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Neonatal perirhinal cortex lesions impair monkeys’ ability to modulate their emotional responses

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Abstract
The medial temporal lobe (MTL) is a collection of brain regions best known for their role in perception, memory, and emotional behavior. Within the MTL, the perirhinal cortex (PRh) plays a critical role in perceptual representation and recognition memory, although its contribution to emotional regulation is still debated. Here, rhesus monkeys with neonatal perirhinal lesions (Neo-PRh) and controls (Neo-C) were tested on the Human Intruder (HI) task at 2 months, 4.5 months, and 5 years of age, to assess the role of the PRh in the development of emotional behaviors. The HI task presents a tiered social threat to which typically developing animals modulate their emotional responses according to the level of threat. Unlike animals with neonatal amygdala or hippocampal lesions, Neo-PRh animals were not broadly hyper- or hypo-responsive to the threat presented by the HI task as compared to controls. Instead, Neo-PRh animals displayed an impaired ability to modulate their freezing and anxiety-like behavioral responses according to the varying levels of threat. Impaired transmission of perceptual representation generated by the PRh to the amygdala and hippocampus may explain the animals’ inability to appropriately assess and react to complex social stimuli. Neo-PRh animals also displayed fewer hostile behaviors in infancy and more coo vocalizations in adulthood. Neither stress reactive nor basal cortisol levels were affected by the Neo-PRh lesions. Overall, these results suggest that the PRh is indirectly involved in the expression of emotional behavior, and that effects of Neo-PRh lesions are dissociable from neonatal lesions to other temporal lobe structures.

Keywords
social; stress; anxiety; rhesus macaque; development

Introduction
Structures within the medial temporal lobe (MTL) are highly interconnected, and activity within the MTL is known to contribute to perceptual processing, learning, memory, and the
regulation of emotional behaviors. Within the MTL, the perirhinal cortex (PRh), comprised of Brodmann’s areas 35 and 36, is positioned between sensory association cortices and subcortical structures (the hippocampus and amygdala). Its functions have been assessed in a variety of model systems as well as humans. Studies in rodents (Bang & Brown, 2009a; Bang & Brown, 2009b; Barker, Bird, Alexander, & Warburton, 2007; Barker et al., 2006), primates (Alvarado & Bachevalier, 2005; Bachevalier, Nemanic, & Alvarado, 2015; Meunier, Cirilli, & Bachevalier, 2006), and humans (Barbeau, Pariente, Felician, & Puel, 2011; Dougal, Phelps, & Davachi, 2007; Haskins, Yonelinas, Quamme, & Ranganath, 2008; Mundy, Downing, Dwyer, Honey, & Graham, 2013) have all demonstrated a critical role of the PRh in perceptual processes and various memory-related functions. In spite of this wealth of knowledge, much less is known about role of the PRh in emotional behavior.

The PRh is anatomically connected to the amygdala, cingulate cortex, and hippocampus (Lavenex, Suzuki, & Amaral, 2004; Pitkänen, Pirkkanen, Nurminen, & Ylinen, 2000), brain areas important for social and emotional behavior (Cacioppo, 2002; Ledoux, 1998). Thus, the PRh is a likely candidate to be involved in emotional processing as has been suggested by several rodent studies. Lesion studies have demonstrated that the PRh is necessary for certain, but not all, fear conditioning paradigms (Furtak, Allen, & Brown, 2007; Kent & Brown, 2012; Rosen et al., 1992; Schulz, Fendt, Richardson, & Schnitzler, 2004). The necessity of PRh function for normal emotional expression has been further supported by anxiolytic effects of PRh lesions (Schulz-Klaus, 2009) and anxiogenic effects of PRh kindling (Hannesson et al., 2005). Furthermore, experiments in monkeys have allowed for a more refined analysis of behavioral changes following PRh damage. Monkeys with separate or combined damage to the entorhinal and perirhinal cortices show increased defensive behaviors and decreased submissive behaviors to social stimuli, along with reduced approach behaviors to rewarded objects (Meunier & Bachevalier, 2002). Yet, they display normal emotional behavior to non-social stressors, such as a rubber snake (Chudasama, Wright, & Murray, 2008; Meunier et al., 2006). Also rhinal-lesioned animals did not attenuate their fear responses over repeated exposures with social stimuli. Their persistence to abnormally react to emotionally charged stimuli could be due to the impaired recognition memory often observed after perirhinal lesions. However, no correlation was found between a lack of habituation and memory performance (Chudasama et al., 2008). Other studies have reported that rhinal lesions are insufficient to cause emotional impairments (Zola-Morgan, Squire, Clower, & Alvarez-Royo, 1991), suggesting that the role of the PRh in the expression of emotional behavior is subtle and likely depends on the specific task used to test the animal. Emotional stress responses are associated with significant changes in hypothalamic-pituitary-adrenal (HPA) axis functioning, which would suggest that abnormal emotional responses following lesions to the PRh would also affect HPA axis reactivity. Yet, lesion studies in adulthood have shown that damage to the amygdala (Kalin et al., 2004; Machado and Bachevalier, 2008) or hippocampus, but not perirhinal, impact cortisol secretion (Sapolsky, Zola-Morgan, & Squire, 1991). Thus, the role of the PRh in emotion regulation and HPA axis functioning in adult animals needs to be further investigated.

One important factor to be considered is that the role of PRh in emotion regulation has been gathered exclusively in adult animals, in whom the behavioral repertoire and the

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hypothalamic-pituitary-adrenal (HPA) axis are fully matured. As such, the contribution of the PRh in the development of species typical emotional behavior and HPA axis stress reactivity is still unknown. It is possible that early damage to the PRh results in altered development or reorganization of the brain networks recruited during emotional processing and stress. For example, our earlier developmental studies have demonstrated that both neonatal lesions of the hippocampus and amygdala yielded significant, but opposing, changes in emotional reactivity and cortisol stress response (Raper, Wilson, Sanchez, Machado, & Bachevalier, 2013b; Raper, Wilson, Sanchez, Payne, & Bachevalier, 2017). Given the strong connections between the PRh and the hippocampus and amygdala, it is possible that neonatal perirhinal lesions (Neo-PRh) could impact the development of normal behavioral and neuroendocrine responses to emotionally charged stimuli. To test this proposal, the current study used the Human Intruder (HI) paradigm to examine the development of emotional behavior and the HPA-axis after neonatal PRh lesions in rhesus monkeys. Animals were tested on the HI paradigm during infancy at 2-months and 4.5-months of age, and again in adulthood (5 years of age). HPA-axis functioning was also assessed in adult animals by measuring basal cortisol levels as well as changes in cortisol levels prior to and after the HI test. Behavioral and neuroendocrine changes after neonatal PRh lesions were then compared to those found after neonatal lesions of either the amygdala or the hippocampus (Raper et al., 2013b; Raper et al., 2017). Findings have been previously presented in abstract form (Ahlgrim, Raper, Johnson, & Bachevalier, 2017; Johnson, Raper, & Bachevalier, 2012).

