Published in final edited form as:

Horm Behav. 2013 April ; 63(4): 646–658. doi:10.1016/j.yhbeh.2013.01.010.

Sex-dependent role of the amygdala in the development of emotional and neuroendocrine reactivity to threatening stimuli in infant and juvenile rhesus monkeys

Jessica Raper^{a,b,*}, Kim Wallen^{a,b}, Mar M. Sanchez^{b,c}, Shannon B. Z. Stephens^{a,b}, Amy Henry^b, Trina Villareal^b, and Jocelyne Bachevalier^{a,b}

Jessica Raper: jraper@emory.edu; Kim Wallen: kim@emory.edu; Mar M. Sanchez: mmsanch@emory.edu; Shannon B. Z. Stephens: ssteph3@emory.edu; Amy Henry: amyrfh@gmail.com; Trina Villareal: tjone24@emory.edu; Jocelyne Bachevalier: jbachev@emory.edu

^aDepartment of Psychology, Emory University, 36 Eagle Row, Atlanta GA 30322

^bYerkes National Primate Research Center, 954 Gatewood Rd NE, Atlanta, GA 30329

^cDepartment of Psychiatry & Behavioral Sciences, Emory University, 101 Woodruff Circle, WMB suite 4000, Atlanta GA 30322

Abstract

Amygdala dysfunction and abnormal fear and stress reactivity are common features of several developmental neuropsychiatric disorders. Yet, little is known about the exact role the amygdala plays in the development of threat detection and emotional modulation. The current study examined the effects of neonatal amygdala lesions on defensive, emotional, and neuroendocrine reactivity of infant rhesus monkeys reared with their mothers in large species-typical social groups. Monkeys received either bilateral MRI-guided ibotenic acid amygdala (Neo-A; $n = 16$) or sham (Neo-C; $n = 12$) lesions at 24.8 ± 1.2 days of age, or served as behavioral control (Neo-BC; $n = 3$). Defensive and emotional responses were assessed using the Human Intruder Paradigm as infants and as juveniles (2.5 and 12 months of age, respectively), whereas neuroendocrine reactivity was only examined during the juvenile period. As infants, Neo-A animals expressed similar levels of freezing and hostile behaviors as compared to controls, whereas during the juvenile period Neo-A animals expressed significantly less freezing compared to controls. Interestingly, the sex of the infant modulated the behavioral effects of neonatal amygdectomy, leading to different patterns of behavior depending on the sex and lesion status of the infant. Unlike controls, Neo-A infants did not modulate their behavioral responses based on the salience of the threat. The impact of neonatal amygdectomy increased with age, such that Neo-A juveniles exhibited fewer emotional behaviors and increased cortisol response to the stressor as compared to controls. These data indicate that the amygdala plays a critical role in the development of both emotional and neuroendocrine reactivity as well as the expression of sexually dimorphic emotional expression.

© 2012 Elsevier Inc. All rights reserved.

*Corresponding author: Jessica Raper, Ph.D., Yerkes National Primate Research Center, 954 Gatewood Rd NE, Atlanta, GA 30329, jraper@emory.edu, Phone: 404-727-8334.

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.

Keywords

sex difference; amygdala; emotion; cortisol; testosterone

Studies in adult rodents, monkeys, and humans have shown that the amygdala plays a critical role in detecting potential dangers and modulating ones behavioral and physiological responses to threats encountered (Aggleton, 2000). In adult monkeys, amygdala lesions had conflicting effects on emotional reactivity. In some studies amygdectomy reduced freezing, fearful, and hostile behaviors toward an unfamiliar conspecific or human (Meunier, et al., 1999; Kalin, et al., 2001, 2004; Machado & Bachevalier, 2008), whereas other studies using unfamiliar humans as stimuli reported no effects of lesions (Kalin, et al., 2001; Izquierdo, et al., 2005). Additionally, studies in adult monkeys and rodents have also demonstrated that the amygdala has an excitatory influence on the neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis stress response through indirect projections to the paraventricular nucleus of the hypothalamus (Mason, et al., 1959; Kalin, et al., 2004; Machado & Bachevalier, 2008; see review Herman, et al, 2003; Myers, et al., 2012). However, in those studies, the amygdala, hypothalamus and their connections are already mature at the time of the experimental manipulation, leaving open the question of the role of the amygdala in the development of threat detection, and modulation of emotional and HPA axis responses towards potential threats.

In rhesus monkeys, the amygdala has a protracted development, increasing in volume from birth to 2 years of age (Payne, et al., 2010; Chareyron, et al., 2012), a period coinciding with behavioral development and increased social competency. For example, infant rhesus monkeys appear to lack fear and defensive behaviors for about two-three months following birth (Mendelson, 1982; Mendelson, Haith, & Golman-Rakic, 1982; Kalin, Shelton, & Takahashi, 1991) with context-appropriate responses to social signals and threats emerging during this refinement of cortical-amygdala projections (Freese & Amaral, 2009) and increased myelination of amygdala connections (Chareyron, et al., 2012). Few studies have examined the role of the amygdala in the development of threat detection and emotional modulation. These studies have shown that early amygdala damage yields abnormal threat detection and inappropriate reactivity to objects and social partners (Thompson, 1981; Prather, et al., 2001; Bauman, et al., 2004; Bliss-Moreau, et al., 2010; Raper, et al., 2012a). Another study has also investigated HPA axis reactivity to stress (Raper, et al., 2012a) using the Human Intruder paradigm in surrogate peer-reared rhesus monkeys with neonatal amygdala lesions. These data showed that animals with neonatal amygdectomies expressed the species-typical defensive behaviors (i.e., freezing or hostility), but lacked the ability to appropriately modulate those behaviors based on the level of threat posed by the human intruder. The magnitude of this effect increased with age. Additionally, neonatal amygdala lesions resulted in a blunted cortisol response to the intruder stressor. These data suggest that the amygdala is essential to adjust animals' behavioral and neuroendocrine responses according to the level of threat presented by the intruder. However, the examination of stress reactive HPA axis response was only examined in adulthood (Raper, et al., 2012a), long after the damage to the amygdala, leaving open the question of the amygdala's influence on the HPA axis during juvenile development.

The amygdala is a sexually dimorphic structure varying in size and in the distribution of androgen receptors in males and females (Pomerantz & Sholl, 1987; Micheal, Rees, & Bonsall, 1989; Abdelgardir, et al., 1999). Thus, the amygdala is potentially an important brain structure in the expression of sexually dimorphic behaviors and neuroendocrine function (McClure, et al., 2004; Zosuls, et al., 2009; Saint-Maurice, et al., 2011). A few animal studies have investigated sex differences in threat detection, indicating elevated

fearful and hostile behavior expression in females compared to males (Mason, et al., 1960), but no sex difference on the duration of freezing (Kalin, et al., 1998) in response to an unfamiliar human. Most studies examining sex differences the emotional behaviors of rhesus monkeys have been conducted after puberty when gonadal hormones are high in circulation (Mason, et al., 1960; Hadidian, 1980; Troisi, et al., 1990). However, sex differences in other behaviors (e.g. play, mounting) have been found before puberty, when the hypothalamic-pituitary-gonadal (HPG) axis is quiescent (Wallen, 1996, 2005). Therefore, the expression of behavioral sex differences may be under the activational influence of circulating gonadal hormones for some behaviors but not others.

Infant male rhesus monkeys undergo a transient activation of the HPG axis from birth to four months of age, called the postnatal testosterone (T) surge (Robinson & Bridson, 1978; Mann, et al., 1989). It is unknown if circulating T levels during the postnatal T surge influence the expression of behavioral sex differences during the Human Intruder paradigm. Additionally, no studies have examined how the sexes may differ in the effects of early amygdala damage. Thus, the current study examined the effects of neonatal neurotoxic amygdala lesions on threat detection, modulation of defensive behaviors and HPA axis stress reactivity in both male and female rhesus monkeys. Animals were tested in infancy during the postnatal T surge (at 2.5 months), and again during the juvenile period (at 12 months) when the T surge had subsided, but much prior to puberty. We also assessed how this infant surge in gonadal hormones or gonadal quiescence during the juvenile period affected sex-dimorphisms in emotional responses. Lastly, the monkeys in this study were reared by their mothers in a large multimale, multifemale age-graded social groups consisting of 85 to 100 group members. Some behavioral sex differences are only evident under more limited social rearing conditions (Wallen, 1996), thus it was possible that our animals would show no difference in responsiveness to the intruder, as a result of their highly enriched social experience.

Methods

Subjects

Thirty-one infant rhesus monkeys (*Macaca mulatta*) from middle-ranking multiparous mothers, which were living in long term age-graded groups of monkeys whose social structure duplicated that seen in naturally occurring populations were used in this study. The mothers rank in the social hierarchy was defined through a combination of historical records and observations of agonistic interactions, and rank categories were defined by the proportion of matriline that fell within the upper, middle, or lower third of the hierarchy. Mother-infant pairs and other members of the social groups lived in large outdoor compounds (38m × 39 m) with attached heated and cooled indoor areas at the Yerkes National Primate Research Center (YNPRC) Field Station (Lawrenceville, GA) of Emory University. Mother-infant pairs were temporarily removed from this social group 3 days prior to surgical procedures and remained out of the social group for 8 to 14 days before being reintroduced. Subjects received neonatal neurotoxic lesions of the amygdala (Neo-A; males = 9, females = 7), and sham operations (Neo-C; males = 6, females = 6) at an average of 24.8 ± 1.2 days of age, or served as behavioral control (Neo-BC; males = 2, females = 1). All neuroimaging and surgical procedures were performed at the YNPRC Main Station (Atlanta, GA) and have been described in detail elsewhere (Raper, et al., 2012b) and will be briefly summarized below. Subjects' emotional reactivity to a Human Intruder paradigm (Kalin, et al., 1991) was assessed at 2.5 months and 12 months of age.

