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Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats

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Abstract

Structural and social aspects of an environmental system can influence the physiology and behavior of animals occupying that system. This study examined the physiological effects of environmental enrichment (EE) with Kong® Toys® and Nestlets® on stress-responsive hormones of the hypothalamic–pituitary–adrenal (HPA) axis under basal and mild stress conditions in singly housed, jugular vein-cannulated, male and female rats.

Animals of both sexes housed with EE had significantly lower baseline adrenocorticotropic hormone (ACTH) and corticosterone (CORT) concentrations compared to those housed without EE. ACTH responses to the mild stress of saline injection were significantly lower in female rats housed with EE.

Interaction with the Kong Toys and Nestlets appears to have provided the rats with a diversion from monotonous cage life, resulting in lower HPA axis activity before and after mild stress. These results are important because low, stable baselines are essential for accurately discerning pharmacological and other influences on the HPA axis.

Keywords: ACTH; CORT; Environmental enrichment; HPA axis; Stress

1. Introduction

Stress is a variable that often is overlooked in research on laboratory animals. It is representative of a living condition that is unfavorable and damaging to the organism's homeostasis or adaptive state (Martini et al., 2000). Slight changes in a laboratory animal's environment can alter its behavior during an experiment. Factors such as caging, social disruption, restraint, transport, noise, routine cleaners, and lighting can significantly impact the behavior of laboratory animals (Barnard and Hou, 1988; Jain and Baldwin, 2003). Adaptive responses to these factors include adverse physiological and behavioral consequences, as well as alterations in neuroendocrine and autonomic systems (Martini et al., 2000).

Stress reduction may be achieved by enriching an animal's environment with devices that promote its normal instinctive tendencies. Environmental enrichment (EE) can be defined as using objects to improve the quality of life of the caged animal, thus distracting the animal from an otherwise monotonous environment. For example, rats tend to tear materials such as cellulose paper or straw into small pieces to create a nest (Scharmann, 1991). Nestlets®, commercially available, 2 × 2-in. squares composed of sterilized, pulped, virgin cotton fiber, provide an animal with a soft, warm, nest-like environment. Animals housed with Nestlets are less aggressive, gain more weight, and are easier to handle—behaviors compatible with reduced stress (Hobbs et al., 1997). Another object for EE is the Kong® Toy, a 2- to 3-in.-long rubber toy originally designed in larger sizes for dogs. Kong Toys augment an animal's natural affinity for gnawing.

The hypothalamic–pituitary–adrenal (HPA) axis is an important modulator of the stress response. HPA activity is reflected peripherally by plasma concentrations of adrenocorticotropic hormone (ACTH) and corticosterone (CORT), released from the anterior pituitary and adrenal cortex, respectively. Conditions such as chronic cannula implanta-

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tion for serial blood sampling necessitate that animals usually housed in a group be housed individually after surgery. Both current housing conditions and previous housing experience can influence ACTH and CORT in male and female rats: Female rats housed in a group were shown to have lower CORT baselines than female rats housed individually, whereas male rats housed in a group had higher CORT baselines than male rats housed individually (Brown and Grunberg, 1995). In a follow-up study from the same laboratory, individually housed male rats that had been previously housed in a group had higher CORT baselines compared to male rats that had not been previously housed in a group (housed individually) (Brown and Grunberg, 1996).

Because our experiments incorporate singly housed, jugular vein-cannulated male and female rats into pharmacological dosing studies (e.g., Rhodes et al., 2001a,b, 2002), alleviating potential stress from single housing is imperative. The present study therefore attempted to reduce the stress of individual housing and resulting basal HPA axis activity by providing EE with Nestlets and Kong Toys. We also examined HPA responses to the mild stress of saline injection.

2. Materials and methods

2.1. Animals

Eight-week-old, jugular vein-cannulated, male and female Sprague–Dawley rats weighing 200–225 g (Taconic Farms, Germantown, NY, USA) were housed singly in a standard, shoebox-style polycarbonate cage (24 × 46 × 20 cm) with woodchip bedding, a wire-top lid and filter-top plastic cap 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). Male animals were housed within sight, hearing, and smell of other male animals. Likewise, female animals were housed within sight, hearing, and smell of other female animals. Intermingling of cages of animals of each sex housed with or without enrichment insured that temperature and humidity were random variables. Laboratory rat chow and water were available ad libitum. Animals were allowed 3–4 days to acclimate to the housing conditions; during this time they were handled daily for 2–3 min and, on the day before blood sampling experiments, administered an intraperitoneal injection of physiological saline (SAL, 1 ml/kg) to acclimate the animals to the procedure of intraperitoneal injection. Experiments were performed between 0900 and 1300 h to minimize the influence of circadian variations in plasma hormone concentrations. All experiments were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with National Institutes of Health guidelines.