2. Methods

2.1. Animals

Subjects were ten rhesus macaque monkeys (Macaca mulatta) that were surrogate-peer reared in a socially enriched environment designed to promote species-specific socio-emotional well-being (Rommeck, Capitanio, Strand, & Mccowan, 2011; Sackett, Ruppenthal, & Davis, 2002). Animals received either bilateral MRI-guided ibotenic acid lesions of the PRh (Neo-PRh; males = 3, females = 3) or sham operations (Neo-C; males = 2, females = 2) at 10–12 days of age. All surgical procedures and behavioral testing were conducted at Yerkes National Primate Research Center, and all procedures were approved by the Institutional Animal Care and Use Committee for Emory University.

2.2. Rearing

Animals were surrogate-nursery peer reared and housed so as to promote species-specific social behavior using procedures established by Sackett et al. (2002). Rearing methods and partner housing have been previously described (Goursaud & Bachevalier, 2007; Raper et al., 2013b). The major factors contributing to species-typical behavior in surrogate-peer rearing were extended care and interactions provided by human caregivers, socialization with age-matched peers, and extensive cognitive testing throughout development. Briefly, all animals were individually housed in cages with radiant incubators and contact comfort as infants until 1 month of age. During this time, the cages were positioned to allow somatosensory contact with other infants. Animals were transferred to a larger quad cage and individually housed at 3 months of age. The larger cages allowed visual and physical
contact between pairs of adjacent animals through the large central mesh separating the
cages. At 7 months, animals were housed in quads, then moved to pair housing at 12 months
of age.

Care of infants was given by a principal human caregiver, who interacted with the infants 6
hours per day, 5 days per week. A familiar human caregiver interacted with the animals on
weekends for 2–4 hours per day. Starting at one month of age, infants socialized with three
other age- and sex-matched peers for 3–4 hours, 5 days per week in a large enclosure.
Infants were hand-fed Similac formula until 3–4 weeks of age. Banana pellets (190 mg, P.J.
Noyes, Cleveland, OH) were added to supplement the diet when animals were able to self-
feed. Diet was changed to Purina monkey chow enriched with fresh fruit at 3 months of age.

2.3. Neuro-imaging

Subjects underwent a magnetic resonance imaging (MRI) scan immediately before surgery
to determine stereotaxic coordinates for the lesions. They were anesthetized with Ketamine
Hydrochloride and Xylazine (10 mg/kg of 7:3 Ketamine Hydrochloride, 100 mg/mL and
Xylazine, 20 mg/mL, i.m.). Animals were then intubated, given an intravenous drip
dextrose and 0.45% sodium chloride) for hydration and their head fixed in a stereotaxic
apparatus. Animals were placed on a heating pad to keep the animal at normal body
temperatures, and maintained under general anesthesia (isoflurane, 1–3% to effect) during
scanning and surgical procedures. Coronal images were taken using a 5-cm surface coil. The
first of two sets was used to determine stereotaxic coordinates for the injection site (3D T1-
weighted fast spoiled gradient (FSPGR)-echo sequence, TE = 2.6 ms, TR = 10.2 ms, 25°
flip angle, contiguous 1 mm sections, 12 cm FOV, 256 × 256 matrix), and the second was
used to measure edema as a marker of cell death from the neurotoxic lesion (Fluid
Attenuated Inversion Recovery (FLAIR) sequence, TE = 140 ms, TR = 10,000 ms, inversion
time (TI) = 2200 ms, contiguous 3 mm sections, 12 cm FOV, 256 × 256 matrix). Post-
surgical FLAIR images were obtained 6–8 days after surgery and compared to pre-surgical
scans to quantify the lesions.

2.4. Surgery

Surgical procedures have been previously described in Zeamer, Richardson, Weiss, and
Bachevalier (2015). After the scanning procedures, the animals were kept under anesthesia
in the stereotaxic apparatus and brought to the surgical suite. Briefly, aseptic surgery was
performed and animals were monitored by veterinary staff until recovery. The skin on the
head was cleaned and skin and connective tissue underneath were cut and retracted to
expose the cranial bone. Two craniotomies (1 cm wide x 2.5 cm long) were made bilaterally,
approximately above the injection sites. The dura was cut, and a Hamilton syringe was
lowered to the coordinates determined by the pre-surgical T1 scan by a Kopf electrode
manipulator (David Kopf Instruments, Tujunga, CA). At 3 sites bilaterally along the antero-
posterior axis of the PRh (Brodman areas 35 and 36), 0.4 μL ibotenic acid (Biosearch
Technologies, Novato, CA, 10 mg/mL in PBS, pH 7.4) was injected at 0.4 μL/min.
Dexamethazone sodium phosphate (0.4 mg/kg, i.m.) and cefazolin (25 mg/kg, i.m.)
treatment were initiated 12 hr before surgery and was continued through 7 days post-surgery.
Acetaminophen (10 mg/kg, p.o.) was administered 4 times per day for three days post-
surgery. Sham operated controls underwent the same procedure described above, but no needle was lowered after the dura opening.

2.5. Lesion Verification

Histological verification of lesion sites is not available because the animals are currently participating in other experiments. However, the extent of damage was analyzed using previously described procedures (Málková, Lex, Mishkin, & Saunders, 2001; Nemanic, Alvarado, Price, Jackson, & Bachevalier, 2002), in which areas of hypersignal – showing edema – were identified from the one-week post-surgical FLAIR images and plotted onto a normal monkey brain atlas (Bachevalier, unpublished atlas). Image J® was used to measure the area of damage to both the PRh and adjacent structures (amygdala, hippocampus, and entorhinal cortex). Lesion extent was quantified as a percent damage in a given structure for each animal divided by the volume of that structure in a normally developing monkey.

3. Experiment 1

Development of emotional reactivity in infancy was measured with the Human Intruder (HI) task. Compared with other tests of emotional reactivity, the HI task is better suited for longitudinal testing of monkeys, since monkeys do not seem to habituate to repeated exposures to the task (Kalin & Shelton, 1998). The HI task is designed to assess emotional reactivity to a social stressor (Kalin, 1993), and the modulation of emotional behavior to different levels of social threat based on the gaze direction of an unfamiliar human [reviewed in (Coleman & Pierre, 2014)]. In addition, the HI task has been shown to rapidly activate the HPA axis and stimulate the release of glucocorticoids (Jahn et al., 2010; Kalin, Larson, Shelton, & Davidson, 1998; Kalin, Shelton, Davidson, & Kelley, 2001; Raper et al., 2013a; Raper et al., 2013b). As such, the HI task enables the measurement of both behavioral and physiological reactivity to emotionally charged stimuli. We have previously reported that modulation of defensive and emotional behaviors (i.e. freezing, hostility, etc.) emerged by 4.5 months of age in surrogate-peer reared control monkeys (Raper et al., 2013b). Thus, we predicted that neonatal PRh lesions would impair typical development of emotional behavior, and that the resulting abnormal behavior would be observable by 4.5 months of age.

