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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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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.

Keywords

Environmental enrichment; HPA Axis; Mecamylamine; Nicotine; Sexual Diergism; Withdrawal

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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 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 200–225g (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 (1ml/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 hours. 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 approximated a Gaussian distribution such that no transformations were required (log-transformation did not alter the statistical results).

3. RESULTS

3.1 Behavioral Observations

Observable nicotinic effects following single-dose or continuous NIC such as sedation, flaccid posture, and loss of body tone, and observable anticholinergic behaviors following MEC such as agitation, teeth chatter, ataxia, eye blinks, and ptosis (facial/eyelid dropping) were absent in all groups.

Both males and females consistently chewed the Kong® Toys and shredded the Nestlets®, suggesting that these objects stimulated natural gnawing and nesting behaviors. It appeared that the females were more active in shredding the Nestlets® and sleeping on or next to them. There appeared to be no difference in Kong® Toy utilization between the sexes. Animals living in EE also were easier to handle during acclimation periods and experiments. Vocalizations and other observable behaviors (e.g. exploratory behavior and rearing) did not appear to differ between groups, suggesting that these behaviors were not influenced by the housing conditions.

3.2 HPA Axis Hormone Responses

3.2.1 ACTH—Fig. 1 shows plasma ACTH concentrations (pg/ml) following SAL (Fig. 1A and B) and increasing doses of NIC (Fig. 1C–F) in males and females housed with or without EE. Note the expanded ordinate scale in Fig. 1A and B (0–100 pg/ml) vs. the scale in Fig. 1C–F (0–800 pg/ml). NIC stimulated ACTH in both sexes in a dose-response fashion. Females showed greater ACTH responses than males, as indicated by highly significant main effects of drug ($F(3,145) = 77.2$; $p<0.0001$) and sex ($F(1,145) = 41.4$; $p<0.0001$). EE significantly lowered responses to SAL, as indicated by its highly significant main effect ($F(1,46) = 31.45$; $p<0.0001$). EE attenuated male ACTH responses at 0.5 mg/kg NIC doses. Multiple comparisons between groups housed with or without EE and between sexes are indicated in Fig 1.

Fig. 2 shows the effects of EE on plasma ACTH concentrations (pg/ml) following single doses of SAL and NIC (0.3 mg/kg) administered with and without continuous NIC infusion. Note the expanded ordinate scale in Fig. 2B, D F, H (0–350 pg/ml) vs. the scale in Fig. 2A, C, E, G (0–700 pg/ml). For the experiments in Fig. 2, SAL was given at −20 min, followed by SAL (1 ml/kg) or NIC (0.3 mg/kg) at 0 min. Fig. 3 shows the effects of EE on plasma ACTH concentrations (pg/ml) following single doses of SAL and NIC (0.3 mg/kg) administered with MEC pretreatment with and without continuous NIC infusion. For the experiments in Fig 3, MEC (5 mg/kg) was given at −60 min, followed by SAL (1 ml/kg) or NIC (0.3 mg/kg) at 0 min. For the experiments in Figs. 2B, D, F, and H and 3B, D, F and H, NIC was infused for two weeks via osmotic mini-pumps prior to single-dose SAL or NIC challenge.

EE appeared to attenuate ACTH responses in most of the male groups (Fig. 2A and B; Fig. 3A, B, D) and female groups (Fig 2E, F, G, H; and Fig 3H). In addition to the significant effect of EE previously mentioned in the SAL groups, there also was a significant main effect of EE on the SAL challenge preceded by a two-week NIC infusion ($F(1,33) = 4.83$; $p = 0.04$) for both males and females (Fig 2B, F). As shown in Fig. 3B, D, F, and H, EE also attenuated ACTH responses in both the male and female withdrawal groups (administered MEC and single-dose SAL or NIC following two-week NIC infusion), as indicated by a significant main effect of EE ($F(1,34) = 7.50$; $p = 0.01$). In contrast, baseline ACTH and ACTH responses of females housed with EE were significantly elevated in animals acutely administered MEC and NIC (Fig. 3G). Multiple comparisons between groups with or without EE and between sexes are indicated in Figs. 2 and 3.

