



## Differential responses toward conditioned and unconditioned stimuli, but decreased hypothalamic-pituitary-adrenal axis responsiveness in neonatal hippocampal lesioned monkeys

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### ABSTRACT

The hippocampus is important for long-term memory storage, but also plays a role in regulating the hypothalamic-pituitary-adrenal (HPA) axis and emotional behaviors. We previously reported that early hippocampal damage in monkeys result in increased anxious expression and blunted HPA responses to an acute stressor. Here, we further probe their responses toward aversive stimuli (conditioned and unconditioned) and evaluate HPA axis dysfunction. Responses toward social, innate, and learned aversive stimuli, fear potentiated acoustic startle, and pituitary-adrenal function were investigated in 13 adult rhesus monkeys with neonatal hippocampal lesions (Neo-Hibo=6) and controls (Neo-C=7). Neo-Hibo monkeys' responses depend on the type of unconditioned stimulus, with increased anxiety behaviors toward social and learned, but decreased reactivity toward innate stimuli. Neo-C and Neo-Hibo monkeys exhibited similar performance learning conditioned cues and safety signals. Neo-Hibo monkeys were less sensitive to HPA axis stimulation, potentially suggesting adrenal fatigue. Current findings suggest that the hippocampus plays a large role in regulating not only anxiety behaviors, but also the HPA-axis, a neural system crucial to regulate how we respond to the world around us. These data have important clinical significance considering that many developmental neuropsychiatric disorders exhibit altered hippocampal structure and function, emotional and HPA axis dysregulation.

Studies of the hippocampus have historically focused on its role in forming new memories for long-term storage (Squire et al., 2018; Larimore, 2017). Although not widely recognized, the hippocampus is involved in regulating emotional reactivity and the hypothalamic pituitary adrenal (HPA) axis (Squire et al., 2018; Larimore, 2017; Jacobson and Sapolsky, 1991; Kalin, 2002; Buchanan et al., 2004, 2009; Lyons et al., 2007; Kubarych et al., 2001; Knutson et al., 2013; Yang and Wang, 2017). Regulation of emotional reactivity is a process that allows us to control the value and intensity of fear expressed in a given situational context and social norm. Disruption of this process, however, leads to excessive and pervasive fear that interferes with normal functioning and has been associated with several neuropsychiatric disorders, such as anxiety disorders and post-traumatic stress disorder (PTSD). The

hippocampus may play an important role in this regulatory process (Fitzgerald et al., 2018; Ji and Maren, 2005). In fact, hippocampal damage in adult rodents, monkeys, and humans results in decreased freezing/dampened fear, impaired contextual fear memory, increased approach toward aversive stimuli, and increased anxiety/tension behaviors (Buchanan et al., 2009; Chudasama et al., 2009, 2008; Machado and Bachevalier, 2008), as well as altered HPA axis responses to stressors with either prolonged cortisol secretion (see review (Herman et al., 2003)) or a lack of cortisol response to a psychological stressor (Buchanan et al., 2009; Tuvnes et al., 2003). Evidence for the role of the hippocampus in emotional responses and HPA axis function thus far has largely been investigated using lesions acquired during adulthood when the hippocampus and neuroendocrine systems are already fully

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developed. Considering the prolonged maturation of the hippocampus (Payne et al., 2010), it is important to understand its contribution to the maturation of emotional regulation and neuroendocrine responses.

Few studies have begun to assess whether the hippocampus plays a critical role in emotional and neuroendocrine development. Bliss-Moreau and colleagues demonstrated that rhesus monkeys with neonatal hippocampal lesions exhibited a decline in emotional expressions toward aversive stimuli or social partners from infancy to adulthood (Bliss-Moreau et al., 2017, 2011b, 2011a, 2010), had spared Pavlovian fear learning (Antoniadis et al., 2007), but displayed more stereotypies with age (Bliss-Moreau et al., 2017; Bauman et al., 2008). In response to an acute stressor, we found that neonatal hippocampal-lesioned monkeys exhibited increased anxious and self-directed behaviors (Raper et al., 2017). To date only two studies have examined the impact of early hippocampal lesions on HPA axis functioning, although there appears to be no difference during infancy (Goursaud et al., 2006), neonatal hippocampal lesions resulted in blunted cortisol stress response in adulthood (Raper et al., 2017). Combined these data suggest that neonatal hippocampal lesions have an age-dependent effect on emotional reactivity and HPA axis regulation.

