Published in final edited form as:

Physiol Behav. 2007 January 30; 90(1): 125–132. doi:10.1016/j.physbeh.2006.09.012.

Associations between behavior, hormones, and Fos responses to novelty differ in pre- and post-pubertal grass rats

Colleen M. Novak^{*,1}, David B. Parfitt², Cheryl L. Sisk³, and Laura Smale^{3,4}

¹Endocrine Research Unit, Mayo Clinic and Mayo Foundation, Rochester, MN 55905

²Department of Psychiatry, University of Rochester Medical Center, Rochester, NY 14642

³Departments of Psychology and Neuroscience Program, Michigan State University, East Lansing, MI 48824

⁴Department of Zoology, Michigan State University, East Lansing, MI 48824

Abstract

As animals progress from one developmental stage to the next, the nature of the challenges they face can change in systematic ways, as do the mechanisms that enable them to deal effectively with them. Here we examined the changes in the behavioral patterns and neuroendocrine mechanisms associated with exposure to a novel environment before and after the transition from a pre- to a post-pubertal stage of development in the unstriped Nile grass rat (*Arvicanthis niloticus*), a murid rodent that appears to live in family groups in the wild. We introduced juvenile (28 day old) and adult (60 day-old) grass rats to a novel glass aquarium where they were kept for one hour; controls were maintained in their home cages during this time. Animals were then killed, blood was sampled, and plasma corticosterone and testosterone were measured. Brains were collected and processed for immunohistochemical detection of Fos. Although behavior in the novel environment did not differ as a function of age, corticosterone secretion and Fos expression in a variety of stress-related brain regions were increased by the manipulation to a greater extent in the juveniles compared to the adults. The data suggest a pattern of development in which a novel environment that elicits the same levels of exploratory behaviors in young and adult animals triggers a greater response in stress-related brain regions as well as corticosterone secretion in the more vulnerable young ones.

Keywords

Arvicanthis niloticus; unstriped Nile grass rat; stress; fear; puberty; development

Over the course of life span development, the various challenges encountered by an animal change, as do the animal's responses to them. For example, young prepubertal animals don't engage in the myriad behaviors associated with reproduction, but they do have a greater need to explore and learn about their environment. Young animals are also typically more vulnerable to a greater range of potentially dangerous stimuli than are older animals. The overall balance between avoidance and exploration of a novel environment may thus shift as an animal develops from a pre- to a post-pubertal state. This type of change occurs in male *Rattus*

*Corresponding author Dr. Colleen Novak, Endocrine Research Unit, 200 First Street SE, Saint Marys Hospital, Joseph 5-194, Mayo Clinic and Mayo Foundation, Rochester, MN 55901, Phone: (507) 255-0316, Fax: (507) 255-4828, Email: E-mail: novak.colleen@mayo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

norvegicus, in which exposure to a novel environment causes a decrease in social interaction with an unfamiliar conspecific, and this effect is more pronounced in older compared to younger animals [1]. A similar phenomenon also occurs in mice, in which exploration of a novel environment is elevated in pre-pubertal compared to post-pubertal animals [2]. The development of responses to a novel environment may not be the same across species as age-related patterns of vulnerability, as well as the demands for exploration, may differ. For example, dispersal from the natal home range is a high risk behavior that requires increased exploration and a decreased fear response, and it occurs at quite different developmental stages in different species [3]. A better understanding of how fundamental processes involved in exploratory behavior and stress mechanisms change across the lifespan warrants examination of how this occurs in additional species, particularly those that face demands that differ from those confronted by mice and laboratory rats.

In this study, we examined the development of behavioral, endocrine, and brain responses to a novel environment in pre- and post-pubertal *Arvicanthis niloticus* (also referred to here as “grass rats”). *A. niloticus* is a diurnal species with a social system quite different from those more typically used in studies of the development of fear and stress reactivity. These territorial rodents typically live in family groups consisting of one adult immigrant male, one or more related adult females, and one or two generations of offspring [4,5]. In some respects, therefore, the social environments in which these animals develop suggest that grass rats could represent a better model of the human condition than do mice and lab rats. These family groups of grass rats live together in an underground nest from which animals emerge during the day to foray into the environment along runways that radiate outwards from the nest, to which they rapidly retreat when they are threatened. Adult males appear to leave their natal groups, a step that involves new challenges and dangers associated with a novel environment. In this study we evaluated the hypothesis that juveniles and adults exhibit different behavioral and endocrine responses to a novel environment. We also used Fos, the protein product of the immediate early gene *c-Fos*, to evaluate how neural circuits involved in fear, anxiety, and stress respond before and after puberty to the experience of the novel environment.