3.2.2 CORT—Fig. 4 shows plasma CORT concentrations (ng/ml) following SAL (Fig. 4A and B) and increasing doses of NIC (Fig. 4C–F) in males and females housed with or without EE. Note the expanded ordinate scale in Fig. 4A and B (0–800 ng/ml) vs. Fig. 4C–F (0–1000 ng/ml). Overall, baseline CORT responses were elevated in females compared to males. NIC stimulated CORT in both sexes, with little effect of dose other than a more prolonged CORT response following the highest NIC dose in females. Females showed greater CORT responses than males, as indicated by highly significant main effects of drug

(F (3,145) = 28.1; $p<0.0001$) and sex (F (1,145) = 236.7; $p<0.0001$). EE significantly lowered responses to SAL, as indicated by a significant main effect of EE (F(1,46) = 6.93; p = 0.01). EE appeared to attenuate baseline hormone responses before the 0.3 mg/kg NIC dose, as well as responses following the 0.3 mg/kg NIC dose in females only, as indicated by a significant EE × Sex interaction (F(1,33) = 15.43; p = 0.0005). Multiple comparisons between groups with or without EE and between sexes are indicated in Fig. 4.

Fig. 5 shows the effects of EE on plasma CORT concentrations (ng/ml) following single doses of SAL and NIC (0.3 mg/kg) administered with and without continuous NIC infusion. For these experiments, SAL was given at −20 min, followed by SAL (1 ml/kg) or NIC (0.3 mg/kg) at 0 min. Fig. 6 shows the effects of EE on plasma CORT concentrations (ng/ml) following single doses of SAL and NIC (0.3 mg/kg) administered following MEC pretreatment, with and without continuous NIC infusion. For these experiments, MEC (5 mg/kg) was given at −60 min, followed by SAL (1 ml/kg) or NIC (0.3 mg/kg) at 0 min.

As with ACTH, EE appeared to attenuate the CORT responses in most of the male (Fig. 5A and B; Fig. 6A, B, C, D) and female groups (Fig. 5E, F, G; Fig. 6E and H). In addition to the significant effect of EE previously mentioned in the SAL groups, EE marginally lowered the CORT responses of female animals administered SAL after continuous NIC (Fig. 5F). EE had variable effects on baseline CORT concentrations in single-dose groups, but paradoxically raised baseline CORT concentrations in most continuous NIC groups. As shown in Fig. 6A and E, there was a significant main effect of EE on male and female animals pretreated with MEC and administered SAL (F(1,36) = 7.34; p = 0.01). EE also appeared to attenuate CORT responses in females administered MEC and single-dose NIC following a two-week NIC infusion (Fig. 6H). The CORT responses of males acutely administered MEC and NIC were attenuated by EE (Fig. 6C); similar to their ACTH responses, however, female CORT responses were significantly elevated by EE (Fig. 6G), as indicated by a significant EE × Sex interaction (F(1,33) = 7.62; p = 0.01). Multiple comparisons between groups with or without EE and between sexes are indicated in Figs. 5 and 6.

4. DISCUSSION

This appears to be the first study to explore the effects of EE on sexually diergic HPA responses to single-dose NIC, continuous NIC infusion, and MEC induction of NIC withdrawal. Our results highlight sex differences in the effects of EE on HPA axis activity of rats in a pharmacological paradigm modeling acute NIC administration, NIC habituation, and NIC withdrawal. In many cases, EE lowered stress-responsive HPA axis hormones in rats experiencing NIC habituation and withdrawal, suggesting that EE may be useful for stress reduction in animal models of NIC addiction.