The current project is part of a longitudinal study investigating cognitive (Glavis-Bloom et al., 2013; Heuer and Bachevalier, 2011a, 2011b; Zeamer and Bachevalier, 2013; Zeamer et al., 2010) and socio-emotional development (Raper et al., 2017; Goursaud and Bachevalier, 2007) in rhesus monkeys with neonatal hippocampal lesions. The present study focused on responsiveness to conditioned and unconditioned (social and nonsocial) aversive stimuli and on probing the pituitary-adrenal dysfunction previously detected in these same animals. We predicted that monkeys with neonatal hippocampal lesions would display decreased responsiveness toward unconditioned aversive stimuli, but spared fear potentiated startle response based on previous literature (Bliss-Moreau et al., 2011a, 2010; Antoniadis et al., 2007; Raper et al., 2017). We also predicted that early hippocampal damage would impair negative feedback inhibition of the HPA axis resulting in long-term alterations of pituitary and adrenal functioning.

## Methods and materials

The methods and procedures most relevant to understanding the behavioral and neuroendocrine assessments are provided below. Complete detailed methods for rearing, neuroimaging, neurosurgery, and estimation of lesion extent have been previously published (Glavis-Bloom et al., 2013; Heuer and Bachevalier, 2011a, 2011b; Zeamer and Bachevalier, 2013; Zeamer et al., 2010; Goursaud and Bachevalier, 2007; Meng et al., 2014, 2016) and are also located in Supplemental Materials. Surgical procedures were performed at the University of Texas Health Science Center (UTHSC, Houston, TX), whereas behavioral testing and neuroendocrine measures during adulthood was performed at the Emory National Primate Research Center (ENPRC, Atlanta, GA). At both institutions, animals were housed under a 12 h light/dark cycle and all procedures were approved by the respective Institutional Animal Care and Use Committees of the UTHSC and of Emory University.

## Subjects

Thirteen adult rhesus monkeys (*Macaca mulatta*) were tested longitudinally between 4 and 10 years of age (Fig. 1). Animals received bilateral neonatal neurotoxic lesions of the hippocampus (Neo-Hibo; 2 females, 4 males) or sham operations (Neo-C; 4 females, 3 males) between 1 and 3 weeks of age. The extent of hippocampal cell loss (see Fig. 2 and Supplemental Table 1) varied from bilateral and roughly symmetrical in Neo-Hibo-2 and -3 (L-R: 53.6%–79% and 63.4%–42.0%) to mostly unilateral in Neo-Hibo-1 and -4 (L-R: 65.6%–23.8% and 33.9%–69.9%, respectively), whereas Neo-Hibo-6 had minor bilateral hippocampal cell loss located in the uncus (L-R: 23.8%–7.5%).

## Approach avoidance task

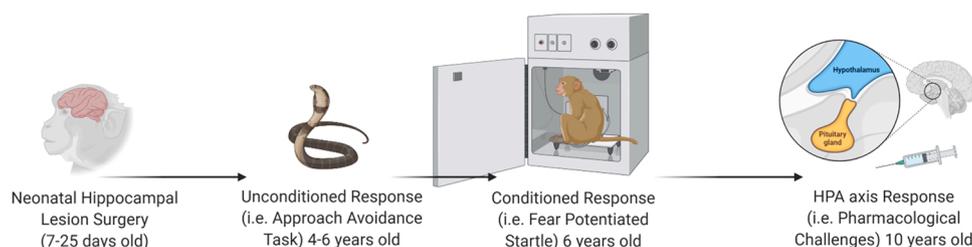
Between 4 and 6 years of age, responses toward unconditioned stimuli, novel nonthreatening or potentially aversive objects, were examined using the Approach Avoidance Task following previously published protocols (Machado et al., 2009; Medina et al., 2020), and will be briefly summarized below. Due to prior experience (Kazama et al., 2012; Kazama and Bachevalier, 2012, 2013), animals were already acclimated to testing in a Wisconsin General Testing Apparatus (WGTA) where the current testing took place.

Sixteen inanimate objects were presented, eight were intended to be aversive or potentially threatening emotional valence, whereas the remaining eight were intended to be neutral items of similar size and shape. The aversive items were specifically selected to be either items that the animals innately feared (rubber snake, spider) (Chudasama et al., 2009; Mineka, 1987; Mineka et al., 1980; Izquierdo et al., 2005), items common to the nonhuman primate laboratory that can elicit fear (capture net and handling gloves), or items with a social component of direct eye contact (girl doll, Mr. Potato Head, Elmo, and SpongeBob toys) (Bliss-Moreau et al., 2011b, 2010; Machado et al., 2009; van Hooff, 1967; Chevalier-Skolnikoff, 1973). An example item from each aversive category can be seen in Fig. 3.

