory rat chow and water were available *ad libitum*. The stage of estrous cycle in the females was uncontrolled and thus was considered a random effect; we have shown the influence of estrous cycle stage on HPA activity to be of considerably less magnitude than the changes produced by NIC [46]. Prior to experimentation, animals were allowed 4–5 days to acclimate to the housing conditions and blood sampling via routine flushing of their cannulae. Experiments were performed between 0900 h and 1300 h to minimize circadian variations in plasma hormone concentrations. Seven to 15 rats per sex were tested in each group, housed either with or without EE. N's for each group are reported in the figure legends. All experiments were approved by the Allegheny-Singer Research Institute Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

2.2. Drug administration

All doses of NIC (nicotine hydrogen tartrate salt; Sigma, St. Louis, MO, USA) were calculated and reported based on the weight of the free base and chosen to discriminate sex differences [38] and minimize noxious side effects and non-specific activation of the HPA axis [53]. Immediately before each experiment, NIC was freshly prepared in saline (SAL). On experiment days, all single-doses of NIC or SAL were administered as IP injections after baseline blood samples were collected. To investigate the effects of single-dose NIC alone and following continuous NIC, animals were administrated SAL (1 ml/kg) at −20 min, followed by SAL (1 ml/kg) or NIC (0.3 or 0.5 mg/kg) dissolved in SAL at 0 min; animals therefore always received two injections. To investigate the effects of single-dose MEC alone and following continuous NIC, animals were administered MEC (mecamylamine HCl; 5 mg/kg; Sigma, St. Louis, MO) at −60 min, followed by SAL (1 ml/kg) or NIC (0.3 mg/kg) dissolved in SAL at 0 min. Dosing time and concentrations of NIC and MEC were based on pharmacokinetic parameters and previous studies in rats [33,34,38,54–56], as supported by a review on NIC dose selection for in vivo studies [57].

2.3. Continuous NIC administration

For the continuous NIC groups, Alzet® osmotic mini-pumps (model 2002) were surgically implanted into a 1 cm opening in the peritoneal cavity under pentobarbital (35–40 mg/kg) anesthesia. Before implantation, the mini-pumps were primed by injecting them with NIC solution (105 mg/ml) and placing them into 37 °C physiological SAL for a minimum of 4 h. Following insertion of the mini-pumps, the abdominal muscles were closed with a non-interrupted 4–0 absorbable suture, and the skin was closed with wound clips. Surgery time was 10–15 min. Based on the initial NIC dose (105 mg/ml) and the osmotic rate of the pumps, rats were delivered a continuous infusion of NIC at approximately 4.5 mg/kg/day (the approximate exposure of a smoker who uses one-half to one pack per day) for 14 days [56,58–60]. We did not measure plasma NIC concentrations, because the stability of NIC in the osmotic mini-pumps and the steady-state NIC concentrations in plasma following pump implantation are reliable and reproducible [56,58–62].

2.4. Environmental enrichment (EE)

Upon arrival from the supplier, rats were randomly assigned to two groups. One group was singly housed under standard laboratory conditions, and the second group was singly housed with EE, by having both Kong® Toys (Bio-Serv®, Frenchtown, NJ, USA) and Nestlets® (Ancare Corp., Bellmore, NY, USA) present in the cages. Kong® Toys remained in the cages throughout the duration of the experiment; fresh Nestlets® were presented with each change of bedding, every 4–5 days.

2.5. Blood sampling

The two-person procedure for blood sampling from cannulated animals was used. One person gently contained the animal, which remained calm subsequent to daily handling, while the other person collected the blood sample. Each sampling was completed in less than 1 min. To maintain cannula patency and acclimate the animals to the sampling procedures, twice each week the stainless-steel cannula plug was removed, the heparin-polyvinylpyrrolidone (PVP) (100 IU/ml) lock solution (Sigma, St. Louis, MO) aspirated, and 0.1 ml buffered normal SAL injected, followed by replacement of 0.02 ml lock solution. A similar procedure was followed for blood sampling: The PVP lock solution was aspirated, and 300–325 μl blood was withdrawn into a 1 ml tuberculin syringe, immediately transferred into microcollection tubes, and stored on ice. A replacement solution of buffered normal SAL (37 °C) equal to the amount of blood withdrawn was infused through the cannula, the cannula injected with 0.02 ml lock solution, and the stainless-steel plug reinserted. The plasma was separated by centrifugation, quickly frozen at −80 °C, and stored until hormone analyses. Baseline blood samples were collected before and after administration of SAL or MEC. Four additional blood samples were collected at 10, 20, 40, and 60 min after SAL or NIC.

2.6. Hormone assays

Plasma $ACTH_{1-39}$ was determined in singlet by a highly specific immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA, USA & Diasorin, Stillwater, MN, USA). Inter- and intra-assay coefficients of variation were less than 8%, and the minimum detectable ACTH concentration was 1.7 pg/ml. Plasma CORT was determined in duplicate by a highly specific radioimmunoassay (ICN Pharmaceuticals, Costa Mesa, CA); antibody cross-reactivities were less than 0.5% for all other steroids. Inter- and intra-assay coefficients of variation were less than 10%, and the minimum detectable CORT concentration was 3.5 ng/ml.

2.7. Statistical analysis

Group N's varied, owing to insufficient sample for analysis of both hormones from some of the animals because of surgical complications and loss of cannula patency. Data are presented as mean ± standard error of the mean (SEM). Changes in plasma hormone concentrations following drug treatments were calculated for each animal as the post-drug change from its own baseline. Between-group comparisons of drug treatments used the SAL-treated group as control and were assessed by a three-way (Sex × Drug × Time) analysis of variance (ANOVA). Between-group comparisons of EE and sex also were assessed by a three-way (EE × Sex × Time) ANOVA. Time was a within-groups factor for all analyses. Where appropriate, post hoc pairwise comparisons were made to determine locations of significance (designated as "e" for environmental enrichment and "s" for sex on the figures) with Fisher's LSD tests. Significance was considered as $p < 0.05$. All data sufficiently

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