Imaging and surgical procedures

Magnetic Resonance Imaging (MRI) Procedure—The day of surgery, infants were removed from their mother, sedated (Ketamine hydrochloride, 100mg/ml), intubated, anesthetized with isoflurane (1–2% to effect), their head was shaved and secured in a nonferromagnetic stereotaxic apparatus. Infants received an intravenous drip of 0.45% dextrose and sodium chloride to maintain hydration and vital signs (heart rate, respirations, blood pressure, expired CO₂) were monitored throughout the procedures. Two MRI sequences were obtained using a Siemens 3.0T/90 cm whole body scanner and a 3" circular surface coil. First, a T1-weighted scan (spin-echo sequence, echo time [TE] = 11ms, repetition time [TR] = 450ms, contiguous 1mm section, 12cm field of view [FOV], 256×256 matrix) was acquired in the coronal plane and used to determine the coordinates of injection sites in the amygdala. Additionally, three fluid attenuated inversion recovery (FLAIR) scans (3D T2-weighted fast spoiled gradient [FSPGR]-echo sequence, TE = 2.6ms, TR = 10.2ms, 25° flip angle, 12 cm FOV, 256×256 matrix) were obtained in the coronal plane at 3.0 mm (each offset of 1 mm posteriorly) throughout the brain. These MR sequences were repeated for Neo-A animals 7–10 days after the surgical procedure, to accurately localize the areas of edema and estimate the extent of lesion.

Surgical Procedure—After imaging, the infants were kept anesthetized and brought to the surgical suite where they were prepared for aseptic surgical procedures. The scalp was disinfected with Nolvasan solution and a local anesthetic (Bupivacaine 0.25% concentration, 1.5ml) was injected subcutaneously along the midline to reduce the pain during skin incision. The skin and connective tissue were gently displaced laterally, two small bilateral craniotomies were made in front of bregma and above the amygdala, and the dura was cut and retracted to expose the brain.

Neo-A animals received injections of ibotenic acid (PH 7.8–7.9, 10 mg/ml concentration) in 6–8 sites within the center of the amygdala using 10µl Hamilton syringes. Needles were lowered simultaneously in both hemispheres and a total of 0.6–0.8 µl of ibotenic acid was manually injected at a rate of 0.2µl/minute. After each injection, needles were left in place for a 3-minute period to minimize the spread of neurotoxin during needle retraction.

At the completion of the injections, the dura was closed with silk sutures, the craniotomies were covered with Surgicel NU-KNIT (absorbable hemostat), and connective tissues and skin were closed. The animal was placed in a temperature controlled incubator ventilated with oxygen until fully recovered from anesthesia. All animals received banamine (1mg/kg for 3 days), dexamethasone (0.5mg/kg for 3 days) and antibiotic (rocephin, 25mg/kg for 7 days) after surgery to prevent pain, edema, and infection, respectively.

Neo-C animals received the same surgical procedures except no needle was lowered and no injections were given. Animals in the behavioral control group (Neo-BC) were not transported to the YNPRC Main Station, but were separated from their mother, sedated (Ketamine hydrochloride, 100mg/ml), their head was shaved, and scalp was disinfected with Nolvasan Solution, but no surgery was performed. They also received the same “post-surgical” medications as did the Neo-C and Neo-A animals. Twenty-four hours after separation they were reunited with their mother and a week later received a follow-up separation as did the Neo-C and Neo-A animals.

Lesion Verification—Estimation of the extent of intended and unintended damage for Neo-A animals was made using pre- and post-surgical MR images (Malkova, et al, 2001; Nemanic, et al, 2002). The T1 images were used to identify the borders of each structure and FLAIR images were used to visually identify extent of hypersignals, which were then plotted onto corresponding coronal drawings from a normalized infant rhesus monkey brain

(J. Bachevalier, unpublished atlas) using Adobe Photoshop software. Image-J® (version 1.44, <http://rsbweb.nih.gov/ij/>) program was used to measure the surface area (in pixels squared) containing hypersignals in amygdala and surrounding structures (entorhinal and perirhinal cortex and hippocampus). The volume of the amygdala damage was then divided by the normal volume of the amygdala (obtained from the template brain in the same manner) and multiplied by 100 to estimate a percentage of the total damage volume. The same procedure was applied to estimate potential damage to structures adjacent to the amygdala.

Lesion Extent

Based on post-surgical MR images, an average of 81.3% amygdala damage was obtained across both hemispheres, but varied from case to case (Table 1). Twelve cases received substantial damage to the amygdala in both hemispheres (right: M = 84.82%; left: M = 92.80%). One case received only moderate bilateral damage (Neo-A-F5: right: 61.6%, left: 58.4%) and two cases had more asymmetrical amygdala damage (Neo-A-F3: right 100%, left 32.2%; Neo-A-M4: right 50.5%, left 84.9%). For one case (Neo-A-F1), the damage was restricted to the right hemisphere (right 82.3%, left 0%). The extent of unintended damage to the perirhinal and entorhinal cortices, anterior portion of the hippocampus, and tail of the putamen were negligible in 13 cases. In two cases, there was moderate damage to the entorhinal cortex in the right hemisphere (Neo-A-F1: 18.3%; Neo-A-F3: 21.8%). Moderate damage to the tail of the putamen was seen in two cases (Neo-A-F5 and Neo-A-F6). Figure 1 illustrates the extent of bilateral amygdala damage in a representative case (Neo-A-M5) as shown by the location and extent of hypersignals seen in the post-surgical FLAIR images.

Human Intruder Paradigm

Thirty subjects (Neo-C n=12; Neo-BC n=2; Neo-A n=16) were tested as infants at 81 ± 10 days of age and given their dependence on their mother at this early age, all mother-infant pairs were trained for quick capture from the social group (see Raper, et al., 2012b). Mothers would carry infants into the indoor area, and once inside, mothers were trained to enter a transfer box with their infant. The pair was then placed into a cage from which the infants could be separated from their mother for behavioral testing. All subjects were re-tested again at 12 months of age, except five subjects (two Neo-C males, one Neo-A female, and two Neo-A males) that were not tested due to illness unrelated to their experimental procedures, and an additional Neo-BC male was tested at this age (see Suppl. Table 1). Thus, only 26 subjects were tested as juveniles (Neo-C n=10; Neo-BC n=3; Neo-A n=13). Juvenile monkeys were independently trained to quickly separate from their social group without their mothers, and once inside they were trained to voluntarily enter a transfer box.

For behavioral testing, at both ages, animals were transported to a novel testing room, and transferred to a stainless steel cage with one wall made of clear plexi-glass to allow video recording. The Human Intruder paradigm lasted 30-min and consisted of three conditions (Alone, Profile [referred to as “No Eye Contact” in other publications], Stare) presented in the same order for all animals. At each age, the experimenter wearing a rubber mask depicting a male face with cuts around the eyes to allow the monkey to view the experimenter’s eyes were used as the Intruder. Different masks were used at each age. Thus, the animal first remained alone in the cage for 9-min (Alone condition), then the intruder entered the room, and sat two meters from the test cage for 9-min while presenting his/her profile to the animal (Profile condition). After the Profile, the intruder left the room and the animal remained in the cage alone for 3-min, after which the intruder re-entered the room and made direct eye contact with the animal for 9-min (Stare condition). Immediately following the test, monkeys were reunited with their mother and social group.

Animals' emotional reactivity during the Human Intruder paradigm was video recorded and later coded using a detailed ethogram (Table 2). Digital videos were coded using the Observer XT program (Noldus, Inc., Netherlands) by one experimenter. Since the experimenter was not blind to the animals' treatment, videos were coded without identifying information about the subject, which was revealed after coding was complete. Cohen's Kappa was used to assess the intra- and inter-rater reliability on four videos. The experimenter had an average intra-rater reliability of Cohen's Kappa = 0.98; and an average intra-rater reliability of Cohen's Kappa = 0.845 with other trained experimenters who coded the videos.

Hormone assessment

At both test ages, blood samples were collected on all animals, immediately after being removed from their social group and prior to transport to the testing room, and used to assess basal hormonal levels. As described above, animals were trained for quick capture from the social group and blood samples were collected on unanesthetized subjects within 10 minutes of initial group disturbance, when the experimenters first enter the social group (see Sanchez, et al., 2010; Raper, et al., 2012a). During infancy, blood was collected prior to Human Intruder paradigm testing for another experiment to assess basal cortisol during infancy (Raper, et al., 2012b), and due to the animals small size no post-stress blood sample could be collected. Infants were wrapped in a fleece cloth and gently held while a second researcher took a blood sample via femoral venipuncture. As juveniles, both a basal sample was collected as well as a post-stress sample immediately after the human intruder task. Juveniles were trained to voluntarily present a leg through a hole in a modified housing cage, such that one researcher gently held their leg while a second researcher took a blood sample via saphenous venipuncture. All blood samples were collected in pre-chilled plastic 2 ml vacutainer tubes containing EDTA (3.6mg) and immediately placed on ice. Samples were centrifuged at 3,000 rpm for 15 minutes in a refrigerated centrifuge (at 4°C). Plasma was pipetted into sterile cryovials and stored at -80°C until assayed.

All assays were performed by the YNPRC Biomarker Core Laboratory (Atlanta, GA). During infancy, because of small plasma volumes, samples were only assayed for cortisol and testosterone, whereas juvenile plasma samples were assayed for ACTH, cortisol, and testosterone. Plasma concentrations of ACTH were assayed in duplicate by radioimmunoassay (RIA) using commercially available kits (DiaSorin, Inc., Stillwater, MN). The sensitivity of the DiaSorin assay was 7.10 pg/ml and intra- and inter-assay coefficients of variation in each assay were < 9.2%. Plasma concentrations of cortisol were assayed using liquid chromatography – mass spectroscopy (LC-MS). LC-MS analyses were performed via reverse phase chromatography on an LTQ-Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA). Quantitation was achieved using a deuterated cortisol internal standard (CDN Isotopes, Cortisol-9,11,-12,12-d4). The assay range was 2.5–60 ug/dl with intra- and inter-assay coefficients of variation < 8%. Plasma testosterone levels were also assayed in duplicate by R.I.A using commercially available kits (DSL kit: Diagnostic Systems Laboratories, Webster, TX). The sensitivity of the DSL assay was 0.05ng/ml and intra- and interassay coefficients of variation were <7%.