3.1. Human Intruder Paradigm: During infancy (2 and 4.5 months of age)

At 2 months and 4.5 months after individual animal’s date of birth, all subjects were tested on the HI task at 0700 (light-on) in a novel testing room. Subjects were transferred to a stainless steel cage (53 cm x 53 cm x 55 cm). The cage side facing the experimenter was made of clear lexan plastic. Cage height was such that the subject was level with the experimenter’s face. The HI paradigm lasted 43 minutes and consisted of four 10-minute conditions (Alone 1, Profile, Stare, Alone 2) and a three-minute break between the second and third conditions.

Animals were tested on two consecutive days during which the order of Profile and Stare was counterbalanced across subjects between the two days. For each day, the animal first remained alone in the cage for 10 minutes (Alone 1) to acclimate to the environment and
obtain a baseline level of behavior. Then, the intruder (experimenter wearing a human male rubber mask) entered the room, stood two meters from the test cage while presenting his/her profile to the animal for 10 minutes (Profile condition). The intruder then left the room while the animal remained in the cage alone for a 3-min period, after which the intruder re-entered the room and stared directly at the animal for 10 minutes (Stare condition). Finally, the intruder left the room leaving the animal alone for another 10 minutes (Alone 2). A different rubber masks was worn by the intruder at each age of testing, so that the same intruder was seen at each age across all subjects in our laboratory (Raper et al., 2013a; Raper et al., 2013b; Raper et al., 2017). The intruder wore a rubber mask to provide a uniform stimulus to each subject. Previous experiments carried out with and without masks have elicited similar behavioral profiles from monkeys across all ages (Kalin & Shelton, 1989, 1998; Raper et al., 2013b; Raper et al., 2017).

The animal’s behavior was videotaped during all conditions and later coded using a previously described ethogram (Raper et al., 2013a; Raper et al., 2013b) (see Table 2) and the Observer XT 10 software (Noldus Inc.). Specific behavior within each of four categories was independently coded and the combined for each category. Two trained experimenters coded all of the videotapes and had an inter-rater reliability of Cohen’s Kappa = 0.81 and an average intra-rater reliability of Cohen’s Kappa = 0.97.

### 3.2. Data Analysis

At each age, preliminary analyses were first performed to compare emotional reactivity during the two Alone conditions (Alone 1 & Alone 2). Repeated measures ANOVA (Group X Testing Day) revealed no significant main effects or interactions. Therefore, data from the two Alone conditions over the two days of testing were averaged to create a single Alone condition at each age. Similarly, data on the Profile and Stare conditions from the first testing day were compared to data obtained on the second testing day using repeated measures ANOVA (Group X Testing Day). Again, no significant differences were detected between the two days of testing and, for each animal, a single measure combining the scores from the two Profile and the two Stare conditions were calculated. Thus, for the final analyses (see below), the combined scores of each animal for each of the 3 conditions (Alone, Profile, Stare) for each behavioral category were used. Behavioral data was transformed using a natural log plus one constant (LnX + 1) to achieve normality, based on the Kilmogorov-Smirnov (K-S) Test.

For each behavioral category, group differences were examined using repeated measures ANOVAs with Group (control, Neo-PRh) and Condition (Alone, Profile, Stare) as between-subject factors, and age (2-month, 4.5-month) as the repeated measure. Additionally, the ability of Neo-PRh animals to modulate their emotional behavior across conditions was examined using a test of equivalence (Rogers, Howard, & Vessey, 1993; Wellek, 2003). Test of equivalence was used to examine whether differences found in behavior between two conditions (Alone vs. Profile and Profile vs. Stare) within each group were small enough to be considered equivalent. Statistical difference between conditions was tested with a confidence interval of 95% and an equivalence interval of $\Delta = \pm 1$. Any condition difference falling within the equivalence interval of $\Delta = \pm 1$ or including $\Delta = 0$ indicates that the
difference is not meaningful and the conditions can be treated as equivalent/similar. As such, for any contrast whose confidence interval extends past ±1 and does not include zero, the behavior in the given conditions will be described as meaningfully different and not equivalent. For those behaviors that were found to be different between Neo-PRh and control groups, Pearson correlation coefficients were calculated to examine the relationship between behavioral expression and lesion extent. The only analyzed regions were the PRh and the entorhinal cortex, because those were the only two regions with substantial average damage (see results on Extent of Lesion).

3.3. Results

3.3.1. Extent of lesion—Lesion extent of the PRh was comparable between all six cases, with bilateral damage ranging from 67.07% to 83.34% (average: 73.6%; Table 1). Unintended damage extended bilaterally to the entorhinal cortex (average: 20.57%), and was minimal to area TE (average: 2.15%), the hippocampus (average: 0.81%), and the amygdala (average: 2.48%). Reconstructions of the PRh lesions on all six cases were reported in (Zeamer et al., 2015). Figure 1A displays MR images of a representative case (Neo-PRh-1) taken before and after surgery. Additional post-lesion MR images (Figure 1B) are shown for cases Neo-PRh-2 (adapted from Weiss and Bachevalier (2016), and Neo-PRh-6 (adapted from Weiss, White, Richardson, and Bachevalier (Submitted-b)). Other previously published cases include Neo-PRh-3 (Zeamer et al., 2015) and Neo-PRh-4 (Weiss, Nadji, & Bachevalier, 2015).

3.3.2. Effects of Neo-PRh lesions on Human Intruder reactivity in infancy

Coo vocalizations: Infant monkeys often emit coo vocalizations when separated from their cagemates and caregiver, and coo vocalizations are the most prevalent behavior during the Alone condition of the HI task. Early PRh damage did not impact the emission of coo vocalizations during infancy (Condition: F[2,24]=0.49, p=0.62, ηp²=0.04; Group: F[1,24]=0.96, p=0.34, ηp²=0.04; Age: F[1,24]=0.65, p=0.43, ηp² = 0.03; see Figure 2A). No interaction effects between any factors reached significance.