2.2. Environmental enrichment

Two environments were used. One group of animals was singly housed under standard laboratory conditions. A second group of animals had both Kong Toys (Bio-Serv, Frenchtown, NJ, USA) and Nestlets (Ancare, Bellmore, NY, USA) in their cages. Kong Toys remained in the cage for the duration of housing. A new Nestlet was presented when the bedding from each cage was changed every 3 days.

2.3. Blood sampling

The two-person procedure for blood sampling from cannulated animals was used: One person gently contained the animals that typically remained calm after daily handling for 3–4 days before experimentation, while the other person collected the blood sample. Sampling was completed in less than 1 min. During experimentation, the stainless-steel cannula plug was removed, the heparin–polyvinylpyrrolidone (PVP; 100 IU/ml) lock solution (Sigma, St. Louis, MO, USA) was aspirated, and 300–350 μl blood was withdrawn into a 1-ml tuberculin syringe, immediately transferred into microcollection tubes, and stored on ice. A replacement solution of warm, buffered physiological SAL equal to the amount of blood withdrawn was infused through the cannula, the cannula was injected with 0.02 ml lock solution, and the stainless-steel plug was reinserted.

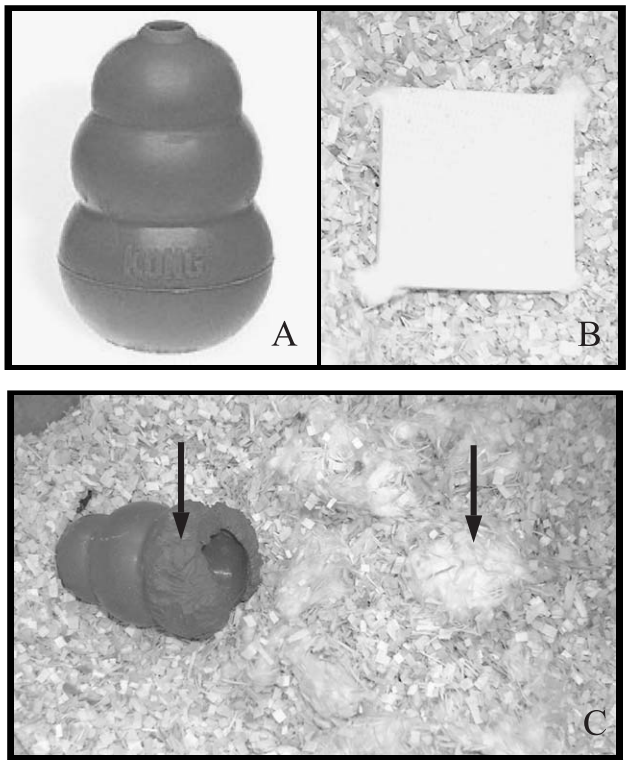

Fig. 1. Environmental enrichment. Examples of Kong Toys and Nestlets before (A, B) and after (C) presentation to singly housed male and female rats.

The plasma was separated by centrifugation, quickly frozen at −80 °C, and stored until hormone analyses. Baseline blood samples were collected at −25 and −15 min before SAL injection. The two samples were averaged to yield a mean baseline at −20 min. SAL (1 ml/kg) was administered intraperitoneally to animals after the baseline blood samples were collected. Additional samples were collected 10, 20, 40, and 60 min after SAL administration.

2.4. Hormone assays

Multiple assays were performed; each assay contained random samples from all experimental groups. Plasma samples were analyzed in singlet for ACTH and in duplicate for CORT. ACTH was determined by a highly specific immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA, USA) (Raff and Findling, 1989). Inter- and intra-assay coefficients of variation were 6.2% and 3.8%, respectively. The minimum detectable ACTH concentration was 2.6 pg/ml. CORT was analyzed by RIA with materials obtained in kit form (ICN Pharmaceuticals, Costa Mesa, CA, USA). Antibody specificities compared to CORT were less than 0.5% for all other steroids. Inter- and intra-assay coefficients of variation were less than 10%. The minimum detectable CORT concentration was 2.3 ng/ml.