Data Analysis

At each age, preliminary analyses were first performed to compare the two groups of control animals (i.e., behavioral control group [Neo-BC] and the sham-operated group [Neo-C]). Repeated measures ANOVA (Group X Condition) revealed no significant main effects or interactions. Therefore, data from both groups were combined to create a single control group (Neo-C) for all subsequent analyses. During infancy, there were 14 Neo-C animals (males = 7; females = 7) and 16 Neo-A animals (males = 9; females = 7) tested (see Suppl

Table 1). During preadolescence, three females had to be dropped from hormonal and behavioral analyses due to illness or injury at the time of testing (one Neo-C female, two Neo-A females), reducing the sample size to 12 Neo-C animals (males = 6; females = 6) and 11 Neo-A animals (males = 7; females = 4; see Suppl Table 1).

Behavioral analysis—Prior to analysis, Kolmogorov-Smirnov (K-S) tests were performed to examine the normality of behavioral data. When behaviors were not normally distributed they were transformed using a natural log plus constant to obtain normality. The impact of early amygdala damage on the expression of defensive and emotional behaviors toward the Human Intruder was examined separately at each age using a Repeated Measures ANOVAs with Group (Neo-C, Neo-A) and Sex as between subjects factors, and Condition (Alone, Profile, Stare) as the within subjects factor. Interactions were examined with post-hoc one-way ANOVAs, whereas the one-way Repeated Measures ANOVA's were used to examine animals' ability to modulate behaviors across the three conditions.

Discriminant function analyses were conducted separately for each age (infancy, juvenile), to test whether cortisol and behavioral expression during the Human Intruder task could accurately classify individual animals by Group (Neo-C, Neo-A). Items included in the discriminant function analyses were coo vocalizations, freezing, hostility, anxiety, stereotypy and self-grooming behaviors as well as cortisol levels (pre- and post-stressor). Behavioral items were selected based on previous studies that demonstrated behavioral alterations after amygdala damage (Newman, & Bachevalier, 1997; Kalin, et al., 2004; Bauman, et al., 2008; Raper, et al., 2012a). To test if the discriminant function categorized individuals better than would occur by chance, we used Press's Q statistic (Hair, et al., 2009) calculated as follows:

$$\text{Press's } Q = [N - (nK)]^2 / N(K - 1)$$

N = total sample size

n = number of observations correctly classified

K = number of groups

Press's Q is distributed as a Chi-square with K – 1 degrees of freedom.

Hormonal analyses—A preliminary analysis was performed to ascertain if the time from disturbance until collection of the blood sample had any influence on pre-stress cortisol levels. Two Hierarchical Linear Model (HLM) Regression analyses were performed on pre-stress cortisol and ACTH levels separately. The amount of time it took to collect the blood sample explained a significant portion of the variance in pre-stress cortisol levels ($R^2 = 0.20$, $F(1,20) = 3.57$, $p = 0.047$), but not in pre-stress ACTH levels ($R^2 = 0.10$, $F(1,20) = 1.08$, $p = 0.36$), thus the variable of time to collect the sample was used as a covariate in subsequent analyses of group differences for cortisol levels only.

Changes in cortisol and ACTH levels were assessed using repeated measures ANOVA with Group (2) and Sex as between subjects factor and Time (pre- vs post-stressor) as the within subjects repeated measures. Post hoc analyses to further examine interactions effects were performed with One-way ANOVA's.

Correlations—In a previous publication, we examined potential group differences in testosterone (T) levels, and found no differences between groups (Raper, et al., 2012b). In this study, we investigated potential relationships between T levels and behavior during the Human Intruder paradigm at each age. Partial correlations were performed for T levels and

behavior correcting for the amount of time it took to collect the blood sample. Four animals were excluded from analyses during infancy because their T level (Neo-C male = 1) or total yawning (Neo-C male = 1; Neo-A female = 1; Neo-A male = 1) was two standard deviations above the group mean, thus a total of 26 animals were included in the infant correlation analyses (see Suppl Table 1). During the juvenile period, three animals (Neo-C male = 1; Neo-C female = 1; Neo-A female = 1) exhibited yawning behavior two standard deviations above the group mean, thus to avoid bias they were excluded from analysis leaving a total of 23 animals in the juvenile correlation analysis (see Suppl Table 1).

The relationship between the extent of amygdala damage and behavior during the Human Intruder paradigm was also examined using Pearson correlations. Lastly, the relationship between the extent of amygdala lesion and hormone levels (i.e. testosterone, cortisol) were examined with partial correlations to correct for the amount of time it took to collect the blood sample. All analyses were conducted with SPSS 16 for Windows, a $p < 0.05$ was considered significant, and effect sizes (eta squared or Cohen's d) were calculated.

Results

Neonatal amygdala lesions and emotional reactivity in infancy

Alone condition—When removed from the colony and place in a novel environment infant monkeys emit coo vocalizations to re-connect with their group and this behavior was observed in animals of both groups. Thus, during the Alone condition, all infant monkeys emitted significantly more coo vocalizations as compared to the other two conditions (Condition: $F[2,52]=4.3$, $p=0.019$, $\eta^2=0.14$; Figure 2). Neo-A infants also emitted more coos throughout all conditions compared to Neo-C infants (Group: $F[1,26]=3.9$, $p=0.05$, $\eta^2=0.13$). Moreover, Neo-C infants engaged in more cage exploration during the Alone condition than did Neo-A animals (Group X Condition: $F[2,52]=3.9$, $p=0.03$, $\eta^2=0.13$; see Figure 2), they also modulated their cage exploration based on the presence or gaze direction of the human intruder (Condition effect: $F[2,12]=17.08$, $p<0.001$, $\eta^2=0.74$; $F[2,12]=5.02$, $p=0.03$, $\eta^2=0.46$, Neo-C females and males respectively), but Neo-A infants did not (Condition effect: $F[2,12]=2.79$, $p=0.10$, $\eta^2=0.32$; $F[2,16]=2.92$, $p=0.08$, $\eta^2=0.26$, Neo-A females and males respectively). Interestingly, females of both groups explored the cage more than did males (Sex: $F[1,26]=5.1$, $p=0.032$, $\eta^2=0.17$; Figure 2).

Profile Condition—When the intruder's profile was presented, both groups displayed increased freezing behavior (Condition: $F[2,52]=35.8$, $p<0.001$, $\eta^2=0.58$) and there were no group differences. However, the frequency of fearful defensive behaviors varied according to the infants' group, sex, and the condition (Group X Sex X Condition: $F[2,52]=3.4$, $p=0.04$, $\eta^2=0.12$; see Figure 2). Neo-C males exhibited a linear decline from Alone to Profile to Stare condition, but Neo-C females had more fearful defensive behaviors during the Profile condition than in the other two conditions (see Figure 2). Thus, Neo-C animals displayed a clear modulation of fearful defensive behaviors across conditions that differed between the sexes, but similar modulation was not seen in Neo-A animals. Unlike Neo-C males, Neo-A males exhibited similar levels of fearful defensive behaviors across all conditions, and significantly more fearful defensive behaviors during the Profile and Stare conditions as compared to Neo-C males ($F[1,15]=5.98$, $p=0.028$, $\eta^2=0.30$; $F[1,15]=8.15$, $p=0.013$, $\eta^2=0.37$, respectively). Similarly, unlike Neo-C females, Neo-A females expressed more fearful defensive behaviors during the Alone and a trend in the Stare conditions ($F[1,13]=5.07$, $p=0.044$, $\eta^2=0.30$; $F[1,13]=4.10$, $p=0.06$, $\eta^2=0.25$, respectively). Taken together, these data suggest that neonatal amygdala lesions altered the ability to modulate fearful defensive behaviors based on the contextual information in the environment (i.e. presence or gaze direction of the intruder).

Stare Condition—The most salient threat to a monkey occurs when the intruder stares directly at the monkey and both groups exhibited increased hostile responses to this threat (Condition: $F[2,52]=23.4$, $p<0.001$, $\eta^2=0.47$) with no differences between groups. In contrast, frequency of infant scream vocalizations varied depending on lesion status, sex, and Human Intruder condition (Group X Sex X Condition interaction: $F[2,52]=5.15$, $p=0.009$, $\eta^2=0.17$). As illustrated in Figure 2, Neo-C infants of both sexes emitted more screams during the Stare condition than the other two conditions (Condition: $F[2,12]=8.69$, $p=0.005$, $\eta^2=0.59$; $F[2,12]=16.1$, $p<0.001$, $\eta^2=0.73$, respectively). Although Neo-A males exhibited the same modulation of screams as control males (Condition: $F[2,16]=7.05$, $p=0.006$, $\eta^2=0.47$), Neo-A females did not (Condition: $F[2,12]=2.16$, $p=0.16$, $\eta^2=0.27$) and emitted significantly more screams during the Alone condition than Neo-C females ($F[1,13]=6.47$, $p=0.026$, $\eta^2=0.35$; see Figure 2).

Infant monkeys regardless of neonatal treatment expressed more anxious behaviors, tooth grinding, and yawning during the Stare condition than during the Alone and Profile conditions (Condition: $F[2,52]=84.7$, $p<0.001$, $\eta^2=0.77$; $F[2,52]=106$, $p<0.001$, $\eta^2=0.80$; $F[2,52]=15.5$, $p<0.001$, $\eta^2=0.37$, respectively). Interestingly, the expression of anxious and tooth grinding behaviors differed depending on the infants' group and sex (Group X Sex interaction: $F[1,26]=5.0$, $p=0.034$, $\eta^2=0.16$; $F[1,26]=4.2$, $p=0.05$, $\eta^2=0.14$), such that Neo-C males exhibited more anxiety and tooth grinding compared to Neo-A animals or Neo-C females (see Figure 2). There was also a significant increase in yawning that differed according to the sex of the animals (Sex: $F[1,26]=4.1$, $p=0.05$, $\eta^2=0.14$), such that males expressed more yawns than females in both groups. Lastly, self-grooming increased as the salience of the threat increased (Condition: $F[2,52]=9.01$, $p<0.001$, $\eta^2=0.26$). Again, these behaviors tended to differ according to the sex of the infant (Sex X Condition: $F[2,52]=2.88$, $p=0.06$, $\eta^2=0.10$) with females of both groups exhibiting more self-grooming as compared to males ($F[1,13]=6.04$, $p=0.03$, $\eta^2=0.34$). No significant differences were found between groups for affiliative or stereotypic behaviors.