Freezing: When faced with an unfamiliar human, monkeys often adopt the defensive behavior of freezing, with the maximal amount of freezing typically occurring during the Profile condition (Kalin & Shelton, 1989). As expected, freezing increased between Alone and Profile, and decreased between Profile and Stare conditions (Condition: F[2,24]=10.66, p<0.01, ηp²=0.47; see Figure 2B). There was no effect of Group or Age (F[1,24]=0.45, p=0.45, ηp²=0.02; F[1,24]=0.71, p=0.41, ηp²=0.03, respectively). No interaction effects between any factors reached significance. At both ages, control animals successfully modulated freezing between Alone-Profile (2-month: t[3]=5.08, p<0.01; 4.5-month: t[3]=3.91, p=0.01) and Profile-Stare conditions (2-month: t[3]=6.97, p<0.01; 4.5-month: t[3]=4.40, p=0.01, see Figure 2C), and the test of equivalence showed the differences between conditions to be meaningful and not equivalent. In contrast, Neo-PRh animals did not modulate freezing between conditions at 2-months of age (Alone-Profile: t[3]=0.77, p=0.24; Profile-Stare: t[3]=1.84, p=0.06) and the test of equivalence revealed that the contrasts were equivalent for all conditions (see Figure 2C). At 4.5 months of age, Neo-PRh animals only exhibited a meaningful difference between Profile-Stare conditions (Alone-
Profile: t[3]=2.21, p=0.04; Profile-Stare: t[3]=2.57, p=0.02, as the test of equivalence showed behavior to be equivalent between Alone-Profile but not equivalent for the Profile-Stare (see Figure 2C). These results demonstrate that, as compared to control animals that were able to modulate their freezing behavior at both ages in response to the changing level of threat across the conditions of the Human Intruder task, the Neo-PRh animals did so only between the Profile and Stare conditions at 4.5 months of age.

**Hostile Behavior:** The Stare condition is the most salient and direct threat, which elicits hostile behavior from the animal (Kalin & Shelton, 1989). Both groups exhibited increased hostile behaviors during the Stare condition (Condition: F[2,24]=31.96, p<0.01, $\eta_p^2=0.73$), but Neo-PRh animals expressed less hostile behaviors compared to controls (Group: F[1,24]=14.17, p<0.01, $\eta_p^2=0.37$, see Figure 2D). No interaction effects between any factors reached significance. At both ages, controls (2-month: t[3]=3.66, p=0.02; 4.5-month: t[3]=3.15, p=0.03) and Neo-PRh animals (2-month: t[3]=3.43, p=0.01; 4.5-month: t[3]=2.59, p=0.02) exhibited significant differences in hostile expression between Profile-Stare, and the test of equivalence revealed behavior between Profile-Stare to not be equivalent in 4.5-month controls and 2- and 4.5-month Neo-PRh. In contrast, only 2-month controls exhibited significant differences in hostile expression between Alone-Profile (Control: 2-month: t[3]=3.82, p=0.02; 4.5-month: t[3]=3.15, p=0.07; Neo-PRh: 2-month: t[3]=1.65, p=0.08; 4.5-month: t[3]=0.12, p=0.45). Therefore, both groups modulated their hostile behaviors according to the level of threat presented by the intruder, although Neo-PRh animals expressed less hostility overall.

**Anxiety-like behavior:** Species-typical anxiety-like behaviors (e.g. scratching, yawning, etc.) are most commonly elicited during the Stare condition, the most salient threat (Kalin & Shelton, 1989). Both groups expressed increased anxiety-like behavior during the Stare condition (Condition: F[2,24]=23.04, p<0.01, $\eta_p^2=0.66$), with no difference between Groups (F[1,24]=0.77, p=0.39, $\eta_p^2=0.03$) or effect of Age (F[1,24]=0.73, p=0.40, $\eta_p^2=0.03$, see Figure 2E). No interaction effects between any factors reached significance. Interestingly, at 2 months of age controls did not differ in the expression of anxiety-like behavior between conditions (Alone-Profile: t[3]=2.08, p=0.06; Profile-Stare: t[3]=1.67, p=0.10). By 4.5 months, controls exhibited a significant difference in anxiety-like behavior between the Profile and Stare conditions (Alone-Profile: t[3]=1.35, p=0.14; Profile-Stare: t[3]=5.98, p<0.01) and test of equivalence showed behavior in these conditions were not equivalent (see Figure 2F). Neo-PRh animals exhibited the opposite pattern with a significant difference in anxiety-like behaviors between the Profile-Stare at 2 months (Alone-Profile: t[3]=1.32, p=0.12; Profile-Stare: t[3]=9.10, p<0.01) and equivalence tests revealed that Profile-Stare conditions were not equivalent. By 4.5 months, although the expression of anxiety-like behavior in Neo-PRh animals’ differed between Profile-Stare, the test of equivalence showed both contrasts to be equivalent (Alone-Profile: t[3]=1.20, p=0.14; Profile-Stare: t[3]=2.11, p=0.04). Therefore, controls exhibited a developmental increase in their ability to modulate their anxiety-like behavior expression according to the level of threat in the environment, whereas Neo-PRh animals appeared to exhibit a decline of anxiety-like behavioral modulation with age.
3.3.3. Relationship between extent of lesion and behavioral changes in infancy—Pearson correlations were computed to determine whether the magnitude of behavioral deficits was related to the extent of damage to either the PRh or entorhinal cortices. No significant correlations were found between PRh or entorhinal cortex damage and any behavior (i.e. freezing, anxiety-like, or hostility) at 2-months or 4.5-months (data not shown).

4. Experiment 2

Results from Experiment 1 reveal that animals with Neo-PRh lesions exhibited less hostility and were unable to modulate their freezing and anxiety-like behaviors during infancy as compared to controls. These group differences suggest that early damage to the PRh did not abolish the development of species typical defensive and emotional behaviors, but that the lesions altered the ability to shift emotional behavior expression based on the magnitude of the threat presented by the human intruder. To investigate whether changes in emotional reactivity after Neo-PRh lesions persisted in adulthood or whether the effects dissipated with further maturation, all animals were retested on the HI paradigm in adulthood (5 years after animals’ date of birth). Furthermore, to assess whether neonatal damage to the PRh altered the development of the HPA-axis stress reactivity, basal cortisol levels were examined on a baseline (non-stressor) day as well as immediately prior to and after the HI task (acute stressor).

4.1.1. Human Intruder Paradigm: during adulthood

The HI paradigm was altered for adult subjects to optimize the test of stress reactive cortisol response. Specifically, for adult monkeys, the HI paradigm consisted of three 9-minute sessions separated by one break period of approximately 3 minutes, for a total testing duration of 30 minutes. All subjects were tested once on a single day and conditions were presented in the same order: Alone (1), Profile (2), and Stare (3). The animal’s behavioral reactivity was video recorded and coded using the same ethogram that was used for Experiment 1 (Table 2) and Observer XT 10 software (Noldus, Inc.). Three experimenters unaware of treatment groups independently scored all the videos, having an inter-rater reliability of Cohen’s Kappa = 0.86, and an average intra-rater reliability of Cohen’s Kappa = 0.96.