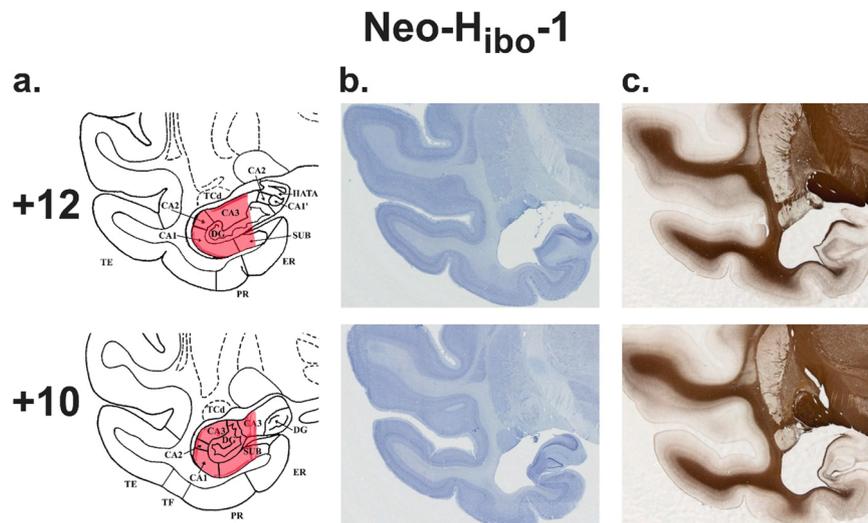
Neutral/aversive stimulus pairs were presented daily, without replication, to measure the animals' emotional reactivity without the influence of experience or habituation. A seedless red grape was paired with each of the items to motivate their approach. A given pair of neutral and aversive objects was presented within a block of four 1-min trials: (a) Baseline Trial—nothing presented on the test tray, (b) Grape Only—grape presented in the center food well, (c) Neutral Item and (d) Aversive item—neutral/aversive item was positioned 2 cm behind the grape in the center food well. Two four-trial blocks occurred each day, and each trial was separated by a 30-s intertrial interval. During each trial, animals could take or manipulate the grape and object freely. To control for circadian effects on the animals' motivation, testing was done between 1000 hr and 1200 hr, and testing order was randomly generated and counterbalanced between groups.

## Behavioral measures

Animals' responses toward the neutral/aversive stimuli were coded



**Fig. 1.** Schematic of the study design. First infant rhesus monkeys received selective bilateral lesions of the hippocampus at 7–25 days of age. At 4–6 years of age, monkeys were tested for their responses toward unconditioned and conditioned stimuli. At 10 years of age, responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis was examined in the same monkeys. Imaged created in BioRender.



**Fig. 2.** Hippocampal lesion extent for a single case (Neo-Hibo-1): Atlas drawing outlining the extent to damage in pink (a), Corresponding photomicrographs of 50  $\mu\text{m}$  coronal sections of the left hippocampus stained for cell bodies with Nissl (b), and fiber tracts with silver impregnation (c).

using a detailed ethogram (Supplemental Table 2) and The Observer XT 10 software package (Noldus Inc., Netherlands) by one experimenter blinded to the animals' treatment. Prior to coding, the experimenter reached an average inter-rater reliability of Cohen's Kappa = 0.90 with another experimenter; and intra-rater reliability Cohen's Kappa = 0.96.

#### Fear-potentiated startle

At 6 years of age, 12 of the 13 monkeys (Neo-C, 3 females, 3 males; Neo-Hibo, 4 females, 2 males) were tested on AX+/BX- Fear-Potentiated Acoustic Startle Paradigm. All methods have been previously described in detail (Kazama et al., 2012).

Monkeys were seated in a primate chair positioned on a platform connected to a load cell (Med Associates, St. Albans, VT) in a sound attenuated chamber with automated delivery of unconditioned and conditioned stimuli. Two unconditioned stimuli (US) were a 700 msec jet of compressed air (100 PSI) projected via four air jet nozzles directed at the face of the monkey, and a startle stimulus consisting of a 50 msec burst of white noise (5 msec rise-decay time), which varied in intensity (range: 95–120 dB). There were three conditioned stimuli (CS) identified as cues A, B, and X. First, the visual CS was a 4 s light generated by an 8 W fluorescent bulb (100  $\mu\text{sec}$  rise time, 700-foot lamberts). Second, an auditory CS was produced by a white noise generator and bandpass filtered, with both the low and high passes set at 2 kHz (24 dB/octave attenuation), at an intensity of 65 dB. Third, a tactile CS was generated by a quiet computer fan that produced a gentle airflow directly onto the monkey's head. The cue assignments (A, B, or X) were pseudo-random and counter-balanced across groups.