Neonatal amygdala lesions effects on emotional reactivity in juveniles

Group differences were still present at twelve months of age (see Figure 3), but there were fewer sex differences in the emotional responses in the two groups.

Alone Condition—Although the groups did not differ in the amount of cage exploration, Neo-A animals continued to emit more coos throughout all conditions compared to controls (Group: $F[1,19]=4.14$, $p=0.05$, $\eta^2=0.18$; see Figure 3). Juvenile monkeys of both groups exhibited more stereotypies (e.g. pacing) and more affiliative behaviors (including coo vocalizations) during the Alone condition compared to the other conditions (Condition: $F[2,38]=7.38$, $p=0.002$, $\eta^2=0.28$; $F[2,38]=4.41$, $p=0.02$, $\eta^2=0.19$, respectively).

Profile Condition—The impact of neonatal amygdala lesions at this juvenile period became more apparent when the intruder presented his/her profile. Thus, as compared to infancy during which both groups exhibited similar levels of freezing, juvenile Neo-A animals now exhibited significantly less freezing than did Neo-C animals (Group X Condition: $F[2,38]=6.69$, $p=0.003$, $\eta^2=0.26$; see Figure 3). However, only Neo-C subjects modulated their freezing based on both the presence and gaze direction of the human intruder (Alone vs Profile: $F[1,10]=21.7$, $p=0.001$, $\eta^2=0.69$; Profile vs Stare: $F[1,10]=27.7$, $p<0.001$, $\eta^2=0.74$). By contrast, Neo-A juveniles only modulated their freezing based on the absence/presence of the human intruder (Alone vs Profile: $F[1,9]=16.2$, $p=0.003$, $\eta^2=0.64$), and exhibited similar levels of freezing regardless of the gaze direction of the intruder (Profile vs Stare: $F[1,9]=3.5$, $p=0.09$, $\eta^2=0.28$). Additionally, both groups exhibited an increase in fearful defensive behaviors depending on the presence and gaze direction of the

intruder (Condition: $F[2,38]=10.63$, $p<0.001$, $\eta^2=0.36$; see Figure 3). However, whereas Neo-C juveniles exhibited more fearful defensive behaviors in the Profile as compared to the other two conditions (Alone vs Profile: $F[1,10]=12.17$, $p=0.006$, $\eta^2=0.55$; Profile vs Stare: $F[1,10]=15.45$, $p=0.003$, $\eta^2=0.61$), Neo-A juveniles did not (Alone vs Profile: $F[1,9]=1.59$, $p=0.23$, $\eta^2=0.15$; Profile vs Stare: $F[1,9]=3.9$, $p=0.06$, $\eta^2=0.31$). Thus, during preadolescence, early amygdala damage has impacted what would be considered “predator avoidance” behaviors in the wild (Kalin, et al., 1991, 2005).

Stare condition—Juvenile monkeys of both groups exhibited a large and significant increase in hostility (Condition: $F[2,38]=51.97$, $p<0.001$, $\eta^2=0.73$), although the magnitude of this increase was less in Neo-A animals than Neo-C animals (Group: $F[1,19]=5.71$, $p=0.027$, $\eta^2=0.23$, see Figure 4). Additionally, both groups expressed more scream vocalizations, yawning, and anxiety during the Stare condition as compared to the other two conditions (Condition: $F[2,38]=7.18$, $p=0.002$, $\eta^2=0.27$; $F[2,38]=9.2$, $p=0.001$, $\eta^2=0.33$; $F[2,38]=27.1$, $p<0.001$, $\eta^2=0.59$, respectively; Table 3). However, groups did not differ in their expression of scream vocalizations or yawning (Group: $F[1,19]=0.03$, $p=0.88$, $\eta^2=0.001$; $F[1,19]=3.73$, $p=0.07$, $\eta^2=0.16$, respectively), but Neo-A animals expressed significantly fewer anxious behaviors and tooth grinding (Group: $F[1,19]=5.26$, $p=0.033$, $\eta^2=0.22$; $F[1,19]=9.72$, $p=0.006$, $\eta^2=0.34$, respectively, Figure 3). The sex difference in yawning found in infancy was no longer present during preadolescence (Sex: $F[1,19]=1.17$, $p=0.29$, $\eta^2=0.06$).

Similar to infancy, juvenile monkeys exhibited increased self-grooming behaviors during the Stare condition compared to the other conditions (Condition: $F[2,38]=4.21$, $p=0.022$, $\eta^2=0.18$), and females of both groups exhibited more self-grooming compared to the males (Sex: $F[1,19]=14.46$, $p=0.001$, $\eta^2=0.43$; see Figure 3).

Neonatal amygdala lesions impact on HPA axis stress reactivity

At one year of age, neonatal amygdectomy produced profound changes in neuroendocrine stress response. Thus, as shown in Figure 4B, Neo-A animals exhibited greater cortisol levels during the post-stress sample as compared to Neo-C animals (Group: $F[1,18] = 7.79$, $p = 0.012$, $\eta^2 = 0.30$). There was also a significant Group X Sex X Time interaction for cortisol levels ($F[1,18] = 4.31$, $p = 0.05$, $\eta^2 = 0.19$), indicating that females with neonatal amygdectomies had the highest cortisol levels after exposure to the human intruder. By contrast, both groups demonstrated significant increase in ACTH level from pre- to post-stressor (Time: $F[1,19] = 13.28$, $p = 0.002$, $\eta^2 = 0.41$, Figure 4A), but there was no significant main or interaction effects of Group or Sex ($F[1,19] = 0.59$, $p = 0.45$, $\eta^2 = 0.03$; $F[1,21] = 0.20$, $p = 0.65$, $\eta^2 = 0.01$, respectively). The significant difference seen in cortisol, but not ACTH, is likely due to the differences in the timing of secretion, regulation (e.g., negative feedback) and clearance for the two hormones (Fink, 2010). There were no significant correlations between the extent of amygdala lesion and hormone levels.

Discriminant function analysis

A discriminant function analysis was performed to test whether the expression of coo vocalizations, freezing, hostility, anxious, self-grooming and stereotypic behaviors during the Human Intruder paradigm, as well as cortisol levels (infancy: pre-stressor; juvenile: pre- and post-stressor) accurately classified individual animals into those with an intact amygdala and those with neonatal amygdala lesions. During infancy, the overall Wilks's Lambda was not significant ($\Lambda = 0.66$, $\chi^2 [7, N=30] = 10.1$, $p = 0.19$) indicating that groups could not be discriminated based on these factors. In contrast, as juveniles, the overall Wilks's Lambda was significant ($\Lambda = 0.32$, $\chi^2 [8, N=23] = 19.4$, $p = 0.013$) indicating a strong discrimination between groups, accounting for 82% of the total variance. Table 3 shows the percentage of

subjects correctly classified by the juvenile discriminant function. The function correctly classified 91.3% of the subjects, which differed significantly from chance (Press's $Q = 15.7$, $df = 1$, $p < 0.001$). Specifically, the expression of behaviors that best predicted group classification were hostility ($r = 0.47$), post-stress cortisol level ($r = 0.41$), anxious behaviors ($r = 0.39$), duration of freezing during the Profile condition ($r = 0.37$), coo vocalizations ($r = 0.30$), and pre-stress cortisol levels ($r = 0.27$). Self-grooming ($r = 0.09$) and stereotypic behaviors ($r = 0.05$) did not account for a significant amount of variance and were not predictive of group classification. This analysis demonstrates that the expression of these four behaviors and cortisol levels accurately discriminates animals with neonatal amygdala damage from those with a functional amygdala, but only later in juvenile development and not in infancy.

Correlations between Behavior, Testosterone, and extent of amygdala damage

Previous studies have demonstrated a sex difference in yawning, which is influenced by testosterone (T) in post-pubertal old world monkey (Phoenix, et al., 1973; Hadidian, 1980; Troisi, et al., 1990; Graves & Wallen, 2006). Given the sex difference found in yawning during infancy in the current study, the relationship between T levels and total amount of yawning were examined using partial correlations to control for the blood sample collection time. There was a significant, but moderate positive relationship ($pr[23] = 0.42$, $p = 0.02$; see Figure 5A) between yawning and testosterone levels in infancy, but this relationship disappeared in juveniles ($pr[20] = 0.23$, $p = 0.16$; Figure 5B), at a time when the postnatal testosterone surge in males had subsided and sex differences in yawning had disappeared. Thus, during infancy, sex differences in yawning appeared linked to circulating testosterone levels that may indicate activation of this behavior by testosterone at this early developmental time.

To further investigate the sex difference in yawning behavior, a repeated measures ANOVA was conducted for yawning with Group and Sex as between subjects and Age (infant, juvenile) as repeated factors. There was a significant Sex X Age interaction ($F[1,18] = 4.87$, $p = 0.04$, $\eta^2 = .21$), such that males exhibited a decrease in yawning from infancy to juvenile, whereas the expression of yawning did not change with age in females (see Figure 5C). The sex difference in yawning between infant males and females was very large (Cohen's $d = 1.66$), whereas it became small and nonsignificant among juveniles (Cohen's $d = 0.19$). Since yawning appeared to be activated by infant T we further analyzed yawning in males, which showed an infant T increase, using a repeated measures ANOVA that revealed a significant effect of Age ($F[1,11] = 5.24$, $p = 0.04$, $\eta^2 = .32$). Both Neo-C and Neo-A males exhibited more frequent yawns as infants than as juveniles, yet, Neo-A males exhibited lower yawning as compared to Neo-C males at both ages (Group: $F[1,11] = 4.94$, $p = 0.048$, $\eta^2 = .31$), suggesting either a lower social reactivity or lowered sensitivity to T, and Neo-A and Neo-C males did not differ in infant levels of T (Raper, et al., 2012b).