4.1.2. Blood Collection for Neuroendocrine Function Assessment

Animals were trained to voluntarily present a leg to the experimenter for blood collection from the saphenous vein. The first and third samples were collected within 10 minutes of the experimenter entering the housing room. Collecting samples using this established protocol has been shown to reflect basal levels and prevent inflated cortisol levels from the disturbance (Blank, Gordon, & Wilson, 1983; Raper et al., 2013b). The initial collection occurred at lights on (0700) immediately after removing the animal from the housing room and immediately prior to the beginning of the HI task. A second sample was taken immediately after the task ended, before the animal was returned to the housing room (~0730). Finally, a third sample was taken approximately 45 minutes after the animal was returned to the home cage to assess HPA axis regulation and negative feedback control.
Two days prior to the HI stressor, this blood sampling procedure was mirrored for each animal at the same time of day to account for possible perturbations of the HPA axis due to handling. These samples were taken at Lights-on (0700), +30 minutes, and +75 minutes. Blood samples were collected and stored in pre-chilled 2-mL vacutainer tubes containing 3.6 mg EDTA and immediately placed on ice. After collection, samples were centrifuged at 3000 rpm for 15 min while refrigerated (4°C). Plasma samples were pipetted into cyrovials for storage at -80°C until assayed.

4.1.3. Plasma Cortisol Assays

All assays were performed by the YNPRC Biomarker Core Laboratory. Plasma samples were assayed for cortisol 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 μg/dl with intra- and inter-assay coefficients of variation <10%.

4.2. Data Analysis

Prior to statistical analysis, normality of the data was examined using Kilmogorov-Smirnov (K-S) Test. Only coo vocalizations were not normally distributed, and those data were transformed using the same natural log constant (LnX + 1) as used in Experiment 1 for the infancy data. As with the data from infancy, the Neo-PRh and control groups were of different sizes (6 and 4 animals, respectively), sphericity was not assumed in any analysis and the degrees of freedom were Huynh-Feldt corrected (corrected degrees of freedom are available upon request).

To determine the potential long-term effects of early PRh damage, repeated measures ANOVAs were conducted with Group (control, Neo-PRh) as between-subjects factor and Condition (Alone, Profile, Stare) as the within-subjects factor with repeated measure. As in Experiment 1, the test of equivalence was used for each group to examine whether differences in behavior between two conditions (Alone vs. Profile and Profile vs. Stare) were small enough to be considered equivalent. Statistical difference between conditions was tested with a confidence interval of 95% and an equivalence interval of Δ = ±1. Any condition difference falling within the equivalence interval of Δ = ±1 or including Δ = 0 indicates that the difference is not meaningful and the conditions can be treated as equivalent/similar. As above, contrasts of behavior between conditions whose confidence interval extends past ±1 and does not include zero will be described as meaningfully different and not equivalent.

Pearson correlation coefficients were used to examine the relationship between behavioral expression and lesion extent as in Experiment 1 during infancy.

Changes in the cortisol levels were assessed using repeated measures ANOVA with Group (2) as between-subject factors and Time (pre-, post-, and 45min post-stressor or Lights-On, +30min, +75min) as the within subjects repeated measure. Significance level was set at p<0.05 for all analyses and effect sizes were calculated using partial eta squared.
4.3. Results

4.3.1. Effects of Neo-PRh lesions on Human Intruder reactivity in adults (Figure 3)

**Coo Vocalization:** During adulthood, animals did not display a significant effect of Condition (F[2,16]=0.42, p=0.64, $\eta^2_p=0.05$) for coo vocalizations on the HI task. Control animals displayed very few coo vocalizations mainly in the Stare condition, whereas animals with Neo-PRh lesions emitted more coo vocalizations than controls across all conditions (Group: F[1,8]=5.66, p=0.05, $\eta^2_p=0.41$; see Figure 3A). The interaction effect of Group X Condition was not significant. Thus, although control animals showed a typical decrease in coo vocalizations from infancy to adulthood (Frequency: 3.27±0.45 to 0.19±0.47) (Kalin & Shelton, 1998), the decrease in frequency of coo vocalizations for the PRh group from infancy to adulthood was much less pronounced (Frequency: 3.84±0.37 to 1.64±0.38).

**Freezing:** Freezing behavior showed a significant effect of condition (Condition: F[2,16]=19.43, p=0.001, $\eta^2_p=0.71$), but no effect of Group (F[1,8]=0.27, p=0.62, $\eta^2_p=0.03$; see Figure 3B), and no interaction between Group and Condition. As adults, controls were able to modulate their defensive freezing behavior between Alone-Profile ($t[3]=21.66$, p<0.001) and Profile-Stare ($t[3]=4.15$, p=0.01), and the test of equivalence reveal that the contrasts were not equivalent, indicating meaningful differences in freezing duration. In contrast, freezing among the Neo-PRh animals was significantly different between the Alone-Profile ($t[5]=2.52$, p=0.03) and Profile-Stare ($t[5]=4.15$, p=0.01), yet the test of equivalence demonstrates freezing to be equivalent between conditions (see Figure 3C). Therefore, Neo-PRh animals display a weaker modulation of freezing according to the level of threat as was also seen when the animals were tested in infancy.

**Hostile behavior:** Both groups exhibited increased hostility during the direct threat of the Stare condition (Condition: F[2,16]=9.42, p=0.02, $\eta^2_p=0.54$; see Figure 3D). Neo-PRh animals expressed similar hostility levels compared to controls (Group: F[1,8]=0.41, p=0.54, $\eta^2_p=0.05$). The interaction between Group and Condition was not significant. Hostile behavior did not differ between Alone-Profile conditions for both groups (Control: $t[3]=0.40$, p=0.36; Neo-PRh: $t[3]=0.68$, p=0.26), but significantly differed between the Profile-Stare conditions (Control: $t[3]=6.06$, p<0.01; Neo-PRh: $t[3]=3.74$, p<0.01). Equivalence intervals revealed hostile behaviors were not equivalent between Profile-Stare in either group, indicating a meaningful difference between the conditions (data not shown). Therefore, both groups successfully modulated their hostile behavior based on the level of threat presented by the intruder, and animals with Neo-PRh lesions appeared to normalized their hostile responses in adulthood as compared to infancy.

**Anxiety-like behavior:** Anxiety-like behaviors were expressed more during the Stare condition (Condition: F[2,16]=4.45, p=0.04, $\eta^2_p=0.36$; see Figure 3E), and there was no difference between Groups (F[1,8]=0.001, p=0.97, $\eta^2_p<0.01$). The interaction between Group and Condition was not significant. Unlike the Neo-PRh group, the control group showed a significant difference between Alone-Profile conditions (Control: $t[3]=2.26$, p=0.05; Neo-PRh: $t[3]=0.22$, p=0.42), but the test of equivalence demonstrated anxiety-like behaviors between the Alone-Profile conditions were equivalent for both groups (Figure 3F).
The anxiety-like behavior of control animals was significantly different between the Profile-Stare conditions (t[3]=4.50, p=0.01), and the equivalence test revealed that conditions were not equivalent (see Figure 3F), indicating that controls were able to modulate their behavior in response to a graded increase in threat between the two conditions. In contrast, Neo-PRh-lesioned animals did not show a difference in behavior between Profile-Stare conditions (t[5]=1.69, p=0.08; see Figure 3F). Therefore, Neo-PRH animals were unable to modulate their anxiety-like behavior to respond to incremental changes in threat like their typically developing counterparts; a lack of modulation similar to that already seen when the animals were tested in infancy.