Methods

Animals

In male grass rats, the pubertal rise in testes weight begins around 26 days of age and is completed by 60 days, and testosterone rises steadily from 26 to 40 days of age (unpublished data). In this study, 25 juveniles (28 days old) and 24 adults (60 days old) were individually housed in polycarbonate cages, provided with *ad libitum* access to food and water and a metal food hopper they could use for shelter (referred to as the “home tube”). Animals were kept on a 12:12 light:dark cycle with lights on at 0600 and tests were conducted and samples collected during the light phase of the cycle, between 1130 and 1430. All experiments were performed in compliance with Michigan State University All-University Committee on Animal Use and Care and in accordance with the standard in the National Research Council *Guide for the Care and Use of Laboratory Animals*.

Behavioral testing

On the experimental day, animals were tested using a modified version of the defensive-withdrawal test. At the time of testing, 12 juvenile and 12 adult males were removed from their cages and placed in a novel environment (10 gallon glass aquarium). This transfer was completed by picking up the animals in their home tubes, which were then placed in the novel arena; animals thus had a “secure base” from which to explore the new environment. After being placed in the testing arena, each animal was observed for 10 minutes, during which we recorded the latency to emerge from the home tube, the time spent exploring the novel

environment, and the number of entries back into the home tube. In addition, the number of fecal boli left by each animal was recorded.

After completion of the observation session, each animal remained in the novel environment for an additional 50 min (total of 60 min). We chose to keep the animals in the novel environment so that they would not be handled again prior to euthanasia. This interval was chosen because previous studies have indicated Fos peaks at least 60 min after the onset of the behaviors with which it is associated [6,7]. At this time, each experimental animal was euthanized with an injection of 0.5 ml sodium pentobarbital (i.p.). Blood samples were collected via cardiac puncture into heparinized tubes. Plasma was collected after centrifugation and frozen for later use. Control animals (13 juveniles and 12 adults) that remained undisturbed in their home cages through the test period were euthanized, sampled, and perfused alongside experimental animals.

Hormone levels

Plasma levels of corticosterone and testosterone were measured in each blood sample. Corticosterone was measured using a radioimmunoassay (RIA) kit developed for lab rats (Diagnostics Products Corp, Los Angeles, CA). In the laboratory rat, levels of corticosterone secretion typically undergo a robust circadian rhythm with peak values occurring during the active phase of the day; this appears to be true in grass rats as well, as plasma corticosterone taken from these animals at ZT 22 is higher than from animals sampled at ZT 14 (unpublished data). Testosterone was quantified in plasma samples using the Coat-A-Count Total Testosterone RIA kit developed for humans. Previous work in our laboratory has validated the use of this assay for testosterone measurement in rodents – specifically the Syrian hamster [8]. For both corticosterone and testosterone assessments, all samples were run together in a single assay and the intrassay coefficients of variation (CV) were calculated from internal controls run in duplicate. The intrassay CVs were 9.2% and 22.7% for corticosterone and testosterone. The limits of detectability were 0.1 ng/ml for testosterone and 20 ng/ml for corticosterone.

Fos expression

After blood sampling, each animal was transcardially perfused with 0.9% saline (150 ml, room temperature) followed by 4% paraformaldehyde in saline (150 ml, chilled). After perfusions, brains were removed and placed in the fixative for 1 hour, then transferred to a 20% sucrose solution. After saturation, each brain was sectioned through the preoptic area, hypothalamus, and amygdala at 30 μ m using a freezing microtome.