Examination of potential correlations between the extent of amygdala damage and any of the behaviors examined revealed no significant relationships either during infancy or preadolescence. There were no significant correlations between basal cortisol levels and behaviors during infancy or preadolescence, as well as no significant correlations between post-stress cortisol levels and behavior in juveniles.

Discussion

This study examined the effects of early amygdala damage on emotional reactivity across the infant and juvenile periods. There were six main findings. First, as found in previous studies conducted under markedly different social conditions, neonatal amygdala lesions did not impair the basic expression of emotional or defensive behaviors using the Human

Intruder paradigm (Raper, et al., 2012a). Rather these early lesions altered the magnitude of the expression of emotional behaviors, and in some cases reduced the contextual modulation of these behaviors depending on the presence and gaze direction of the intruder. Second, these data complemented earlier findings by showing that the effects of the neonatal amygdala lesions on emotional reactivity became more pronounced as the animals matured. Third, unlike previous studies (Kalin, et al., 1998), the data also demonstrated the presence of sex differences in the expression and modulation of emotional reactivity, which were surprisingly more prevalent in infants than in juveniles. Fourth, these results indicated intriguing influences of gonadal hormones on expression and modulation of behavior as exemplified by the sex difference in yawning during the male infant postnatal testosterone surge, which was not evident in juveniles after male testicular function stopped. Fifth, contrary to previous studies demonstrating a blunted HPA axis response to a stressor in adult animals with either adult-onset (Kalin, et al., 2004; Machado & Bachevalier, 2008) or neonatal (Raper, et al., 2012a) amygdala lesions, the current results indicated that neonatal amygdala lesions lead to increased cortisol stress responses in juvenile monkeys. Lastly, a suite of emotional behaviors exhibited during the Human Intruder paradigm and cortisol levels accurately discriminated lesioned from non-lesioned in the juvenile stage, but not in infancy, suggesting that a combination of behavioral responses may reflect an amygdalectomy syndrome that would be recognizable to other members in the animal's social groups. Furthermore, the fact that this discrimination was only seen in juvenile monkeys suggests a developmental trajectory in which the consequence of neonatal amygdala damage becomes more pronounced with age or social experience. Together, these results demonstrate that the amygdala plays a critical role in the expression of sexually-dimorphic emotional responses during primate early development and influences the reactivity of the HPA axis in response to stressors.

Amygdala and Emotional Reactivity

As shown in previous studies (Kalin, et al., 1989, 1991; Raper, et al., 2012a), infant rhesus monkeys modulated their defensive and emotional behaviors depending on the gaze direction and magnitude of the threat provided by the human intruder in the first few months after birth. Importantly, early damage to the amygdala did not disrupt the overall development of species typical behaviors. Thus, during infancy, both sham-operated controls and animals with neonatal amygdala lesions exhibited more freezing during the Profile condition and more hostility during the Stare condition. However, as juveniles, the impact of early amygdala damage became more apparent as indicated by a reduction in the production of these behaviors in animals with neonatal amygdala lesions as compared to controls. The current results are in agreement with those of a recent study indicating that neonatal amygdala lesions yielded no changes in freezing at 4.5 months of age, but decreased freezing in adulthood in animals reared under more socially restrictive conditions than used here (Raper, et al., 2012a). This pattern of findings replicated in separate studies indicates that the effects of neonatal amygdalectomy may emerge or become more apparent with development. There are numerous neural developmental changes occurring between infancy through adolescence that could account for these effects. Amygdala volume significantly increases in normal rhesus monkeys from 1 week to 2 years of age (86.49% in males and 72.94% in females; Payne, et al., 2010) and these volume increases are reflected by important cytoarchitectonic changes within the different amygdala nuclei (Chareyron, et al., 2012). There are also developmental changes in the neural pathways involved in the coordination of the reactive stress response (Lidow, et al., 1991; Andersen, 2003). Glucocorticoid receptors increase from infancy to adolescence (Perlman, et al., 2007; Pryce, 2008; Sinclair, et al., 2011b), whereas neurotransmitter (monoaminergic, cholinergic, and GABAergic) receptor density steadily declines and tapers off at puberty (Lidow, et al., 1991). In addition, the impact of the amygdala on emotional reactivity also involves

interactions of the amygdala with other neural systems, such as the orbital and ventromedial prefrontal cortex, which are known to have a more protracted development than the amygdala (Machado & Bachevalier, 2003). Therefore, the enduring effects of amygdala damage on emotional reactivity may have resulted from an impact of the amygdala damage on the normal development of other structures and pathways involved in assessing threat level and coordinating an appropriate behavioral and neuroendocrine response.

Although neonatal amygdala lesions did not disrupt the development of species typical defensive and emotional behaviors, it did impact the magnitude of the expression of emotional behaviors based on the contextual information in the environment. As infants and as juveniles, monkeys with early amygdala damage expressed more coo vocalizations throughout the test and exhibited less cage exploration during the Alone condition in infancy but not in preadolescence. The alterations in cooing and cage exploration are in line with the role of the amygdala in detecting environmental danger and adapting an appropriate behavioral strategy according to the salience of that threat (Davis & Whalen, 2001). The willingness to emit coo vocalizations regardless of the presence or gaze direction of the intruder suggests that amygdalectomized animals had difficulty discerning the difference in threat level between conditions or in modulating their behavioral responses to these conditions. However, it is possible that the lack of modulation resulted from an overall change in the magnitude of the emotional reactivity.

Interestingly, discriminant function analyses indicated that an animals' expression of coo vocalizations, freezing, hostility, anxious behaviors and cortisol levels could predict with great accuracy the group in which the animals belonged. This finding was present at the juvenile stage but not in infancy further supporting the proposal that the impact of early amygdala damage becomes more apparent with age. Additionally, the juvenile data suggest that these four behaviors and cortisol levels can accurately classify animals with amygdala damage from those with a functional amygdala. Interestingly, reduced freezing is the most common finding across previous studies examining the effect of amygdala lesions (Meunier, et al., 1999; Kalin, et al., 2004; Machado & Bachevalier, 2008; Raper, et al., 2012a). In our conditions, with animals continuously living in complex social groups, freezing moderately predicted whether an animal had amygdala damage or not, whereas the expression of hostility, post-stress cortisol level, and anxious behaviors were stronger predictors. Although our discriminant function accurately classified 91.3% of subjects, some caution is appropriate as our sample size ($n=23$), although large for a primate study, is small for discriminant analysis and will need to be verified by future studies. However, the ability to discriminate amygdala-lesioned from intact animals based on a behavioral and hormonal profile could be a valuable tool for future behavioral testing and determining the amygdala's role in behavioral development.

Sex-dependent Role of the Amygdala in Emotional Reactivity

For some emotional responses, the effects of the amygdala lesions varied according to the sex of the animal, indicating an important role of the amygdala in the expression of sexually-dimorphic behaviors. In the case of fearful defensive and scream vocalizations, infant females with neonatal amygdalectomy showed a different pattern compared to control females, exhibiting more of those behaviors in the Alone condition. Previous studies have reported that female macaques express more screaming vocalizations than males (Tomaszycki, et al., 2001; Jiang, Kanthaswamy, & Capitanio, 2012). In fact, infant vocalizations to separation or rejection by their mothers, an emotionally arousing situation, are sexually differentiated with females giving longer and more complex vocalizations than did males (Tomaszycki, et al., 2001). These vocalizations are masculinized in females whose mothers were exposed to elevated testosterone raising the possibility that the sex

differences seen here in emotional vocalizations may reflect sexual differentiation of the amygdala under prenatal androgens.

Adult rhesus monkeys exhibit a sex difference in fearful and hostile behaviors, such that females express more fear grimaces and hostility toward a novel human as compared to males (Mason, et al., 1960). In the current study, infant female controls expressed more fearful defensive behaviors during the Profile condition compared to the other two conditions, whereas control males exhibited a linearly decreasing decline across conditions. Neonatal amygdectomy resulted in an exaggeration of the patterns in fearful defensive behaviors, such that Neo-A females expressed more fearful defensive behaviors as compared to control females. Whereas Neo-A males expressed a similar declining pattern in fearful defensive behavior across conditions as did control males, they exhibited higher frequency of fearful defensive behaviors compared to control males. Thus, the impact of early amygdala damage resulted in an exaggeration in the expression of fearful defensive behaviors during infancy. However, for some behaviors, amygdala damage did not increase, but rather eliminated, the typical sex difference. For example, normally developing male infants exhibited increased anxious behaviors and tooth grinding compared to females, neonatal amygdectomy eliminated this sex difference in infancy, such that Neo-A males expressed similar levels of anxiety and tooth grinding to that of Neo-A and Neo-C females. Taken together these results indicate that the amygdala is an important neural structure involved in the expression of behavioral sex differences for some emotional behaviors.

Neonatal amygdectomy did not impact the expression of all behavioral sex differences, as shown by the presence of overall sex differences in yawning during infancy and self-grooming behaviors as infants and juveniles. Previous studies have shown that the sex difference in yawning emerges after puberty (Hadidian, 1980; Troisi, et al., 1990) and is influenced by testosterone (T) in old world monkey (Phoenix, et al., 1973; Graves & Wallen, 2006). However, in our study this sex difference occurred in infancy, during a developmental stage when males are experiencing the postnatal T surge, a period when gonadal hormones are temporarily active in male infants only (Forest, et al., 1974, 1979; Robinson & Bridson, 1978; Mann, et al., 1989). Thus, the increased yawning in males was positively related with T levels in males, suggesting that this behavioral sex difference is present at birth and under the activational influence of circulating T levels. We are unaware of any other behavior that is activated by neonatal T in rhesus monkeys. Support for the hypothesis that this behavior was activated by T was further provided by the absence of sex difference in yawning as juveniles, at a time when the postnatal T surge has ended and both sexes are in a period of gonadal quiescence prior to puberty. Finally, the fact that early amygdala damage did not impact the sex difference in yawning during infancy suggests that the amygdala is not involved in the sexually dimorphic behavioral expression of yawning.

Another example of a lack of sex-dependent role of the amygdala is provided with self-grooming behaviors during infancy and preadolescence. Juvenile rhesus females exhibit significantly more grooming with partners in their social group (Hassett, et al., 2010), thus the increased self-grooming was expected. Interestingly, early damage to the amygdala did not disrupt this typical sex difference in rhesus monkey females. Nevertheless, additional sex differences in behavior may emerge in these animals after puberty when gonadal hormones are elevated.