For the purpose of assessing baseline acoustic startle, animals were placed in the apparatus and exposed to two days of 60-trial sessions each, composed equally of baseline activity trials (10 trials), and startle noises of varying decibel intensities (95, 100, 110, 115, & 120 dB; 10 trials each), all pseudo-randomly ordered throughout each session. Animals were then tested for pre-pulse inhibition (Heuer et al., 2010) before moving on to the AX+ /BX- paradigm and extinction testing (Kazama et al., 2012) and Supplemental Materials).

#### Pharmacological challenges of the HPA axis

At 10 years of age, the long-term effects of neonatal hippocampal damage on HPA axis function were examined in twelve animals (Neo-C, 4 females, 2 males; Neo-Hibo, 4 females, 2 males). A separate report of blunted diurnal cortisol rhythm and stress hypo-reactivity was already

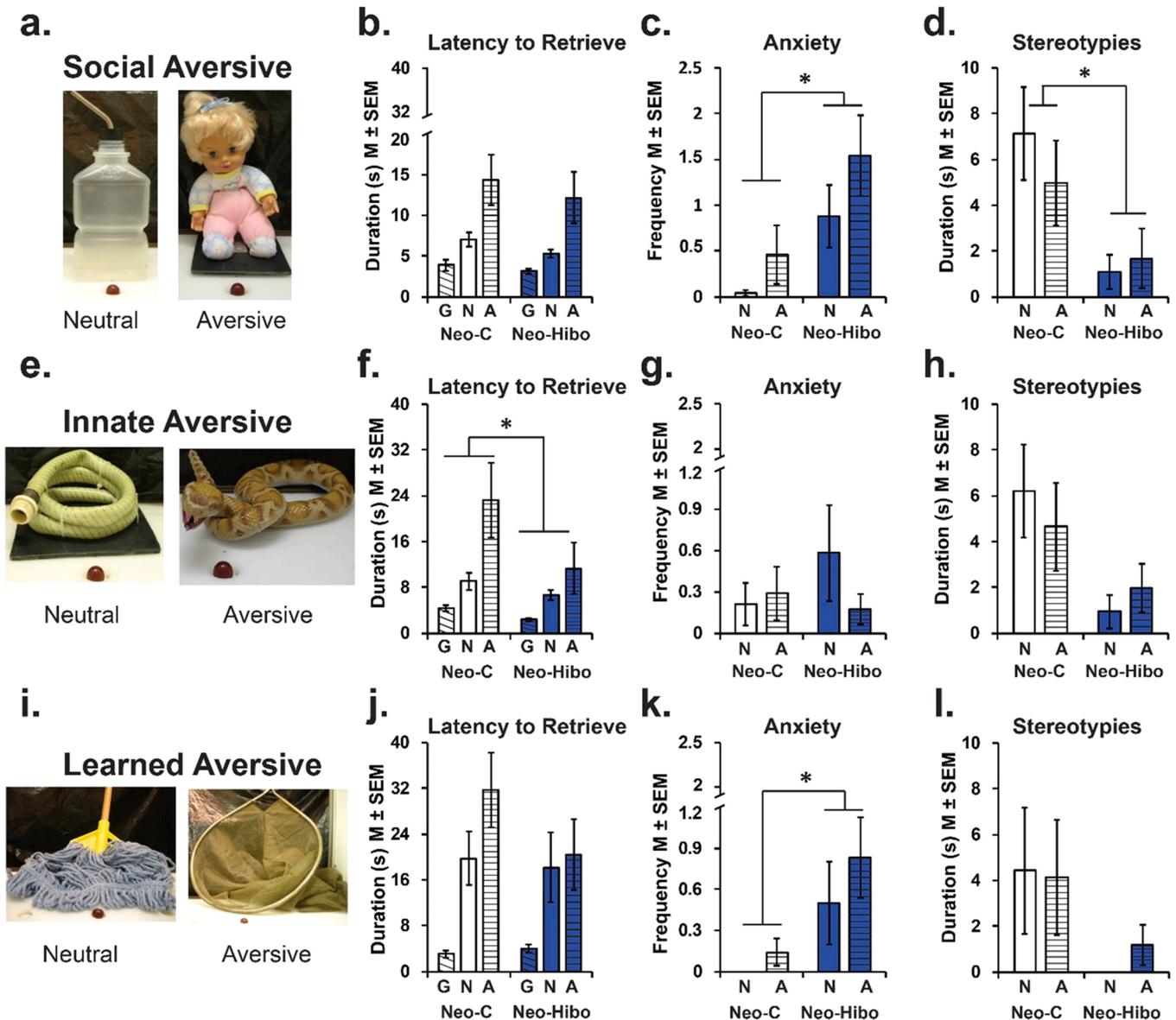
published for these animals at 5–6 years of age (Raper et al., 2017).