2.5. Statistical analysis

Multiple experiments were performed under the same conditions to attain $N = 138$ animals for the baseline analysis. Of these 138 animals, 42 animals were administered SAL at 0 min. All animals from the SAL response groups are included in the baseline analysis. The overall N was necessary to compensate for nonpatent cannula occurrences. Group Ns varied, because there was insufficient sample for the analysis of both hormones from a few of the animals. Statistically significant differences between groups were determined by two-way analysis of variance (ANOVA) (Sex × Environment) for baseline analysis and by three-way ANOVA (Sex × Environment × Time) for SAL-response analysis, with time as a repeated measure. Tukey–Kramer and Fisher's LSD tests were used for post hoc comparisons. Significance was considered as $P < .05$.

3. Results

3.1. Behavioral observations

Animals of both sexes consistently chewed their Kong Toys and shredded their Nestlets (Fig. 1). It appeared that

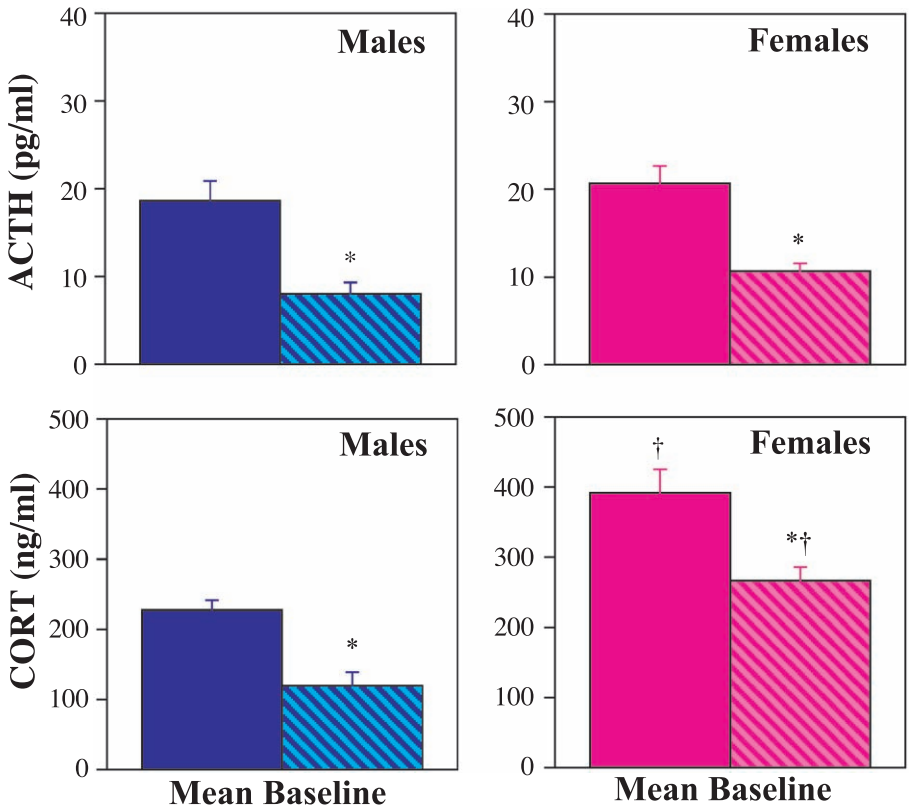

Fig. 2. Baseline ACTH and CORT in male and female rats housed singly with or without environmental enrichment (EE). Each bar represents the mean ± S.E.M. Solid bars = control groups; Striped bars = EE groups. Ns per group for ACTH and CORT, respectively: male control = 48, 44; male EE = 25, 20; female control = 41, 37; female EE = 24, 20. * $P < .05$, difference between EE and control groups; † $P < .05$, difference between males and females.

the females were more active in shredding the Nestlets and sleeping on or next to them. It also was our observation that after the animals experienced living in an enriched environment, they were easier to handle during acclimation periods and experiments. Vocalizations and observable behaviors (e.g., exploratory behavior, rearing) also did not appear to differ between groups, suggesting that these behaviors were not influenced by housing conditions.

3.2. Baseline hormone concentrations

Fig. 2 shows the effects of housing on baseline ACTH and CORT. Both ACTH [$F(1,137) = 24.8$, $P < .0001$] and CORT [$F(1,137) = 18.8$, $P < .0001$] concentrations were significantly lower in the groups housed with EE. For baseline ACTH concentrations, there were no significant sex differences in either the control or the EE groups. For baseline CORT concentrations, there was a significant main effect of sex [$F(1,137) = 34.7$, $P < .0001$], reflected in the significantly higher CORT baselines in females in both the control and the EE groups (Ps $< .05$).