Amygdala and development of the HPA axis stress reactivity

Juvenile monkeys responded to the Human Intruder paradigm with stress-induced elevations in plasma cortisol and ACTH, and the increase in cortisol was greater in animals with neonatal amygdala lesions than in controls. Interestingly, females with neonatal amygdala lesions showed significantly greater cortisol secretion in response to the stressor compared

to all other animals. The stress-induced hyperreactive cortisol response of neonatal amygdalectomized animals was unexpected given that previous lesion studies have reported blunted HPA axis stress response after amygdalectomy (Kalin, et al., 2004; Machado & Bachevalier, 2008; Raper, et al., 2012a). One potential factor that could explain these unexpected findings is the age at which the stress response was examined. All previous lesion studies have examined the effects of amygdala lesions on stress response in adulthood (after puberty), whereas the current study focused on juveniles (before puberty). Puberty is a period of continued development of neural pathways involved in coordinating the HPA axis stress response (Lidow, et al., 1991; Andersen, 2003). For example, prior to puberty neurotransmitter (monoaminergic, cholinergic, and GABAergic) receptor density is steadily declining until tapering off at puberty (Lidow, et al., 1991). In contrast, glucocorticoid receptors (GR) density increases from infancy to adolescence at which time GR expression reaches a plateau and then declines in old age (Perlman, et al., 2007; Pryce, 2008; Sinclair, et al., 2011b). In fact, prepubertal rodents exhibit an exaggerated stress response and delayed return to baseline after stressors compared to adults (Romeo, et al., 2004a, 2004b, 2006), suggesting an immaturity of the HPA axis glucocorticoid negative feedback before puberty. Additionally, primate studies have reported that pre-pubertal female monkeys exhibit greater cortisol response to stressors (Clarke, 1993; Davenport, et al., 2003; Sanchez, et al., 2005). Thus, the elevated cortisol observed after neonatal amygdala lesions, particularly in females, suggests that the neonatal lesions could have impacted the normal development of the HPA axis, including the typical developmental changes in neurotransmitters, neuropeptide and corticosteroid receptor systems, leading to a hyperactive stress response. Further analyses of the stress reactive response of these animals after puberty when the animals reach maturity will demonstrate whether the effects of neonatal amygdala lesions are transitory and will lead to blunted cortisol secretion as it has been shown in previous studies (Kalin, et al., 2004; Machado & Bachevalier, 2008; Raper, et al., 2012a). Unfortunately, similar analysis of HPA axis stress response could not be performed during infancy, limiting our ability for a longitudinal interpretation of our findings.

Despite the exaggerated cortisol stress response, neonatal amygdala-lesioned animals exhibited blunted fear reactivity during the human intruder as reflected by a reduction of freezing in the presence of the intruder as compared to controls. Thus, the data indicate a dichotomy between the effects of neonatal amygdala lesions on fear responses and on HPA axis functioning. This dichotomy could be explained by the different connections of the amygdala with brain systems critical for the production of fear behaviors and those responsible for HPA axis fear reactivity. Thus, the regulation of freezing behaviors requires activation of the periaqueductal gray matter, which receives direct inputs from the amygdala (Kalin, et al., 2001; Walker & Davis, 1997). In contrast, the HPA axis stress response to psychogenic stressors is regulated via different pathways, including indirect projections from the amygdala to the hypothalamic paraventricular nucleus (Herman, et al., 2003; Myers, et al., 2012) and other alternative pathways that could differently impact the HPA axis reactivity in the absence of a functional amygdala. Thus, both the prefrontal limbic cortex and the bed nucleus of the stria terminalis have strong connections with the hypothalamus and both receive strong inputs from the amygdala (Rempel-Clower & Barbas, 1998; Myers, et al., 2012; Radley, 2012). Therefore, in the absence of a functional amygdala, it is possible that the HPA axis reactive stress response could still be stimulated by other structures.

Amygdala and Neuropsychiatric Disorders in Humans

In summary, the present findings inform our understanding on the role of the amygdala on the development of the expression and regulation of emotion. The impact of early amygdala damage is reflected more in the magnitude of the expression of emotional reactivity as

compared to controls. It also appears that amygdala-lesioned animals exhibit a reduced ability to modulate their emotional behavioral responses based on the level of threat presented by the human intruder. Whether this lack of modulation after amygdala lesion is due to an inability to assess the level of threat in the environment, or is merely due to an overall change in the magnitude of emotional reactivity is unknown. Future assessment of emotional reactivity in other tasks will hopefully elucidate this question. Furthermore, this study demonstrates that the amygdala plays a critical role in the expression of sexually-dimorphic emotional response during primate early development. These sex-dependent alterations in defensive and emotional reactivity after neonatal amygdala lesions bear some similarities with sex-dependent symptomatology of developmental neuropsychiatric disorders, such as autism, schizophrenia, and mood disorders (Häfner, 2003; Zahn-Waxler, et al., 2008; Rinehart, et al., 2011; Schumann, et al., 2011). There is growing evidence of aberrant amygdala development and abnormal fear reactivity and emotional dysregulation in children with neuropsychiatric disorders (see review Schumann, et al., 2011). Results from the current developmental nonhuman primate study may shed light on how disruption of normal amygdala function can directly impact emotional behavior in infancy and manifest itself through development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Authors are grateful to Christen Merte, Patrick McFarland, Cassie Lyon, Sara Dicker, and Rebecca Roberts, M.A., for their invaluable assistance running the Human Intruder Paradigm, animal handling, mother-infant reunions, and reintroductions to the social group. We also thank all members of the Bachevalier Laboratory who have helped with the neuroimaging and surgical procedures on the infant monkeys. This research was supported by the National Institute for Mental Health (MH050268). The content is solely the responsibility of the authors and does not necessarily represent the official view of the NIMH, or the National Institutes of Health. The studies were also supported by the Center for Behavioral Neuroscience (NSF IBN 9876754), and Integrated Training in Psychobiology and Psychopathology Fellowship (NIMH T32 MH73525), as well as by the National Center for Research Resources to the Yerkes National Research Center (P51 RR00165; YNRC Base grant), which is currently supported by the Office of Research Infrastructure Programs/OD P51OD11132. The YNPRC is fully accredited by the American for the Assessment and Accreditation of Laboratory Care, International.

References

- Abdelgadir SE, Roselli CE, Choate JVA, Resko JA. Androgen receptor messenger ribonucleic acid in brains and pituitaries of male rhesus monkeys: Studies in distribution, hormonal control, and relationship to luteinizing hormones secretion. *Biol Reprod.* 1999; 60:1251–1256. [PubMed: 10208992]
- Aggleton, JP., editor. *The amygdala. A functional analysis.* 2. New York, NY: Oxford University Press; 2000.
- Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci Biobehav Rev.* 2003; 27:3–18. [PubMed: 12732219]
- Bauman MD, Lavenex P, Mason WA, Capitanio JP, Amaral DG. The development of social behavior following neonatal amygdala lesions in rhesus monkeys. *J Cogn Neurosci.* 2004; 16:1388–1411. [PubMed: 15509386]
- Bauman MD, Toscano JE, Babineau BA, Mason WA, Amaral DG. Emergence of stereotypes in juvenile monkeys (*Macaca mulatta*) with neonatal amygdala or hippocampus lesions. *Behav Neurosci.* 2008; 122:1005–1015. [PubMed: 18823158]
- Bliss-Moreau E, Toscano JE, Bauman MD, Mason WA, Amaral DG. Neonatal amygdala or hippocampus lesions influence responsiveness to objects. *Devel Psychobiol.* 2010; 52:487–503. [PubMed: 20583145]

- Chareyron LJ, Banta Lavenex P, Amaral DG, Lavenex P. Postnatal development of the amygdala: A stereological study in macaque monkeys. *J Comp Neurol.* 2012; 520:1965–1984. [PubMed: 22173686]
- Clarke AS. Social rearing effects on HPA axis activity over early development and in response to stress in rhesus monkeys. *Dev Psychobiol.* 1993; 26:433–446. [PubMed: 8293890]
- Davenport MD, Novak MA, Meyer JS, Tiefenbacher S, Higley JD, Lindell SG. Continuity and change in emotional reactivity in rhesus monkeys throughout the peripubertal period. *Motiv Emotion.* 2003; 27:57–76.
- Davis M, Whalen PJ. The amygdala: vigilance and emotion. *Mol Psychi.* 2001; 6:13–34.
- Einfeld SL, Tonge BJ, Florio T. Behavioral and emotional disturbance in individuals with Williams Syndrome. *Am J Ment Retar.* 1997; 102:45–53.
- Fink, G. Feedback systems. In: Fink, G., editor. *Stress Science: Neuroendocrinology*. New York: Academic Press, Elsevier; 2010. p. 63–75.
- Forest MG, Sizonenko PC, Cathiard AM, Bertrand J. Hypophyso-gonadal function in humans during the first year of life. *J Clin Invest.* 1974; 53:819–828. [PubMed: 4812441]
- Forest MG. Plasma androgens (testosterone and 4-androstenedione) and 17-hydroxyprogesterone in the neonatal, prepubertal, and peripubertal periods in the human and the rat: Differences between species. *J Ster Biochem.* 1979; 11:543–548.
- Freese, JL.; Amaral, DG. Neuroanatomy of the primate amygdala. In: Whalen, PJ.; Phelps, EA., editors. *The Human Amygdala*. New York: Guilford Press, Inc; 2009. p. 1–42.
- Graves FC, Wallen K. Androgen-induced yawning in rhesus monkey females is reversed with a nonsteroidal anti-androgen. *Horm Behav.* 2006; 49:233–236. [PubMed: 16055125]
- Häfner H. Gender differences in schizophrenia. *Psychoneuroendocrinology.* 2003; 28:17–54. [PubMed: 12650680]
- Hadidian J. Yawning in an old world monkey, *Macaca Nigra* (Primates: Cercopithecoidea). *Behaviour.* 1980; 75:133–147.
- Hair, JF.; Black, WC.; Babin, BJ.; Anderson, RE. *Multivariate Data Analysis*. 7. Prentice Hall; New Jersey: 2009. p. 263
- Hassett JM, Rupp HA, Wallen K. Social segregation in male, but not female yearling rhesus macaques (*Macaca mulatta*). *Am J Primat.* 2010; 72:87–92.
- 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 Neuroendocrin.* 2003; 24:151–180.
- Izquierdo A, Suda RK, Murray EA. Comparison of the effects of bilateral orbital prefrontal cortex lesions and amygdala lesions on emotional responses in rhesus monkeys. *J Neurosci.* 2005; 25:8534–8542. [PubMed: 16162935]
- Jiang, J.; Kanthaswamy, S.; Capitanio, JP. Degree of Chinese ancestry affects behavioral characteristics of infant rhesus macaques (*Macaca mulatta*). *J Med Primatol.* 2012. Online ahead of press, <http://doi:10.1111/jmp.12026>
- Kalin N, Shelton SE. Defensive behaviors in infant rhesus monkeys: Environmental cues and neurochemical regulation. *Science.* 1989; 243:1718–1721. [PubMed: 2564702]
- Kalin N, Shelton SE, Davidson RJ. The role of the central nucleus of the amygdala in mediating fear and anxiety in primate. *J Neurosci.* 2004; 24:5506–5515. [PubMed: 15201323]
- Kalin N, Shelton SE, Davidson RJ, Kelley AE. The primate amygdala mediates acute fear but not the behavioral and physiological components of anxious temperament. *J Neurosci.* 2001; 21:2067–2074. [PubMed: 11245690]
- Kalin NH, Shelton SE, Fox AS, Oakes TR, Davidson RJ. Brain regions associated with the expression and contextual regulation of anxiety in primates. *Biol Psychi.* 2005; 58:796–804.
- Kalin N, Shelton SE, Rickman M, Davidson RJ. Individual differences in freezing and cortisol in infant and mother rhesus monkeys. *Behav Neurosci.* 1998; 112:251–254. [PubMed: 9517832]
- Kalin N, Shelton SE, Takahashi LK. Defensive behaviors in infant rhesus monkeys: Ontogeny and context-dependent selective expression. *Child Devel.* 1991; 62:1175–1183. [PubMed: 1756661]