Every fourth section was processed for immunocytochemistry (ICC) for Fos. Brain sections were rinsed 6 times (10 min rinses) in phosphate buffered saline (PBS), then incubated in 5% normal goat serum (NGS; Vector) in PBS with 0.3% Triton-X (PBS-tx) for 80 min. After 3 10-min rinses in PBS, brain sections were incubated in the primary antiserum, rabbit anti-Fos (Santa Cruz) at a concentration of 1:2000 in PBS-tx and 3% NGS with rotation for 18 hours at 4°C. After 3 rinses, tissue was placed in the secondary antiserum, goat anti-rabbit (Vector) at a concentration of 1:200 in PBS-tx and 3% NGS for 80 min. The sections were then rinsed and incubated in the avidin-biotin complex (Vector Elite ABC kit) at a concentration of 10 μ l/ml of each of the avidin and biotin reagents in PBS for 110 min. The sections were rinsed before treatment with the chromagen, 3,3'-diaminobenzidine (DAB; Sigma DAB tablets). Six 10 mg DAB tablets and 1200 μ l nickel chloride (500 mM) were dissolved into 480 ml Tris buffer, and 960 μ l of 30% hydrogen peroxide was added to this solution. The sections were allowed to incubate for 5 min. The tissue was rinsed and mounted onto gelatin-coated slides. The slides were then dehydrated, delipidated, and affixed with coverslips using Permount (Fisher).

Fos-immunoreactive (Fos+) cell numbers were analyzed using an Olympus BX 60 light microscope (40X objective). The number of Fos+ cells in each brain region was counted using an optical grid (250 μm $\times$ 250 μm) placed over the brain region. Subnuclei of the amygdala were analyzed bilaterally, including the central amygdala (Ce), and the anterior and posterior portions of the medial amygdala (MeA and MeP). Cells in the lateral septum (LS) were counted by placing the grid over the “crescent septal nucleus” described in lab rats [9]. The cingulate cortex, paraventricular nucleus of the hypothalamus (PVN, parvocellular region), paraventricular nucleus of the thalamus (PVT; anterior, middle, and posterior PVT were each sampled), and centromedial nucleus of the thalamus (CMT) were also analyzed for Fos+ cell number. Fos+ cell number was counted bilaterally in 2 sections for each brain region, and averages of the two sections from each animal were analyzed. These brain regions were chosen for Fos analysis because (1) they have been shown previously to be responsive to stressors and novelty, (2) we can compare the effects of novelty found here with data from other species describing age-related changes in Fos induction, or (3) these areas are important in the behavioral and hormonal responses to stressors; one additional area, the Ce, was included as a negative control [9-19].

Statistical analyses

Behavioral data from the juveniles and adults were compared with t-tests. Hormone levels and counts of Fos+ cells were subjected to a 2-way analysis of variance (ANOVA), with age (juvenile vs. adult) and testing condition (novel environment vs. control) as the independent variables. Differences between individual groups were determined using a Fisher's PLSD test. Differences were considered statistically significant if $p < 0.05$.

Results

Behavioral testing

No differences were found between adult and juvenile grass rats in their response to a novel environment over 10 minutes (for latency to emerge, $t_{(22)}=0.012$, $p=0.916$; for duration of exploration, $t_{(22)}=-1.001$, $p=0.328$; for number of entries, $t_{(22)}=0.484$, $p=0.633$, and for fecal boli, $t_{(22)}=0.139$, $p=0.891$; all tests were two-tailed). The latency to emerge from the home tube, duration of time spent exploring the novel environment, number of entries back into the home tube, and the number of fecal boli found in the testing arena in juvenile and adult grass rats are presented in Figure 1.

Hormone levels

Mean plasma testosterone concentration was significantly higher in adults compared to juveniles ($F_{(1,41)}=13.616$, $p=0.0007$), but was not influenced by the novel environment ($F_{(1,41)}=0.582$, $p=0.4499$) or by an interaction between these variables ($F_{(1,41)}=0.053$, $p=0.8191$) (see Figure 2). Plasma corticosterone was affected by age ($F_{(1,39)}=8.798$, $p=0.0051$) and environment ($F_{(1,39)}=4.949$, $p=0.0320$), but not by an interaction between these factors ($F_{(1,39)}=2.148$, $p=0.1475$). Post-hoc tests revealed that among juveniles, exposure to the novel environment caused a significant increase in plasma corticosterone concentrations (see Figure 2).