3.3. Responses to SAL injection

Fig. 3 shows the effects of housing on ACTH and CORT responses to SAL injection.

For ACTH, there were significant main effects for environment [$F(1,61) = 46.8$, $P < .0001$] and time [$F(6,61) = 4.7$, $P = .0001$]. Both male and female rats housed with EE had significantly lower absolute ACTH responses to the mild stress of SAL injection ($P < .05$) compared to control rats. Both male and female EE groups had significantly lower ACTH responses compared to the control groups ($P < .01$), because of their lower baseline hormone concentrations. In the female control group, ACTH responses to SAL at +40 min were significantly higher, by 52%, and at +60 min, by 95%, compared to baseline (Ps $< .05$). In contrast, in the female EE group, ACTH responses to SAL were not significantly different at either +40 or +60 min (15% and 48% increases, respectively) compared to baseline.

For CORT, there were significant main effects for environment [$F(1,60) = 10.6$, $P = .0019$], and sex [$F(1,60) = 24.0$, $P < .0001$], as well as for time [$F(6,60) = 21.9$, $P < .0001$].

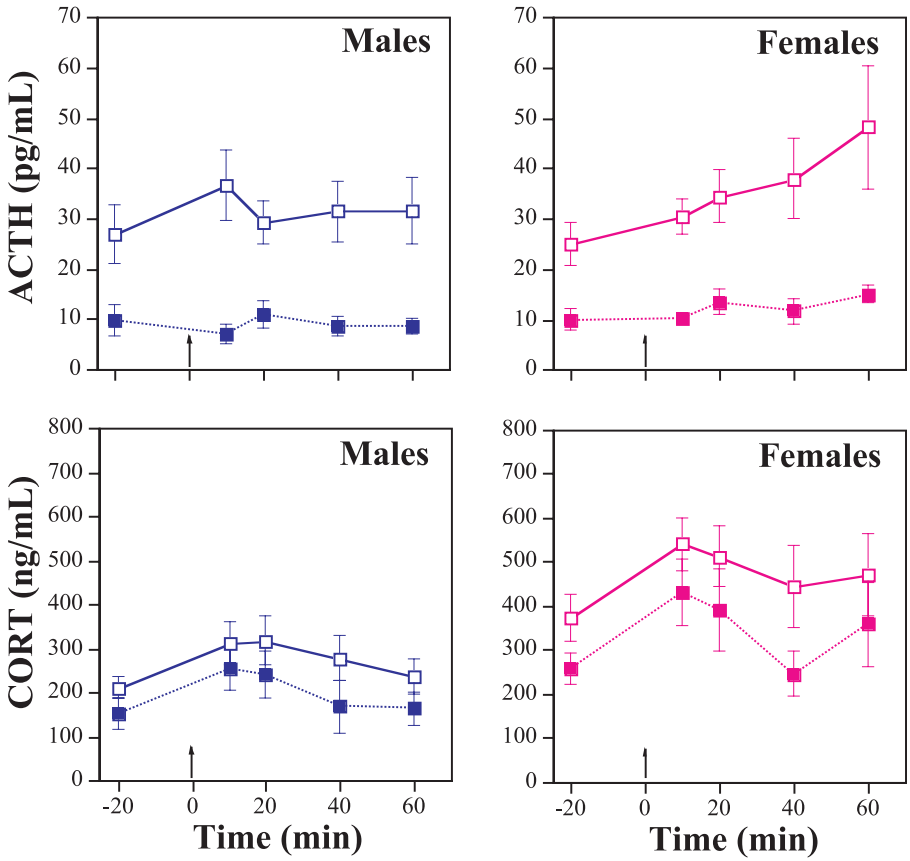

Fig. 3. ACTH and CORT responses to saline (SAL) injection in male and female rats housed singly with or without environmental enrichment (EE). Baseline blood samples collected at −25 and −15 min prior to intraperitoneal injection of SAL (1 ml/kg) were averaged to yield the −20 min baseline value. Postinjection samples were collected at +10, +20, +40, and +60 min. Arrows (↑) indicate time of intraperitoneal SAL injection. Each square represents the mean ± S.E.M. Open squares = control groups; solid squares = EE groups. *N*s per group for ACTH and CORT, respectively: Male control = 13, 13; male EE = 8, 7; female control = 14, 14; female EE = 7, 7.

In both the control and the EE groups, male and female CORT responses to SAL were similar.