- Lidow MS, Goldman-Rakic PS, Rakic P. Synchronized overproduction of neurotransmitter receptors in diverse regions of the primate cerebral cortex. *Proc Nat Acad Sci USA*. 1991; 88:10218–10221. [PubMed: 1658799]
- Machado CJ, Bachevalier J. Non-human primate models of childhood psychopathology: the promise and the limitations. *J Child Psychol Psychi*. 2003; 44:64–87.
- Machado CJ, Bachevalier J. Behavioral and hormonal reactivity to threat: Effects of selective amygdala, hippocampal, or orbital frontal lesions in monkeys. *Psychoneuroendocrinology*. 2008; 33:926–941. [PubMed: 18650022]
- Machado CJ, Emery NJ, Mason WA, Amaral DG. Selective changes in foraging behavior following bilateral neurotoxic amygdala lesions in rhesus monkeys. *Behav Neurosci*. 2010; 124:761–772. [PubMed: 21133532]
- Malkova L, Lex CK, Mishkin M, Saunders RC. MRI-based evaluation of locus and extent of neurotoxic lesions in monkeys. *Hippocampus*. 2001; 11:361–370. [PubMed: 11530840]
- Mann DR, Gould KG, Collins DC, Wallen K. Blockade of neonatal activation of the pituitary-testicular axis: Effect on perpubertal luteinizing hormone and testosterone secretion and on testicular development in male monkeys. *J Clin Endocrinol Metab*. 1989; 68:600–607. [PubMed: 2493038]
- Mason JW. Plasma 17-hydroxycorticosteroid levels during electrical stimulation of the amygdaloid complex in conscious monkeys. *Am J Physiol*. 1959; 196:44–48. [PubMed: 13617434]
- Mason WA, Green PC, Posepanko CJ. Sex differences in affective-social responses of rhesus monkeys. *Behaviour*. 1960; 16:74–83.
- McClure EB, Monk CS, Nelson EE, Zarahn E, Leibenluft E, Bilder RM, Charney DS, Ernst M, Pine DS. A developmental examination of gender differences in the brain engagement during evaluation of threat. *Biol Psychi*. 2004; 55:1047–1055.
- Mendelson MJ. Clinical examination of visual and social responses in infant rhesus monkeys. *Devel Psychol*. 1982a; 18:658–664.
- Mendelson MJ, Haith MM, Goldman-Rakic PS. Face scanning and responsiveness to social cues in infant rhesus monkeys. *Devel Psychol*. 1982b; 18:222–228.
- Meunier M, Bachevalier J, Murray EA, Malkova L, Mishkin M. Effects of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys. *Eur J Neurosci*. 1999; 11:4403–4418. [PubMed: 10594668]
- Michael RP, Rees HD, Bonsall RW. Sites in the male primate brain at which testosterone acts as an androgen. *Brain Res*. 1989; 502:11–20. [PubMed: 2819450]
- Myers B, McKlveen JM, Herman JP. Neural regulation of the stress response: The many faces of feedback. *Cell Mol Neurobiol*. 2012 online ahead of press. doi 10.1007/s10571-012-9801-y.
- Nemanic S, Alvarado MC, Price RE, Jackson EF, Bachevalier J. Assessment of locus and extent of neurotoxic lesions in monkeys using neuroimaging techniques: a replication. *J Neurosci Meth*. 2002; 121:199–209.
- Newman JD, Bachevalier J. Neonatal ablation of the amygdala and inferior temporal cortex alter the vocal response to social separation in rhesus macaques. *Brain Res*. 1997; 758:180–186. [PubMed: 9203547]
- Payne C, Machado CJ, Bliwise NG, Bachevalier J. Maturation of the hippocampal formation and amygdala in *Macaca mulatta*: A volumetric magnetic resonance imaging study. *Hippocampus*. 2010; 20:922–935. [PubMed: 19739247]
- Perlman WR, Webster MJ, Herman MM, Kleinman JE, Weickert CS. Age-related differences in glucocorticoid receptor mRNA levels in the human brain. *Neurobiol Aging*. 2007; 28:447–458. [PubMed: 16540204]
- Phoenix CH, Slob AK, Goy RW. Effects of castration and replacement therapy on sexual behavior of adult male rhesus. *J Comp Physiol Psychol*. 1973; 84:472–481. [PubMed: 4200926]
- Pomerantz SM, Sholl SA. Analysis of sex and regional differences in androgen receptors in fetal rhesus monkey brain. *Devel Brain Res*. 1987; 36:151–154.
- Prather MD, Lavenex P, Mauldin-Jourdain ML, Mason WA, Capitanio JP, Mendoza SP, Amaral DG. Increased social fear and decreased fear of objects in monkeys with neonatal amygdala lesions. *Neuroscience*. 2001; 106:653–658. [PubMed: 11682152]

- Pryce CR. Postnatal ontogeny of expression of the corticosteroid receptor genes in mammalian brains: Inter-species and intra-species differences. *Brain Res Rev.* 2008; 57:596–605. [PubMed: 17916381]
- Radley JJ. Toward a limbic cortical inhibitory network: implications for hypothalamic-pituitary-adrenal responses following chronic stress. *Front Behav Neurosci.* 2012; 6:1–10. [PubMed: 22279431]
- Raper, J.; Wilson, M.; Sanchez, M.; Machado, C.; Bachevalier, J. Pervasive alterations of emotional and neuroendocrine responses to an acute stressor after neonatal amygdala lesions in rhesus monkeys. *Psychoneuroendocrinology.* 2012a. Online ahead of press, <http://dx.doi.org/10.1016/j.psyneuen.2012.10.008>
- Raper, J.; Bachevalier, J.; Wallen, K.; Sanchez, M. Neonatal amygdala lesions alter basal cortisol levels in infant rhesus monkeys. *Psychoneuroendocrinology.* 2012b. Online ahead of press, <http://dx.doi.org/10.1016/j.psyneuen.2012.09.004>
- Rempel-Clower NL, Barbas H. Topographic organization of connections between the hypothalamus and prefrontal cortex in the rhesus monkey. *J Comp Neurol.* 1998; 398:393–419. [PubMed: 9714151]
- Rinehart NJ, Cornish KM, Tonge BJ. Gender differences in neurodevelopmental disorders: Autism and Fragile X Syndrome. *Curr Top Behav Neurosci.* 2011; 8:209–229. [PubMed: 21769728]
- Robinson JA, Bridson WE. Neonatal hormone patterns in the macaque. I. Steroids. *Biology of Reproduction.* 1978; 19:773–778. [PubMed: 105767]
- Romeo RD, Lee SJ, Chhua N, McPherson CR, McEwen BS. Testosterone cannot activate an adult-like stress response in prepubertal male rats. *Neuroendocrinology.* 2004a; 79:125–132. [PubMed: 15103225]
- Romeo RD, Lee SJ, McEwen BS. Differential stress reactivity in intact and ovariectomized prepubertal and adult female rats. *Neuroendocrinology.* 2004b; 80:387–393. [PubMed: 15741744]
- Romeo RD, McEwen BS. Stress and the adolescent brain. *Ann NY Acad Sci.* 2006; 1094:202–214. [PubMed: 17347352]
- Saint-Maurice PF, Welk GJ, Silva P, Siahpush M, Huberty J. Assessing children's physical activity behaviors at recess: a multi-method approach. *Pediatr Exerc Sci.* 2011; 23:585–599. [PubMed: 22109782]
- Sanchez MM, McCormack K, Grand AP, Fulks R, Graff A, Maestripieri D. Effects of sex and early maternal abuse on adrenocorticotropin hormone and cortisol responses to the corticotrophin-releasing hormone challenge during the first 3 years of life in group-living rhesus monkeys. *Devel Psychopath.* 2010; 22:45–53.
- Sanchez MM, Noble PM, Lyon CK, Plotsky PM, Davis M, Nemeroff CB, Winslow JT. Alterations in diurnal cortisol rhythm and acoustic startle response in nonhuman primates with adverse rearing. *Biol Psychi.* 2005; 57:373–381.
- Schumann CM, Bauman MD, Amaral DG. Abnormal structure or function of the amygdala is a common component of neurodevelopmental disorders. *Neuropsychologia.* 2011; 49:745–759.
- Sinclair D, Webster MJ, Wong J, Weickert CS. Dynamic molecular and anatomical changes in the glucocorticoid receptor in human cortical development. *Mol Psychi.* 2011b; 16:504–515.
- Thompson CI. Learning in rhesus monkeys after amygdectomy in infancy or adulthood. *Behav Brain Res.* 1981; 2:81–101. [PubMed: 7225220]
- Tomaszycki ML, Davis JE, Gouzoules H, Wallen K. Sex differences in infant rhesus macaque separation-rejection vocalizations and effects prenatal androgens. *Horm Behav.* 2001; 39:267–276. [PubMed: 11374912]
- Troisi A, Aureli F, Schino G, Rinaldi F, De Andelis N. The influence of age, sex, and rank on yawning behavior in two species of macaques (*Macaca fascicularis* and *M. fuscata*). *Ethology.* 1990; 86:303–310.
- Walker DL, Davis M. Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J Neurosci.* 1997; 17:9375–9383. [PubMed: 9364083]