Fos expression

There were four general patterns of effects of age and testing condition on Fos expression in the different brain regions analyzed. First, in one region, the CeA, no effects of novelty or age were seen, nor was there a significant interaction between these two variables ($F_{(1,44)}=0.038$ for novelty, 6.322 for age, and 0.189 for the interaction, $p<0.05$; Figure 3). Second, in three brain regions (MeP, PVN, and PVT), Fos levels increased in response to novelty in juveniles

but not in adults (Figures 3 and 4). In the MeP, the ANOVA showed a significant interaction between environment and age ($F_{(1,44)}=5.123, p<0.05$), as well as main effects of both age ($F_{(1,44)}=4.423, p<0.05$), and environment ($F_{(1,44)}=15.084, p<0.001$). Specifically, the novel environment significantly increased Fos+ cell number in the MeP in the juvenile but not the adult animals. The same pattern of results was seen for the PVN, where the ANOVA revealed a significant interaction between the factors ($F_{(1,43)}=4.678, p<0.05$), as well as main effects of age ($F_{(1,43)}=10.352, p<0.01$) and novel environment ($F_{(1,44)}=12.646, p<0.001$). In the PVT, significant main effects of both environment ($F_{(1,44)}=12.334, p<0.001$) and age ($F_{(1,43)}=20.856, p<0.0001$) were seen, with no significant interaction between the factors ($F_{(1,43)}=1.269, p>0.05$). Among juveniles, exposure to the novel environment caused a significant increase in Fos expression in the PVT ($p<0.01$), but no difference was seen among the adult animals ($p>0.05$).

In a third set of brain regions (the LS, cingulate cortex, and CMT), baseline levels of Fos were the same in juveniles and adults and Fos rose significantly in both groups, but juveniles exposed to the novel environment showed more Fos expression compared to their adult counterparts. In the cingulate cortex, there was a significant interaction between age and environment on Fos+ cell number ($F_{(1,44)}=9.266, p<0.0001$) and there were main effects of both age ($F_{(1,44)}=9.364, p<0.05$) and novel environment ($F_{(1,44)}=9.266, p<0.05$). Specifically, novelty increased Fos+ cell number in the cingulate region in both juveniles and adults, but the increase was greater in the juvenile grass rats (juveniles vs. adults in the novel environment: $t=4.61, p<0.001$). A similar effect was seen in the LS: a significant interaction ($F_{(1,44)}=6.752, p<0.05$), as well as significant main effects of environment ($F_{(1,44)}=95.985, p<0.0001$) and age ($F_{(1,44)}=15.929, p<0.001$) were found. The novel environment increased Fos expression in the LS in both juveniles and adults, but the increase was to a higher level in the juveniles than in the adults (juveniles vs. adults in the novel environment: $t=4.76, p<0.001$). In the CMT, a significant interaction was also seen between the factors ($F_{(1,44)}=4.432, p<0.05$), as well as significant main effects of age ($F_{(1,44)}=9.828, p<0.01$) and novel environment ($F_{(1,44)}=25.505, p<0.0001$). Again, novel environment significantly increased Fos+ cell number in the CMT in both groups, and there were significantly more Fos+ cells in the juvenile animals compared to the adults exposed to a novel environment (juveniles vs. adults in the novel environment: $t=3.79, p<0.001$).

Finally, increases in Fos expression after novelty were equivalent in juvenile and adult grass rats in one region, the MeA. Here, the number of Fos+ cells increased with novelty ($F_{(1,44)}=37.818, p<0.0001$). Age had no significant effect on Fos+ cell number ($F_{(1,44)}=0.181, p>0.05$), and no significant interaction was found ($F_{(1,44)}=2.126, p>0.05$). Fos+ cell number was increased after exposure to the novel environment similarly in juvenile and adult animals (see Figures 3 and 4).