4.1 Effects of EE on single-dose NIC responses

The sexually diergic, anxiolytic effects of EE support and extend our previous findings [6]. Male and female rats housed with EE appeared to have lower baseline ACTH levels and significantly lower HPA axis responses to the mild stress of SAL injection (Fig. 1A–B). In males, EE appeared to lower ACTH levels to the greatest extent at 0.5 mg/kg NIC doses, but in females, EE lowered ACTH levels at 0.3 mg/kg NIC doses only. As with ACTH, male and female rats housed with EE appeared to have lower baseline CORT levels and lower HPA axis responses to the mild stress of SAL injection (Fig. 4A–B). In females, but not in males, EE lowered CORT responses to the greatest extent at 0.3 mg/kg NIC doses. These results suggest that EE reduced the activating effects of NIC in both males (ACTH) and females (ACTH and CORT), but with different dose sensitivities. The habituation and withdrawal components were carried out with NIC doses of 0.3 mg/kg; at this dose EE

produced greater attenuation of ACTH and CORT responses in females, which likely accounted for the increased efficacy of EE in producing anxiolytic effects in females subjected to habituation and withdrawal.

4.2 Effects of EE on NIC habituation

In continuous NIC groups, which modeled NIC habituation, ACTH responses to continuous NIC were blunted to a greater extent in non-EE-housed males than in non-EE-housed females (Fig. 2D and H). However, in these habituation groups, ACTH responses to continuous NIC were blunted to a greater extent in EE females than in EE males (Fig. 2D and H). CORT responses were reduced by EE in both males and females, although the groups whose levels were attenuated by EE varied between the sexes. Overall, these results suggest that the stress hormone responses of both males and females were reduced by EE, but in a sexually diergic manner. The typically higher ACTH and CORT responses of females appeared to be more sensitive to the anxiolytic effects of EE.

4.3 Effects of EE on MEC responses and NIC withdrawal

In female rats administered single-dose NIC following MEC pretreatment, NIC did not appear to raise ACTH and CORT levels in the non-EE-housed animals. However, NIC did elevate hormone levels to a significantly greater extent in females housed with EE. There are several possible explanations. First, the baseline ACTH and CORT concentrations were greater in the female animals housed with EE (Figs. 3G and 6G), and the post-drug hormone responses may have been higher because of the animals' initial concentrations. However, CORT concentrations in these female animals were only marginally greater in those housed with EE (Fig. 6G), even though post-drug responses were significantly elevated. Second, Coolon and Cain [63], suggested that male rats housed with EE appeared to be less sensitive to the initial behavioral and locomotor effects of MEC during the hypoactive phase (first 15 minutes) following NIC administration. Likewise, in our study, female rats housed with EE appeared to be less sensitive to the inhibitory effects of MEC and acute NIC administration, compared to MEC and acute SAL administration (Figs. 3E, 3G, 6E, 6G). Third, it has been shown that rats housed in impoverished conditions have less CNS acetylcholine (ACh) and an altered number and affinity of CNS ACh receptor sites [64]. If female rats housed with EE in our study had increased ACh and ACh receptors, they may have been less sensitive to nicotinic blockade by MEC and may have shown a greater response to a subsequent single dose of NIC, as we observed (Figs. 3G and 6G).

4.4 Benefits of EE on laboratory animals

An environment to which animals cannot easily adapt may cause stress [65]; therefore, EE may provide multiple homeostatic benefits to laboratory animals. Rats housed in enriched environments performed better in Morris water maze tests in comparison to those in impoverished environments, even under moderate stress [66]. In radial maze tests, mature and aging rats (between 7 and 16 months old) outperformed rats housed in standard laboratory conditions and rats held in impoverished environments [67]. Based upon learning efficiency and energy-conserving behaviors, enriched rats were calculated to have a significantly higher survival rate, regardless of age [67]. Other physiological benefits of EE in rodents include larger forebrains [68], increased granule cell number and hippocampal volume [69], greater amounts of synaptogenesis and formation of dendritic spines in both the cortex and hippocampus [70], and delayed neuron degeneration [70]. The delay of neuron degeneration may be related to proteins involved in neuronal survival, including nerve growth factor and brain-derived neurotrophic factor; EE has been shown to increase production of these factors in the cerebellum [71]. EE also induces downregulation of corticotrophin-releasing factor receptor type 1 (CRFR1) mRNA expression in the amygdala of male and female mice, and CRFR1 appears to be essential for central control of the HPA

axis [72]. Decreased expression of this receptor could have accounted for the anxiolytic effects of EE shown in our study.