4.3.2. Relationship between extent of lesion and behavioral changes in adulthood—Pearson’s correlation coefficients were computed to determine whether the magnitude of behavioral deficits was related to the extent of damage in the PRh and entorhinal cortices as in infancy. No significant correlations were found between the lesion extents and any behavior (freezing, anxiety-like, or coo vocalizations; data not shown).

4.3.3. Effects of Neo-PRh lesions on cortisol reactivity—Although early damage to the PRh impacted the animals’ ability to modulate their emotional responses, it did not impact their cortisol stress response. Both groups exhibited a significant increase in cortisol from pre- to post-stressor and decline from post- to 45min post-stressor (Time: F[2,16]=13.21, p=0.001, ηp²=0.62) with no differences between Groups (F[1,8]=0.002, p=0.97, ηp²=0.001, see Figure 4A). The interaction between Group and Time was not significant. Examination of basal cortisol secretions at the same time of day, under non-stress conditions, also revealed a significant effect of time with a decline in cortisol across the morning (Time: F[2,16]=8.40, p=0.003, ηp²=0.51) with no differences between Groups (F[1,8]=2.38, p=0.16, ηp²=0.23, see Figure 4B). The interaction between Group and Condition was not significant.

5. Discussion
The current data demonstrate, for the first time, that neonatal lesions of the PRh did not alter the expression of typical emotional responses, but did alter their modulation according to the levels of threat displayed by a social stressor. As compared to control animals, whose hostile behavior was most prevalent in the Stare condition in both infancy and adulthood, Neo-PRh-lesioned animals showed fewer hostile behaviors in infancy. This change in emotional regulation dissipated when the animals were tested in adulthood. Also, unlike control animals that showed the species-typical decrease in coo vocalizations from infancy to adulthood, Neo-PRH animals frequently emitted coo vocalizations in adulthood. Further, as compared to controls that showed heightened freezing and anxiety-like behavior in the Profile condition as infants and adults, modulation of these emotional responses was weak at all ages for the Neo-PRh animals. Despite the changes in emotional regulation, basal and reactive cortisol levels were not affected by Neo-PRh lesions. Thus, the current data indicate that the PRh does not contribute to typical HPA axis function or the production of emotional behavior, but does contribute to the development of species-typical modulation of emotional reactivity.
5.1. Neo-PRh lesions impair monkeys’ ability to modulate emotional reactivity

The most striking difference between groups throughout development was the Neo-PRh animals’ inability to modulate freezing and anxiety-like behaviors between conditions. As in other cohorts (Kalin & Shelton, 1989; Kalin, Shelton, & Takahashi, 1991; Raper et al., 2013b; Raper et al., 2017), control monkeys modulated their freezing based on the salience of the threat and gaze direction of the human intruder, although this modulation emerged earlier in animals of the present study (2 months of age) than in animals of previous published studies (Raper et al., 2013b). Given that the degree of Chinese-origin or Indian-origin ancestry among rhesus macaques may influence their emotional reactivity in the HI task (Jiang, Kanthaswamy, & Capitanio, 2013), it is possible that the differences in the genetic backgrounds of the animals between studies are related to the differences in the timing of development of emotional behaviors.

Unlike control animals, Neo-PRh animals had weaker modulation of freezing throughout development, whereas changes in the modulation of anxiety-like behavior were noticed only in adulthood. Yet, the lack of a significant main effect of group on freezing and anxiety-like behavior indicates that the Neo-PRh lesions did not prevent the expression of these behaviors. Rather, the changing threat level of the HI between conditions was not properly perceived and integrated into the behavioral responses of Neo-PRh animals. Data concerning anxiety-like and freezing behaviors are of particular clinical relevance, because these behaviors have been shown to be sensitive to anxiolytic and anxiogenic compounds [reviewed in Coleman and Pierre (2014)].

Rhesus monkeys use coo vocalizations to identify and reconnect with their family and social group members (Hauser, 1991; Pfefferle, Ruiz-Lambides, & Widdig, 2014; Rowell & Hinde, 1962), but coo frequency is known to decrease in the human intruder task as the animals mature (Kalin & Shelton, 1998). A similar trend in coo vocalization was seen in the control animals of this study, whereas animals with Neo-PRh lesions maintained a high frequency of coo vocalizations even in adulthood. Increased cooing in adulthood could be related to the increase in anxiety-like behaviors reported, a finding consistent with the classification of “coos” in the basic measures of primate anxiety/displacement behaviors (Hinde & Rowell, 1962; Jiang et al., 2013; Mcfarland, 1966; Redican, 1975). This increase in cooing behavior may have resulted from a decrease in amygdala drive after the lack of PRh inputs since amygdala activity has been negatively correlated with frequency of separation-induced coo vocalization in adolescent monkeys (Fox et al., 2005) and amygdala lesions increase coos (Kalin, Shelton, & Davidson, 2004; Kalin et al., 2001; Raper et al., 2013a).

Neo-PRh lesions did impact aggressive behaviors during infancy, but this effect dissipated in adulthood. It is likely that the protracted emergence of normal levels of aggressive behaviors in Neo-PRh animals may relate to brain plasticity and redundancy of neural circuits during maturation. Given the critical role of the orbitofrontal cortex in the regulations of aggression (Butter & Snyder, 1972; Butter, Snyder, & Mcdonald, 1970; Izquierdo, Suda, & Murray, 2005; Machado & Bachevalier, 2006) and its more protracted maturation than the PRh (Berger & Alvarez, 1994; Berger, Alvarez, & Goldman-Rakic, 1993; Rodman & Consuelos, 1994; Webster, Bachevalier, & Ungerleider, 1994), it is possible that the orbitofrontal cortex...
could support the modulation of aggressive gestures by adulthood in the absence of a functional PRh.

The complete behavioral responses of Neo-PRh animals supports the idea that the PRh is required for developing the ability to appropriately integrate social stimuli to form an adaptive response. There is no evidence to suggest that the PRh is required for the production and expression of any of the reported behaviors, since Neo-PRh lesions did not produce a systematic group difference in monkeys’ response (i.e. hypo- or hyper-responsive) to the HI task. Although not true for all behaviors, the fact that the group difference in hostile behaviors resolved by adulthood suggests that there is partial functional compensation occurring in surrounding brain regions after neonatal lesions of the PRh (see above). However, Neo-PRh animals still show abnormal emotional behavior in adulthood (e.g. modulation of freezing), suggesting that the circuitry involved in active coping strategies may be uniquely reorganized and recovered. In contrast, the circuitry for passive coping strategies are not compensated for during development.