Discussion

Behavior and hormones

The pattern of results obtained from this study shows that juvenile and adult grass rats were indistinguishable with respect to behavioral indices of stress, but were quite different with respect to neural and endocrine responses to a novel environment. Juveniles and adults took the same amount of time to emerge from shelter when placed in a novel environment and also to explore it, and they returned to their shelters with similar frequencies. In addition, fecal pellets, indicative of stress, were produced at equal rates. This pattern differs from that seen in lab rats and mice, in which fearful behaviors in a novel environment are higher in adults than in younger animals [1,2,20,21]. The differences in the behavior patterns seen between grass rats and the other two species could reflect differences in methodology. Whereas the earlier studies used either the social interaction test or the elevated plus maze to evaluate exploratory

behavior, we examined such behavior in a defensive-withdrawal test where animals were placed in a novel glass aquarium with a familiar enclosure from their home cage. Using the defensive-withdrawal test, Parfitt et al. (2004) [2] found that juvenile C57BL/6 mice emerged into a novel environment more rapidly than their adult counterparts. In the current experiment with grass rats, however, the defensive-withdrawal test was conducted with a familiar shelter with all of the odors of the home cage; an unfamiliar shelter was used in the earlier study with mice. The familiarity of the shelter may have helped buffer the effect of the novel environment on the behavior. Alternatively, the differences may reflect some feature of the natural history of these animals associated with the nature of the challenges with which young and old animals are typically confronted.

While behavior was indistinguishable between the two age groups, hormones were not the same. Levels of circulating testosterone were higher in adult compared to young grass rats, as expected, and were not affected in either group by the environmental manipulation. In contrast, patterns of corticosterone were quite different. Baseline levels of corticosterone were the same in juveniles and adults, but corticosterone increased in response to the novel environment in juvenile but not adult male grass rats (Fig. 1). This age difference could reflect a difference either in the threshold at which a novel environment elicits an adrenal response, or in the time course of the response. For example, both juvenile and adult animals may have experienced an immediate rise in corticosterone, but the hormone may have then declined to baseline levels within the hour among adults but not juveniles. An age difference in termination of the stress response occurs in lab rats, in which prepubertal and adult animals respond to 30 minutes of restraint stress with a similar initial rise in corticosterone but the hormone remains high for the next 60 minutes in juveniles, while it drops to baseline levels by 30 minutes in adults [22]. In one other study of restraint stress in lab rats, however, no difference in time course of the stress response between pre-and post-pubertal animals was observed [23]. The heightened level of adrenal activity seen here in juvenile compared to adult grass rats suggests that although their behavior did not differ in the novel environment, the juveniles became physiologically more prepared for a potential threat. Perhaps this difference in the coupling between behavior and physiology reflects the fact that while young animals are more vulnerable and less experienced than older ones, they must nevertheless explore environments unfamiliar to them. The heightened glucocorticoid levels presumably help prepare them for threats they might encounter.

Fos expression

Exposure to a novel environment did not elicit a Fos response in the CeA of either juveniles or adults, indicating that Fos responses observed in other brain regions were not simply a reflection of generalized activation of the brain by the novel environment. In addition, previous work has shown greater Fos induction within the CeA in response to acute physical stressors such as hemorrhage or immune challenge compared with psychological stressors such as restraint [10,24]. Therefore, the absence of an increase in the number of Fos-ir cells within this brain region in response to the relatively mild stressor used here is not surprising. In most other brain regions examined, however, Fos was elevated in response to the stressor, and juvenile males had a greater response compared to adults. Specifically, the unfamiliar environment induced an increase in Fos in juveniles but not in adults in the MeP, PVN, and PVT. In three other areas--the cingulate cortex, LS, and CMT--the novel environment induced an increase in Fos in both age groups, but to a greater extent in juveniles compared to adults. In the MeA, Fos was induced to a similar extent in the two groups. This overall pattern of results demonstrates that the Fos response to the novel environment, like the hormonal stress response, is greater in juveniles than in adults, even though the behavioral response of juveniles is similar to that of adults.