In some studies, CORT levels have been reported as elevated or unchanged as a result of EE [11, 73–75]. Because EE encompasses a wide variety of novel objects and social housing conditions, these differences could be associated with protocol variations. Rats showed a preference for chewable objects such as the Kong® Toys and Nestlets® used in this study. These, along with other types of EE, such as Nylabones®, allowed the animals to indulge their natural gnawing and tearing tendencies [76], which along with the acclimation process, yielded low stress-responsive hormone baselines during interactions between the animal and researcher. This was demonstrated by our results in both males and females housed with EE and subjected to the mild stress of SAL injection, as well as being supported by our previous findings [6].

4.5 Role of EE in stress reduction

Mounting evidence supports the hypothesis that EE acts an anti-stress mechanism by decreasing the activation of the HPA axis [17, 19, 77, 78]. We propose that EE acted as a coping mechanism for the negative affect or stress associated with NIC withdrawal (Figs. 3D and H; 6D and H). Cocaine relapse in humans [52] and the reinstatement of drug-seeking behavior in rodents [79] have been shown to be elicited by stress and negative affect. Blunting the responses to stress or anxiety brought about by withdrawal and abstinence therefore might aid in preventing relapse. EE also has been shown to eliminate preexisting addiction-related behaviors [80] and cue- and stress-induced reinstatement and craving [81, 82] when made available during periods of forced abstinence. Our results suggest that EE played a role in reducing the stress response during NIC habituation and withdrawal and thereby served as a coping mechanism, resulting in reduced male and female ACTH responses.

In conclusion, EE may be a useful intervention for reducing stress in laboratory animals subjected to NIC habituation and withdrawal. The effect appears to be sexually diergic: EE reduced the higher stress hormone levels of females to a greater extent than males. The introduction of Kong® Toys and Nestlets® to distract rats from an otherwise monotonous cage life thus appeared to act as a coping mechanism during pharmacologically induced NIC withdrawal. The use 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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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

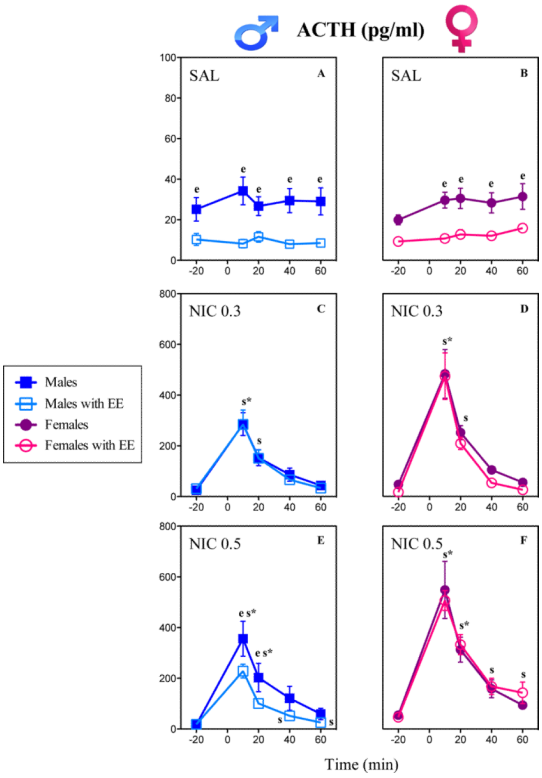

Figure 1. ACTH dose-response to NIC or SAL in male and female rats with and without environmental enrichment

Baseline blood samples were collected at −25 and −15 min to yield an average baseline (−20 min). SAL (1 ml/kg) was given IP at −20 min, and SAL or NIC (0.3, or 0.5 mg/kg) was given IP at 0 min. Additional blood samples were collected at +10, +20, +40, and +60 min. Each bar represents the mean ± SEM of 7 to 15 rats. N's by sex and environment for each group are SAL (M with EE = 9, M without EE = 15, F with EE = 8, F without EE = 15); NIC 0.3 (M with EE = 8, M without EE = 10, F with EE = 7, F without EE = 9); NIC 0.5 (M with EE = 9, M without EE = 9, F with EE = 9, F without EE = 8) *e*, enrichment difference at indicated dose and time ($p < 0.05$); *s,* sex difference at indicated dose and time ($p < 0.05$); * = letter applies to both with and without EE at the indicated time.