- Wallen K. Nature needs nurture: the interaction of hormonal and social influences on the development of behavioral sex differences in rhesus monkeys. *Horm Behav.* 1996; 30:364–378. [PubMed: 9047263]
- Wallen K. Hormonal influences on sexually differentiated behavior in nonhuman primates. *Front Neuroendocrinol.* 2005; 26:7–26. [PubMed: 15862182]
- Zahn-Waxler C, Shirtcliff EA, Marceau K. Disorders of childhood and adolescence: Gender and Psychopathology. *Ann Rev Clin Psychol.* 2008; 4:275–303. [PubMed: 18370618]
- Zosuls KM, Ruble DN, Tamis-LeMonda CS, Shrout PE, Bornstein MH, Greulich FK. The acquisition of gender labels in infancy: implications for gender-typed play. *Dev Psychol.* 2009; 45:688–701. [PubMed: 19413425]

Highlights

- Amygdala's role in the development of emotional reactivity
- Impact of neonatal amygdala lesions assessed using the Human Intruder paradigm
- Animals' sex modulated behavioral effects of neonatal amygdectomy during infancy
- Impact of early amygdala damage became more apparent with age
- Lesions resulted in a dichotomy between behavioral and cortisol stress response

Figure 1.

Two coronal MR images through the amygdala: T1-weighted images in one sham-operated control (Neo-C-1) and Fluid Attenuated Inversion Reversal (FLAIR) images in a representative case with neonatal amygdala lesions (Neo-A-M5). The numerals to the left of each coronal section indicate the distance in millimeters from the interaural plane. Black arrows point to the hypersignal resulting from the cell death from neurotoxic injections.

Figure 2.

Infancy: Average Coo vocalizations, Freezing, Hostile, Cage Exploration, Fearful defensive, Tooth Grinding, and Scream vocalizations during the Alone (A), Profile (P), and Stare (S) conditions of the Human Intruder paradigm for animals with sham operations (Neo-C, open bars) and animals with neonatal amygdala lesions (Neo-A, black bars). * indicates a significant difference from same sex controls ($p < 0.05$). † indicates a trend toward a difference from same sex controls ($p < 0.06$). § indicates a significant sex difference.

Figure 3.

Juvenile: Average Coo vocalizations, Freezing, Hostile, Fearful Defensive, Anxious, Tooth Grinding, and Self-grooming behaviors during the three conditions of the Human Intruder paradigm. # indicates a significant difference between conditions. * indicates a significant difference ($p < 0.05$) between groups. All other abbreviations are the same as in Figure 2.

Figure 4.

Mean $\pm$ SEM of ACTH (A) and cortisol (B) during Human Intruder stressor. Sham operated controls (Neo-C) are represented by squares (males = open squares, females = grey squares), neonatal amygdala lesion animals (Neo-A) are represented by circles (males = black circles, females = grey circles). # indicates a significant increase from pre- to post-stressor. * indicates a significant difference ($p < 0.05$) between groups.

Figure 5.

Correlation between Testosterone and Total Yawning expressed across all three conditions for all animals as infants (A) and as juveniles (B). Mean $\pm$ SEM of Frequency of Total Yawning by Age (C). Sham operated controls (Neo-C) are represented by squares (males = open squares, females = grey squares), neonatal amygdala lesion animals (Neo-A) are represented by circles (males = open circles, females = black circles). # indicates a significant effect of age. § indicates a significant sex difference. * indicates a significant difference ($p < 0.05$) between groups.

Table 1

Intended and unintended damage after neurotoxic lesions of the amygdala

Subjects	Intended Damage				Unintended Damage							
	Amygdala				Hippocampus				Entorhinal			
	Rt%	Lt%	X%	W%	Rt%	Lt%	X%	W%	Rt%	Lt%	X%	W%
Neo-A-F1	82.3	0.0	41.2	0.0	0.0	0.0	0.0	0.0	18.3	0.0	9.2	0.0
Neo-A-F3	100	32.2	66.1	32.2	2.5	0.0	1.2	0.0	21.8	2.9	12.4	0.6
Neo-A-F4	90.9	89.3	90.1	81.1	1.9	0.0	1.0	0.0	12.3	0.0	6.2	0.0
Neo-A-F5	61.6	58.4	60.0	36.0	0.0	0.0	0.0	0.0	1.6	0.0	0.8	0.0
Neo-A-F6	100	97.7	98.8	97.7	2.4	7.9	5.1	0.2	1.3	2.0	1.6	0.03
Neo-A-F7	98.3	99.0	98.6	97.3	4.3	2.1	3.2	0.1	7.3	2.5	4.9	0.2
Mean	88.9	62.8	75.8	57.4	1.9	1.7	1.8	0.05	10.4	1.2	5.9	0.18
Neo-A-M1	100	80.6	90.3	80.6	8.9	9.0	9.0	0.8	8.3	15.8	12.0	1.3
Neo-A-M3	70.3	90.8	80.6	63.9	3.1	5.3	4.2	0.2	2.1	31.4	16.8	0.7
Neo-A-M4	50.5	84.9	67.7	42.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-A-M6	77.3	92.3	84.8	71.3	4.4	0.0	2.2	0.0	0.0	0.3	0.2	0.0
Neo-A-M7	90.9	98.9	94.9	90.0	5.1	0.6	2.9	0.03	1.4	0.0	0.7	0.0
Neo-A-M8	100	87.0	93.5	87.0	6.4	0.4	3.4	0.03	7.1	0.0	3.6	0.0
Neo-A-M9	61.8	93.2	77.5	57.6	0.0	0.0	0.0	0.0	0.0	0.7	0.4	0.0
Mean	78.7	89.7	84.2	70.5	4.0	2.2	3.1	0.15	2.7	6.9	4.8	0.3

L%; percent damage in the left hemisphere; R%; percent damage in the right hemisphere; X%; average damage to both hemispheres; W%; weighted average damage to both hemispheres(W%=L%×R%)/100). Neo-A-F: female amygdala lesion subject, Neo-A-M: male amygdala lesion subject.

Table 2**Behavioral Ethogram**

Category and specific behavior	Measurement	Brief Definition
Fearful Defensive Behaviors	Cumulative Frequency	
Freeze	frequency ^a	Rigid, tense, motionless posture except slight head movement
Crouch	frequency ^a	Whole body or just front limbs bent with head near floor
Withdrawal	frequency	Quick, jerky motion away from intruder (jump back)
Fear Grimace	frequency	Refracted lips, exposed clenched teeth (exaggerated grin)
Hostile Defensive Behaviors	Cumulative Frequency	
Threat Bark Vocalization	frequency	Low pitch, high intensity, rasping, guttural
Threat (facial expression)	frequency	Any of the following: open mouth (no teeth exposed), head-bobbing, or ear flapping
Cage Aggression	frequency	Vigorously slaps, shakes or slams body against cage
Lunge	frequency	A quick, jerky movement toward the intruder
Anxious Behaviors	Cumulative Frequency	
Scratch	frequency	Rapid scratching of body with hands or feet
Body Shake	frequency	Whole body or just head and shoulder region shakes
Tooth Grind	frequency ^a	Repetitive, audible rubbing of upper & lower teeth
Yawn	frequency	Open mouth widely, exposing teeth
Stereotypies	Cumulative Duration	
Pacing	duration	Repetitive motor pattern around the test cage
Motor stereotypy	duration ^b	Repetitive, abnormal voluntary or involuntary motor patterns (swinging, twirling, floating limb)
Self-directed	duration ^b	Sucking thumb, eye poke, self-bite
Affiliative Behaviors	Cumulative Frequency	
Coo Vocalization	frequency	Clear soft, moderate in pitch and intensity, usually “oooooh” sounding
Grunt vocalization	frequency	Deep, muffled, low intensity, almost gurgling sound
Lipsmack	frequency	Rapid movement of pursed lips, accompanied by a smacking sound
Present	frequency	Rigid posture (knees locked) with tail elevated and rump oriented toward intruder
Scream Vocalization	Frequency	High pitch, high intensity screech or loud chirp
Cage Explore	Duration	Calm and inquisitive inspections of cage either by tactile, oral, or visual means
Self-Sooth	Cumulative Duration	
Self-grooming	Duration	Use of hands or mouth to smooth or pick through fur
Self-clasping	Duration ^b	Non-manipulatory enclosing or holding of a limb or body part with arms

List of all behaviors scored, how they are measured and a brief definitions.

^aBehavior for which total duration was also measured.

^bBehavior that was not observed in these subjects.

Table 3

Discriminant function analysis

		Predicted Classification	
		Neo-C	Neo-A
Actual Classification	Neo-C	11 (12)	1 (0)
	Neo-A	1 (0)	10 (11)

Correct classification of 91.3% of original grouped cases based on hostility, anxious behaviors, freezing, and coo vocalizations.