Ours is not the first study to compare Fos-immunoreactivity in response to stress in juvenile and adult animals, and many investigators have examined the stress response in adolescence (reviewed in McCormick and Matthews, 2006,[25]). In rats, Kellogg et al. (1998) [11] have shown that in adult males Fos increases in numerous brain regions after 15 or 120 min of restraint stress whereas stress-induced Fos expression in juvenile males was primarily constrained to the parvocellular paraventricular nucleus; in that study, however, no differences were observed between juveniles and adults[11]. In contrast, Viau et al., (2005) [26] reported increased Fos expression in the parvocellular PVN in 30 compared to 60 day old male rats in response to 30 min of restraint. Similarly, Romeo et al. (2006) [27] have recently demonstrated increased Fos expression in the PVN in juvenile compared to adult male rats following 30 min of restraint stress. Therefore, our results in the grass rat indicating juvenile males have increased neuronal activation after stress compared to adults appear consistent with the patterns seen in the laboratory rat.

A role for the PVT in the mediation of the stress response has been supported by many types of evidence [28-30], and both the PVT and the CMT show an increase in Fos expression after exposure to stressful stimuli in adult lab rats [13,16,31]. This was the case for the CMT in both juvenile and adult grass rats, and in the PVT for juveniles only. The PVT may communicate stress-related signals through its direct projections to the amygdala, a structure critical for the behavioral effects of environmental challenges that induce fear [16]. The heightened responsiveness of the PVT to stress in juvenile relative to adult grass rats raises the possibility that this structure provides different patterns of signals to the amygdala, especially the MeP, in the two groups of animals when they are placed into a novel environment. The two other regions of the amygdala examined here showed different patterns of Fos from that observed in the MeP. The MeA showed increases in Fos expression after the novel environment procedure in both adults and juveniles, while, as noted above, the CeA did not respond to the challenge in either adults or juveniles. Patterns of Fos expression within the different regions of the amygdala in response to a stressor in grass rats were similar in some ways to those seen in adult lab rats. Specifically, as was the case for grass rats, in lab rats the medial amygdala shows extensive Fos expression after a wide variety of stressful procedures [9,31] whereas the central nucleus of the amygdala shows minimal Fos expression in response to an acute physiological stressor in adult rats [9,10,31].

The cingulate cortex and lateral septum are both thought to be involved in emotional regulation [32], and Fos is induced in both of these regions in adult lab rats exposed to stressful stimuli [9,31]. In grass rats, the novel environment led to increases in Fos expression in these regions in both adults and juveniles, but the increase was greater in juveniles (Figure 3). These data provide further evidence that our manipulation does indeed heighten activity of brain regions involved in anxiety and fear. The patterns also suggest that although activity in the cingulate cortex and the septum is increased by exposure to a novel environment in both groups of animals, these regions are more responsive in the younger animals.

General conclusions

This experiment demonstrates that a novel environment can have a greater effect on hormonal and neural indices of stress in juvenile grass rats than in adult grass rats, even when their behavioral responses do not differ. The behavioral patterns seen here may reflect the fact that although young animals are more vulnerable than older ones, they must nevertheless develop exploratory behavior patterns that will enable them to obtain information about their environments. On the other hand, the differences in their neural and endocrine responses may reflect mechanisms that prepare the younger animals to face situations that are a greater threat to them than to adults. It is important to note that there may be other contexts that would represent a greater danger to adults, and would elicit a heightened response in them [1]. The

developmental change in the responsiveness of these neural systems to contexts that represent varying degrees of threat to young and old animals remains an important issue for future investigation.

Acknowledgements

This work was funded by NIH HD26483 and NSF IBN96-02169 to CLS, NIMH MH53433 to LS, and NRSA MH12956 to CMN. The authors thank Terri McElhinny for technical assistance, and Matthew Grober and Michael Black for the use of their computer and imaging equipment.