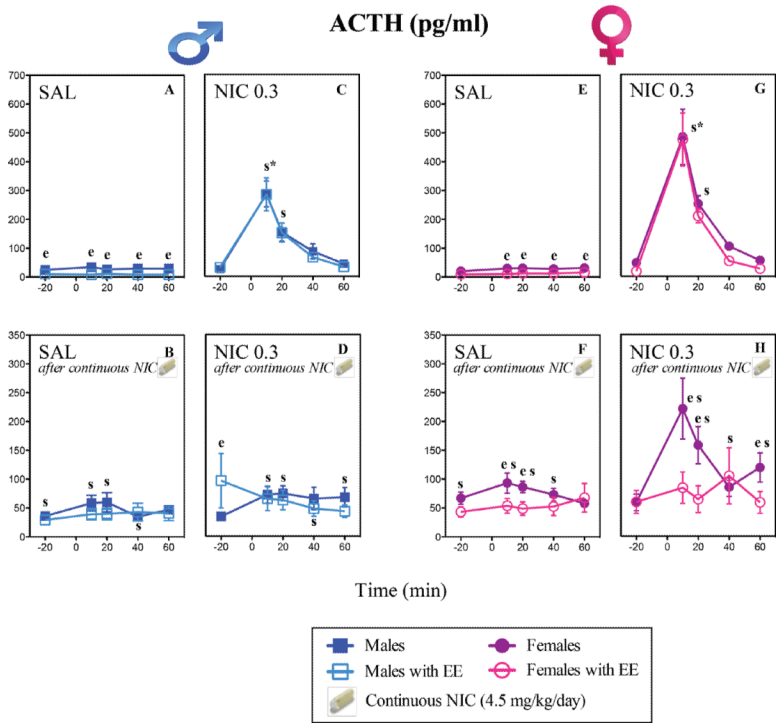

Figure 2. ACTH responses to SAL, and single-dose NIC, alone and following continuous NIC, in male and female rats with and without environmental enrichment

Baseline blood samples were collected at −25 and −15 min to yield an average baseline (−20 min). SAL (1 ml/kg) was given IP at −20 min; and SAL or NIC (0.3 mg/kg) was given IP at 0 min (A–H). NIC was infused continuously for two weeks prior to experimentation (B, D, F, H). Additional blood samples were collected at +10, +20, +40, +60 min. Each bar represents the mean ± SEM of 7 to 15 rats. N's by sex and environment for each group are SAL (M with EE = 9, M without EE = 15, F with EE = 8, F without EE = 15); SAL after continuous NIC (M with EE = 7, M without EE = 10, F with EE = 7, F without EE = 10); NIC 0.3 (M with EE = 8, M without EE = 10, F with EE = 7, F without EE = 9); NIC 0.3 after continuous NIC (M with EE = 7, M without EE = 10, F with EE = 7, F without EE = 10). *e*, enrichment difference at indicated dose and time ($p < 0.05$); *s*, sex difference at indicated dose and time ($p < 0.05$); = continuous NIC (4.5 mg/kg/day) infusion by Alzet® osmotic mini-pump. * = letter applies to both with and without EE at the indicated time.