5.2. The perirhinal cortex and HPA axis function

Both basal and reactive HPA axis activity is dysregulated following adult (Kalin et al., 2004; Machado & Bachevalier, 2008) or neonatal (Raper et al., 2014; Raper et al., 2013b; Raper et al., 2017) lesions to the medial temporal lobe. As such, any manipulation that directly impacts emotional reactivity could be expected to be associated with HPA axis dysfunction. The fact that neither basal nor reactive cortisol levels of the Neo-PRh animals differed from control animals strongly indicated that the PRh is not an emotional center, but that it relays information to and from other emotional centers like the amygdala or hippocampus. Thus, the Neo-PRh lesions indirectly impaired emotional regulation, but did not disrupt activity of the hypothalamus.

5.3. Putative mechanisms of emotional dysregulation

The PRh, one of the last hubs of the ventral visual stream, densely projects to both entorhinal cortex/hippocampal formation and the amygdala (Pitkänen et al., 2000). These PRh-Hippocampus-Amygdala networks are likely involved in emotional regulatory processes. Rodent studies have shown that the PRh contributes to both memory of sensory stimuli (for trace conditioning) and the conjunctive representation of stimuli with many features (for delay conditioning of discontinuous tones, also referred to as stimulus unitization) (Bang & Brown, 2009b; Kent & Brown, 2012; Kholodar-Smith, Boguszewski, & Brown, 2008). Similarly, the primate PRh contributes to perceptual functions like dissociating feature ambiguity (Bartko, Winters, Cowell, Saksida, & Bussey, 2007; Bussey, Saksida, & Murray, 2003), although this PRh function is still debated (Clark, Reinagel, Broadbent, Flister, & Squire, 2011; Levy, Shrager, & Squire, 2005). Social stressors are extremely complex and can be ambiguous, requiring the processing of stimuli that share many visual features that must also be integrated into the appropriate emotional context. Thus, one possible interpretation of the current data is that the lack of PRh functioning impacted the animals’ ability to appropriately integrate all features of the gaze direction of the human intruder with emotional information originating from the amygdala. This view is supported by recent findings on the same Neo-PRh monkeys demonstrating their impaired
recognition memory abilities and decreased familiarity judgements despite normal perceptual discriminations of ambiguous non-social stimuli (Weiss & Bachevalier, 2016; Weiss, Guo, Richardson, & Bachevalier, Submitted-a).

In vitro investigations of the amygdala-PRh-hippocampal network further corroborate this model, indicating how a lack of PRh inputs to the hippocampus can affect hippocampal responses to emotional stimuli. Kajiwara, Takashima, Mimura, Witter, and Iijima (2003) found that electrical stimulation of either the PRh or amygdala in isolation was not sufficient to evoke activity in the entorhinal cortex and dentate gyrus, but joint amygdala-PRh stimulation was sufficient to do so. Those data suggest that disrupted emotional regulation following PRh lesions arises because the information originating from the amygdala may be insufficient to evoke the necessary response in the hippocampus by itself when lacking its complementary inputs from the PRh. In essence, the PRh does not directly drive emotional reactivity, but it enables the binding of complex objects to emotional cues, in order to appropriately distinguish ambiguous stimuli (Suzuki & Naya, 2014). Therefore, PRh lesions impair the ability to accurately integrate emotional valence with recognition of visual stimuli.

This line of reasoning is further supported by the distinct and non-overlapping behavioral and physiological responses to the HI task by neonatal lesions to either the amygdala (Neo-Aibo) (Raper et al., 2013b) or hippocampus (Neo-Hibo) (Raper et al., 2017). Unlike Neo-PRh lesions, reactive cortisol levels in both the Neo-Aibo and Neo-Hibo groups were significantly blunted when compared to control animals, dissociating the PRh from regions that directly influence the development of HPA axis activity. Secondly, emotional changes after Neo-Aibo, Neo-Hibo, and Neo-PRh lesions differed between all groups. Neo-Hibo animals systematically overrated the risk presented by the magnitude of threat during the HI task, suggesting that the hippocampus is necessary for adaptive inhibition of emotional reactivity. Neo-Aibo animals, by comparison, demonstrated decreased freezing and an inability to modulate fearful and anxiety-like behavior. The physiological and behavioral data taken together suggest that the effects of Neo-PRh lesions share similarities with that of Neo-Aibo animals, in that both groups displayed an inability to modulate behavior based on the salience of the threat. Therefore, information from the PRh-amygdala pathway may be the crucial pathway for the modulation of emotional responses.

5.4. Potential impact of nursery rearing on socioemotional behavior

In discussing the impact and implications of these data, it is important to first examine one caveat of developmental research. Brain maturation and behavioral development are the result of complex interactions between genetic and environmental factors, such that changes in these factors may affect normal development. Although control and Neo-PRh animals came from the same genetic pool of monkeys at the YNPRC, rearing condition has been shown to alter brain maturation and socioemotional behavior (Rommeck et al., 2011; Sánchez, Hearn, Do, Rilling, & Herndon, 1998). Therefore, it is possible that the rearing conditions of these animals affected the emotional behavior expression observed. Although we cannot entirely refute this shortcoming, this proposal seems unlikely for several reasons. Firstly, we have previously shown that our surrogate-peer rearing protocol is similar to the
condition of ‘continuous rotation peer rearing’ known to produce behavioral and temperament measures most comparable to those of mother-reared monkeys (Rommeck et al., 2011). Second, our rearing protocol produces species-typical caregiver attachment and developmental pattern in emotional behavior on the HI task and cognitive skills similar to those reported in mother-reared monkeys (Goursaud & Bachevalier, 2007; Kalin et al., 1991; Raper et al., 2013b; Zeamer, Heuer, & Bachevalier, 2010). In addition, our developmental neuroimaging studies indicated that changes in hippocampal volume from infancy to two years of age are remarkably comparable in animals raised in our nursery-enriched protocol (Payne, Machado, Bliwise, & Bachevalier, 2010) and those raised by their mothers in a social group (Hunsaker, Scott, Bauman, Schumann, & Amaral, 2014). Finally, the Neo-Aibo and Neo-Hibo animals discussed earlier have been shown to have distinct and dissociable changes in emotional reactivity as compared to controls. These dissociable results support the conclusion that any behavioral abnormalities observed in the Neo-PRh animals are not a result of rearing conditions, but rather the experimental manipulation.