References Cited

1. Primus RJ, Kellogg CK. Pubertal-related changes influence the development of environment-related social interaction in the male rat. *Dev Psychobiol* 1989;22(6):633–43. [PubMed: 2792573]
2. Parfitt DB, Levin JK, Saltstein KP, Klayman AS, Greer LM, Helmreich DL. Differential early rearing environments can accentuate or attenuate the responses to stress in male C57BL/6 mice. *Brain Res* 2004;1016(1):111–8. [PubMed: 15234259]
3. Smale L, Nunes S, Holekamp KE. Sexually dimorphic dispersal in mammals: patterns, causes and consequences. *Advances in the Study of Behavior* 1997;26:181–250.
4. Blanchong JA, McElhinny TL, Mahoney MM, Smale L. Nocturnal and diurnal rhythms in the unstriped Nile rat, *Arvicanthis niloticus*. *J Biol Rhythms* 1999;14(5):364–77. [PubMed: 10511004]
5. Packer C. Demographic changes in a colony of Nile grass rats (*Arvicanthis niloticus*) in Tanzania. *Journal of Mammology* 1982;64:159–161.
6. Parfitt DB, Newman SW. Fos-immunoreactivity within the extended amygdala is correlated with the onset of sexual satiety. *Horm Behav* 1998;34(1):17–29. [PubMed: 9735225]
7. Sagar SM, Sharp FR, Curran T. Expression of c-fos protein in brain: metabolic mapping at the cellular level. *Science* 1988;240(4857):1328–31. [PubMed: 3131879]
8. Parfitt DB, Thompson RC, Richardson HN, Romeo RD, Sisk CL. GnRH mRNA increases with puberty in the male Syrian hamster brain. *J Neuroendocrinol* 1999;11(8):621–7. [PubMed: 10447800]
9. Duncan GE, Knapp DJ, Breese GR. Neuroanatomical characterization of Fos induction in rat behavioral models of anxiety. *Brain Res* 1996;713(12):79–91. [PubMed: 8724978]
10. Dayas CV, Buller KM, Day TA. Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. *Eur J Neurosci* 1999;11(7):2312–22. [PubMed: 10383620]
11. Kellogg CK, Awatramani GB, Piekut DT. Adolescent development alters stressor-induced Fos immunoreactivity in rat brain. *Neuroscience* 1998;83(3):681–9. [PubMed: 9483552]
12. Wiedenmayer CP, Barr GA. Developmental changes in c-fos expression to an age-specific social stressor in infant rats. *Behav Brain Res* 2001;126(12):147–57. [PubMed: 11704260]
13. Chastrette N, Pfaff DW, Gibbs RB. Effects of daytime and nighttime stress on Fos-like immunoreactivity in the paraventricular nucleus of the hypothalamus, the habenula, and the posterior paraventricular nucleus of the thalamus. *Brain Res* 1991;563(12):339–44. [PubMed: 1786549]
14. Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, Cullinan WE. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol* 2003;24(3):151–80. [PubMed: 14596810]
15. Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci* 1997;20(2):78–84. [PubMed: 9023876]
16. Peng ZC, Grassi-Zucconi G, Bentivoglio M. Fos-related protein expression in the midline paraventricular nucleus of the rat thalamus: basal oscillation and relationship with limbic efferents. *Exp Brain Res* 1995;104(1):21–9. [PubMed: 7621938]
17. Martinez M, Phillips PJ, Herbert J. Adaptation in patterns of c-fos expression in the brain associated with exposure to either single or repeated social stress in male rats. *Eur J Neurosci* 1998;10(1):20–33. [PubMed: 9753110]
18. Emmert MH, Herman JP. Differential forebrain c-fos mRNA induction by ether inhalation and novelty: evidence for distinctive stress pathways. *Brain Res* 1999;845(1):60–7. [PubMed: 10529444]