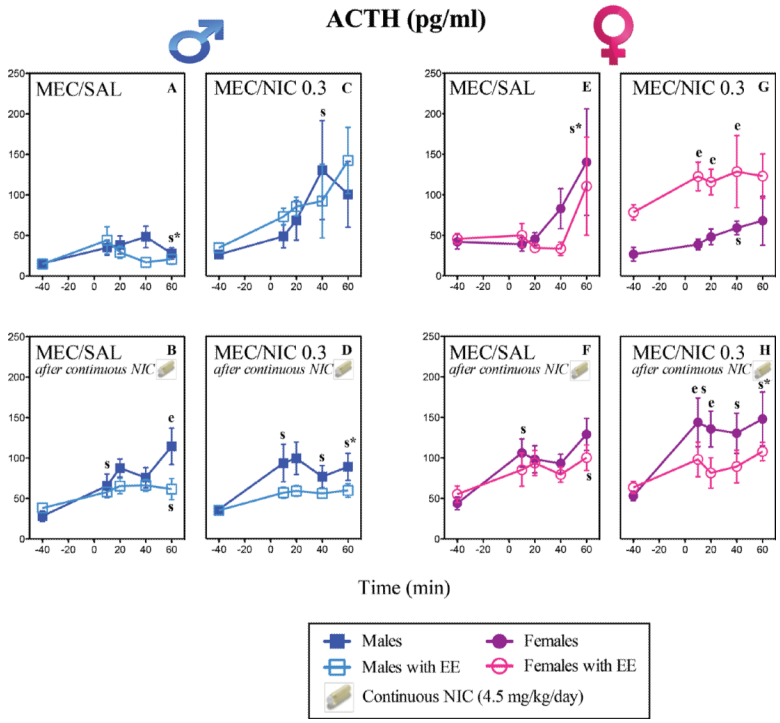

Figure 3. ACTH responses to single-dose MEC, alone, preceding single dose NIC, and following continuous NIC, in male and female rats with and without environmental enrichment

Baseline blood samples were collected at −65 and −15 min to yield an average baseline (−40 min). MEC (5 mg/kg) was given IP at −60 min; and SAL or NIC (0.3 mg/kg) was given IP at 0 min. NIC was infused continuously for two weeks prior to experimentation (B, D, F, H). Additional blood samples were collected at +10, +20, +40, +60 min. Each bar represents the mean ± SEM of 7 to 11 rats. N's by sex and environment for each group are MEC/SAL (M with EE = 11, M without EE = 9, F with EE = 10, F without EE = 7); MEC/SAL after continuous NIC (M with EE = 8, M without EE = 7, F with EE = 8, F without EE = 8); MEC/NIC 0.3 (M with EE = 8, M without EE = 9, F with EE = 9, F without EE = 8); MEC/NIC 0.3 after continuous NIC (M with EE = 8, M without EE = 10, F with EE = 9, F without EE = 8). *e*, enrichment difference at indicated dose and time ($p < 0.05$); *s*, sex difference at indicated dose and time ($p < 0.05$); = continuous NIC (4.5 mg/kg/day) infusion by Alzet® osmotic mini-pump. * = letter applies to both with and without EE at the indicated time.

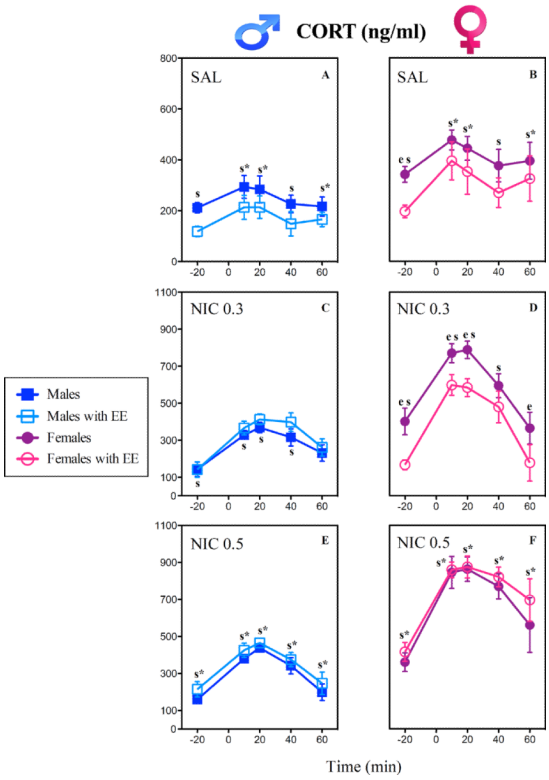

Figure 4. CORT dose-response to NIC or SAL in male and female rats with and without environmental enrichment

See Fig. 1 legend for explanation.