4. Discussion

Social aspects of an environmental system may play a significant role in influencing the physiology and behavior of the animals occupying that system. Because rodents are social animals, isolated housing can produce stress (Barnard and Hou, 1988). Many current studies house rats or mice in grouped environments, which is seemingly beneficial to the animals. Conditions such as cannula implantation for serial blood sampling or drug administration necessitate that animals be singly housed. This prevents group-housed animals from scratching, biting, or gnawing, and thus destroying the cannulae of other animals.

The results of our study support the concept that the structural aspects of an environmental system can influence the physiology and behavior of the animals occupying that system. This is indicated by the significantly lower baseline ACTH and CORT concentrations in the male and female rats housed with EE. Compared to baseline, ACTH responses to SAL injection were significantly lower in female rats housed with EE, but not in males. This may have been related to the mild stress of the SAL injection; in other work, male rats reared with EE (bedding material, shelves, plastic tunnels, hay, ropes, and a hut) had lower ACTH responses to a more severe, 20-min restraint stress than did male rats reared in isolation (Schrijver et al., 2002). The lower ACTH responses to restraint stress may have derived from the greater complexity of the enriched environment. Future studies that determine ACTH and CORT responses to moderate stressors (e.g., footshock or restraint) in singly housed male and female rats with and without Kong Toys and Nestlets may further elucidate the sex differences observed in the present study.

In all our studies with singly housed, jugular vein-cannulated rats, the females had consistently greater CORT baselines and greater absolute CORT responses to SAL and other pharmacological challenges compared to male rats (Rhodes et al., 2001a,b, 2002). Because females typically have higher baseline and stress hormone responses (Rhodes and Rubin, 1999), EE appears to be useful in lowering baseline ACTH and CORT concentrations, as well as ACTH responses to stress, particularly in females. Lower baseline hormone concentrations should produce a greater sensitivity of the animal to the effects of experimental manipulation.

Rats show a distinct preference for chewable objects, allowing them to exhibit their natural tendency to gnaw and tear (Chmiel and Noonan, 1995). Out of numerous forms of enrichment, a block of wood with predrilled holes appeared to stimulate the greatest interest in rats (Chmiel and Noonan, 1995). Little interest was shown in pipes or partitions that would appeal to a wall-hugging tendency, and similarly, no interest was shown for objects that were reasoned to be intriguing to manipulate by the animals (e.g., golf balls, wood dowels, caged peach pit). In another study, Nylabones, comparable to Kong Toys, were actively chewed by many rats because they augmented the gnawing instinct (Watson, 1993). Animals that did not chew the bone occupied their time by moving the bone around the cage. Therefore, providing laboratory rats with EE for chewing will, at the very least, occupy their time as well as allow them to exercise a fundamental instinctive behavior.

EE may provide multiple benefits to animals (Würbel, 2001). An environment to which an animal cannot easily adapt may cause stress (Beaver, 1989; van de Weerd and Baumans, 1995). Male mice housed with EE have lower CORT responses to the stress of cat odor compared to male mice housed under standard conditions (Roy et al., 2001), suggesting that EE can reduce the HPA response to an environmental signal such as cat odor. Other benefits of EE in rodents include larger forebrains and a better-developed brain structure (Beaver, 1989), increased granule cell number and hippocampal volume (Kempermann et al., 1997), enhanced problem-solving and exploratory behaviors (Studelska and Kemble, 1979; Scharmann, 1991), and delayed decline of these physiological and behavioral measures with advancing age (e.g., Fernández-Teruel et al., 2002).

Interactions between animals and the researcher are crucial to maintaining low stress-responsive hormone baselines. Postnatal handling of rat pups produces significantly lower plasma ACTH responses to a variety of stressors compared to nonhandled pups (Viau et al., 1992). In our animals, whatever stress-reducing benefits the acclimation and restraint process provided were significantly augmented by EE. The effects of handling before and after mild stress in the present study are difficult to determine; future studies using cannula extensions whereby blood sampling can occur with minimal contact to the animal (Thrivikraman et al., 2002) would clarify the potential interaction of stress and handling.

In summary, the hormone effects observed in our study are likely due to interaction of the rats with Kong Toys and Nestlets, providing the animals with a diversion from monotonous cage life and resulting in both lower basal HPA hormones and, in the females, reduced ACTH responses to the mild stress of SAL injection. These results are important because low and stable baselines, which can result from an enriched environment, are essential for accurately discerning HPA responses to pharmacological and other challenges.

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