19. Radulovic J, Kammermeier J, Spiess J. Relationship between fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure. *J Neurosci* 1998;18(18):7452–61. [PubMed: 9736664]
20. Brand T, Slob AK. Peripubertal castration of male rats, adult open field ambulation and partner preference behavior. *Behav Brain Res* 1988;30(2):111–7. [PubMed: 3166710]
21. Macri S, Adriani F, Chiarotti F, Laviola G. Risk taking behavior during exploration of a plus-maze is greater in adolescent than in juvenile or adult mice. *Animal Behaviour* 2002;64:541–546.
22. Romeo RD, Lee SJ, Chhua N, McPherson CR, McEwen BS. Testosterone cannot activate an adult-like stress response in prepubertal male rats. *Neuroendocrinology* 2004;79(3):125–32. [PubMed: 15103225]
23. Choi S, Kellogg CK. Adolescent development influences functional responsiveness of noradrenergic projections to the hypothalamus in male rats. *Brain Res Dev Brain Res* 1996;94(2):144–51.
24. Dayas CV, Buller KM, Crane JW, Xu Y, Day TA. Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. *Eur J Neurosci* 2001;14(7):1143–52. [PubMed: 11683906]
25. McCormick C, Mathews I. HPA function in adolescence: Role of sex hormones in its regulation and the enduring consequences of exposure to stressors. *Pharmacology, Biochemistry and Behavior*. 2006in press
26. Viau V, Bingham B, Davis J, Lee P, Wong M. Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat. *Endocrinology* 2005;146(1):137–46. [PubMed: 15375029]
27. Romeo RD, Karatsoreos IN, McEwen BS. Pubertal maturation and time of day differentially affect behavioral and neuroendocrine responses following an acute stressor. *Horm Behav* 2006;50(3):463–8. [PubMed: 16836997]
28. Bhatnagar S, Viau V, Chu A, Soriano L, Meijer OC, Dallman MF. A cholecystokinin-mediated pathway to the paraventricular thalamus is recruited in chronically stressed rats and regulates hypothalamic-pituitary-adrenal function. *J Neurosci* 2000;20(14):5564–73. [PubMed: 10884340]
29. Heilbronner U, van Kampen M, Flugge G. The alpha-2B adrenoceptor in the paraventricular thalamic nucleus is persistently upregulated by chronic psychosocial stress. *Cell Mol Neurobiol* 2004;24(6):815–31. [PubMed: 15672682]
30. Spencer SJ, Fox JC, Day TA. Thalamic paraventricular nucleus lesions facilitate central amygdala neuronal responses to acute psychological stress. *Brain Res* 2004;997(2):234–7. [PubMed: 14706875]
31. Cullinan WE, Herman JP, Battaglia DF, Akil H, Watson SJ. Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience* 1995;64(2):477–505. [PubMed: 7700534]
32. Heimer L, Van Hoesen GW. The limbic lobe and its output channels: Implications for emotional functions and adaptive behavior. *Neurosci Biobehav Rev*. 2005

Figure 1.

Fear-related behavioral measures in response to exposure to a novel environment. No changes were seen between juvenile and adult grass rats in the latency to emerge from shelter, duration of time spent in the open field, number of entries back into the shelter, or the number of fecal boli found in the arena.

Figure 2.

Serum levels of testosterone and corticosterone (ng/ml, mean $\pm$ SEM) in adult and juvenile grass rats. Testosterone was higher in adults compared to juveniles (* $p=0.0007$) and no differences were seen between control animals and those that experienced a novel environment. Exposure to the novel environment significantly increased serum corticosterone levels, but only in the juveniles (** $p<0.05$).

Figure 3.

Mean ($\pm$ SEM) numbers of Fos+ cells in brain regions in juvenile and adult grass rats under control conditions and after exposure to the novel environment; group means are based on averages of counts from the two sections analyzed for each animal. Numbers of Fos+ cells in the central amygdala (CeA) were unaffected by exposure to novel environment or by age. In the anterior portion of the medial amygdala (MeA), Fos expression was significantly greater in both juvenile and adult grass rats exposed to the novel environment compared to controls. In the cingulate cortex lateral septum (LS), and centromedial nucleus of the thalamus (CMT), Fos expression was higher in both juveniles and adults exposed to the novel environment, compared to controls, but the number of Fos+ cells was significantly greater in the juveniles compared to the adults. In the posterior portion of the medial amygdala (MeP), paraventricular nucleus of the hypothalamus (PVN), and paraventricular nucleus of the thalamus (PVT), novelty increased Fos expression only in juvenile grass rats. *significantly different from adult controls ($p < 0.05$); **significantly different from both the juvenile controls and from adults exposed to novel environment ($p < 0.05$).

Figure 4.

Photomicrographs from representative animals of Fos expression in the paraventricular nucleus of the hypothalamus (PVN, top row), the medial amygdala (MeA, middle row), the paraventricular thalamic nucleus (PVT, bottom row), and the centromedial thalamic nucleus (CMT, bottom row). 3V, third ventricle; oc, optic chiasm; bar = 200 μm ; box = 250 $\mu\text{m} \times 250 \mu\text{m}$.