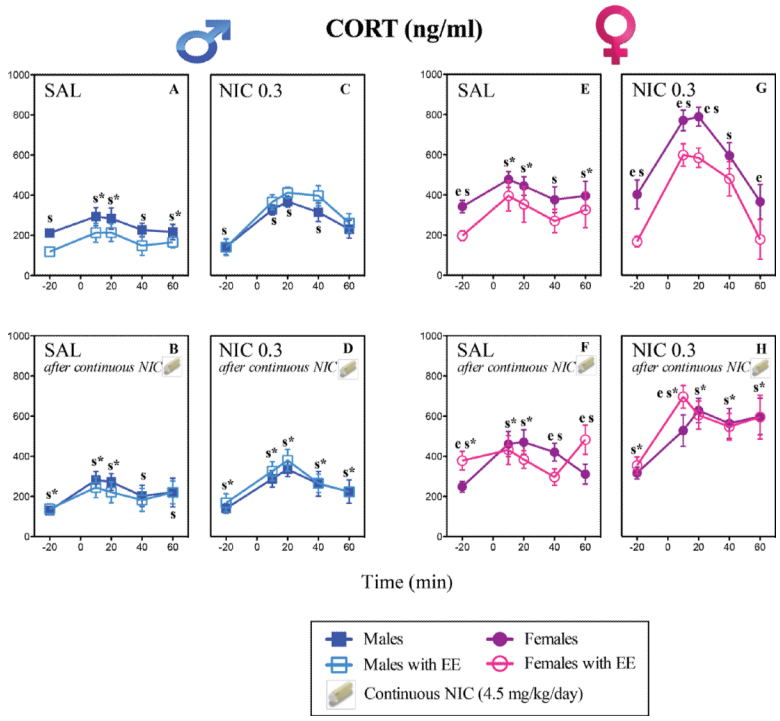

Figure 5. CORT responses to SAL, and single-dose NIC, alone and following continuous NIC, in male and female rats with and without environmental enrichment

See Fig. 2 legend for explanation.

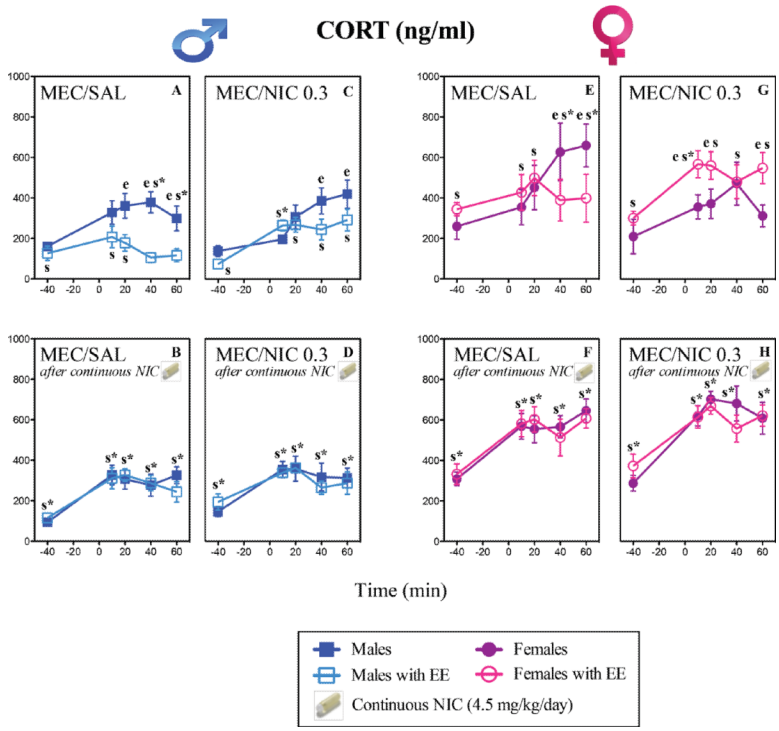

Figure 6. CORT responses to single-dose MEC, alone, preceding single dose NIC, and following continuous NIC, in male and female rats with and without environmental enrichment

See Fig. 3 legend for explanation.