6. Conclusions

Results from the present study suggest that the lack of integration of the emotional valence of sensory stimuli is at the heart of the behavioral abnormalities of Neo-PRh animals to appropriately react to social stressors. Disrupting the information originating from the PRh exerts a profound impact on the propagation of, integration of, and response to that sensory information. Future studies could further the current understanding of the role of the PRh in emotional regulation by extending data from in vitro studies that highlight the interconnectedness of the amygdala, hippocampus, and PRh. Specifically, the relationship between these regions could be explored by recording amygdala and hippocampal activity in Neo-PRh animals (e.g. using depth electrodes or fMRI) during exposure to emotionally salient stimuli. Such electrophysiological and neuroimaging data could be complemented by behavioral studies that investigate the role of the PRh in the development of species-typical emotional responses in a naturalistic environment. In such an environment, real-time social cues must be interpreted correctly and rapidly to trigger adaptive responses within the social group. Such studies would continue to inform why and how complex social cues exert such profound influence on the emotional states of social animals, and how the strong interconnections between PRh-Amygdala-Hippocampus are required for appropriate emotional reactivity to changing social contexts.

Acknowledgments

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Figure 1.
(A) Antero-posterior extent of perirhinal damage in a representative case (Neo-PRh-1). Coronal sections from the pre-surgical T1-weighted MR images (left column), the post-surgical FLAIR MR images (middle column), and reconstruction of the lesion onto matched sections of an infant atlas brain (J. Bachevalier, unpublished atlas). White arrows point to the rhinal sulcus on the T1 images and white shading on the FLAIR images indicates hypersignals from edema. (B) FLAIR images of Neo-PRh-2 [left column] and Neo-PRh-6 [right column]. Abbreviations: rh, rhinal sulcus; amt, anterior medial temporal sulcus.
Images in Figure 1B are adapted from “Object and spatial memory after neonatal perirhinal lesions in monkeys,” by A.R. Weiss and J. Bachevalier, 2016, Behavioural Brain Research, 298(Pt B), p. 213. And “Impaired cognitive flexibility after neonatal perirhinal lesions in rhesus macaques,” by Weiss, A. R., White, J., Richardson, R. L. and Bachevalier, J., Under Review, Developmental Cognitive Neuroscience. Copyright 2016 by authors and with permission from Elsevier Academic Press.
Figure 2.
Emotional behavior during infancy. Mean ± SEM (transformed using an LnX + 1 constant) for coo vocalizations (A), freezing (B), hostility (D), and anxiety-like behavior (E) for control animals (open bars) and animals with neonatal perirhinal lesions (Neo-PRh; black bars). Equivalencies between Alone-Profile and Profile-Stare conditions of the Human Intruder task for normalized freezing (D) and anxiety-like behavior (F) data. The horizontal bars depict 95% confidence intervals of the difference in behavior between conditions (e.g. Alone vs Profile) for both controls (2 months: solid gray lines, 4.5 months: dashed gray lines) and Neo-PRh (2 months: solid black lines, 4.5 months: dashed black lines) groups. Gray vertical lines mark the equivalence interval of Δ = ±1. Confidence intervals that do not include zero are considered different, and those that extend beyond ±1 are considered not equivalent and are also indicated by an asterisk (*). % indicates significant group effect and # indicates significant condition effect.
Figure 3.
Emotional behavior during adulthood. Mean ± SEM for coo vocalizations (A), freezing (B), hostility (D), and anxiety-like behavior (E). Equivalencies between Alone-Profile and Profile-Stare conditions of the Human Intruder task for normalized freezing (D) and anxiety-like behavior (F) data. All other abbreviations and symbols as in Figure 2. Data of coo vocalizations were transformed using an LnX + 1 constant to achieve normality. All other behaviors were normally distributed.
Figure 4.
Cortisol response during adulthood. Mean ± SEM cortisol (μg/dl) response to the Human Intruder stressor (A) and Non-stress day (B) for control animals (open circles and gray lines) and animals with Neo-PRh lesions (black squares and black lines). All other abbreviations and symbols as in Figure 2.
Table 1

Intended and unintended damage after neurotoxic lesions of the perirhinal cortex to select structures.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>PRh</th>
<th>ERh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L %</td>
<td>R %</td>
</tr>
<tr>
<td>Neo-PRh-1</td>
<td>89.76</td>
<td>76.91</td>
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<tr>
<td>Neo-PRh-2</td>
<td>68.16</td>
<td>70.58</td>
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<tr>
<td>Neo-PRh-3</td>
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</tr>
<tr>
<td>Neo-PRh-4</td>
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<td>74.73</td>
</tr>
<tr>
<td>Neo-PRh-5</td>
<td>75.90</td>
<td>66.81</td>
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<tr>
<td>Neo-PRh-6</td>
<td>74.12</td>
<td>80.31</td>
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<tr>
<td>Average</td>
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<td>75.06</td>
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<table>
<thead>
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<th>Subjects</th>
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<th>AMY</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>R %</td>
</tr>
<tr>
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</tr>
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<td>Neo-PRh-4</td>
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</tr>
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<td>Neo-PRh-6</td>
<td>3.22</td>
<td>0.32</td>
</tr>
<tr>
<td>Average</td>
<td>1.12</td>
<td>0.5</td>
</tr>
</tbody>
</table>

L%, percent damage to the left hemisphere; R%, percent damage to the right hemisphere; X%, average damage to both hemispheres; W%, weighted average damage to both hemispheres (W% = (L% × R%)/100); ERh, entorhinal cortex; AMY, amygdala; HF, hippocampal formation. For complete list, see Zeamer et al. (2015).
**Table 2**

Behavioral ethogram. List of behaviors scored, how they are measured and a brief definition.

<table>
<thead>
<tr>
<th>Category and specific behaviors</th>
<th>Measurement</th>
<th>Brief Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coo Vocalization</td>
<td>Frequency</td>
<td>Clear, soft, moderate in pitch and intensity, usually “oooooh” sounding</td>
</tr>
<tr>
<td>Freeze</td>
<td>Duration</td>
<td>Rigid, tense, motionless posture except slight head movement</td>
</tr>
<tr>
<td>Hostile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threat Bark</td>
<td>Frequency</td>
<td>Low pitch, high intensity, rasping, guttural</td>
</tr>
<tr>
<td>Threat</td>
<td>Frequency</td>
<td>Any of the following: open mouth (no teeth exposed), head bobbing, or ear flapping</td>
</tr>
<tr>
<td>Lunge</td>
<td>Frequency</td>
<td>A quick, jerky movement toward the intruder</td>
</tr>
<tr>
<td>Cage Aggression</td>
<td>Frequency</td>
<td>Vigorously slaps, shakes or slams body against cage</td>
</tr>
<tr>
<td>Anxiety-like</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scratch</td>
<td>Frequency</td>
<td>Rapid scratching with hands or feet</td>
</tr>
<tr>
<td>Body Shake</td>
<td>Frequency</td>
<td>Whole body or just head and shoulder region shakes</td>
</tr>
<tr>
<td>Yawn</td>
<td>Frequency</td>
<td>Open mouth widely, exposing teeth</td>
</tr>
<tr>
<td>Tooth Grind</td>
<td>Frequency</td>
<td>Repetitive, audible rubbing of upper and lower teeth</td>
</tr>
</tbody>
</table>