To measure the functioning of the pituitary-adrenal system, all animals received injections of either corticotropin releasing factor (CRF), adrenocorticotropic hormone (ACTH), vehicle (saline), metyrapone, or vehicle (DMSO) following a counterbalanced design for drug order and with a minimum 1-week interval between drug treatments. Due to extensive training animals received previously for unanesthetized blood sample collection from the saphenous vein (Raper et al., 2017), challenges were performed under awake conditions following previously published protocols (Sanchez et al., 2010; Raper et al., 2014).

Briefly, animals were accessed at lights-on (0700 hr), before feeding or other routine care procedures to avoid both meal- or arousal-induced HPA axis activations. Once removed from their home cage, a baseline blood sample (0 min) was taken within 10 min from disturbance. An intravenous bolus of r/h CRH (25  $\mu\text{g}/\text{kg}$ ), ACTH (1  $\mu\text{g}/\text{kg}$ ), or a vehicle solution (10 mM acetic acid/sterile 0.9% saline) was administered into the saphenous vein opposite to that used to draw blood from the animal, and additional blood samples were collected from the femoral vein at 30 and 60 min after the injection. For metyrapone and corresponding vehicle (DMSO) challenges, animals were given an intramuscular injection of metyrapone (30 mg/kg) or vehicle (DMSO) at 0900 hr (after routine care but prior to feeding) followed by a blood sample 2 h later. All blood samples were collected in pre-chilled 2 ml tubes containing EDTA (3.6 mg) and immediately placed on ice. Samples were centrifuged at 3000 rpm for 15 min in a refrigerated centrifuge (at 4  $^{\circ}\text{C}$ ). Plasma was stored at  $-80^{\circ}\text{C}$  until assayed.

#### Hormone assays

All plasma assays were performed by the ENPRC Biomarker Core Laboratory. Plasma samples for CRF/ACTH/vehicle challenges, and metyrapone tests were assayed for cortisol in duplicate 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  $\mu\text{g}/\text{dl}$  with intra- and inter-assay coefficients of variation < 15%. 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 6.40 pg/ml with 4.8% intra-assay coefficients of variation.



**Fig. 3. Behavioral responses toward neutral and aversive stimuli.** (a, e, i) illustrate an example of neutral (N, solid bars) and aversive (A, horizontal striped bars) stimuli across different category types.  $M \pm SEM$  behavioral responses on Social (b-d), Innate (f-h), and learned (j-l) aversive categories. Latency to retrieve the grape (G, diagonal striped bars) reward (b, f, j), frequency of anxiety behaviors (c, g, k), and duration of stereotypies (d, h, l). Blue bars indicate neonatal hippocampal lesions (Neo-Hibo) and white bars indicate sham-operated controls (Neo-C). \* indicates a significant difference between groups ( $p < 0.05$ ). All Rights Reserved for photos copyright © Emory National Primate Research Center, Emory University. See the online article for the color version of the figure.

**Data analyses**

For the Approach Avoidance task, prior to data analysis, Kolmogorov-Smirnov (K-S) tests examined the normality of behavioral data. When behaviors were not normally distributed, they were transformed using a natural log plus constant to obtain normality. For the purposes of interpretation, raw data (means and variance indices) are presented. For trials in which animals did not take the food or explore the objects, the latency data were scored as the maximum length of the trial (60 s). The impact of early hippocampal damage on the response toward neutral/aversive stimuli was examined separately for each aversive category (Social, Innate, Learned) using a General Linear Mixed-Models ANOVA (LMM) with Group (Neo-C, Neo-H) and stimulus condition (grape, neutral, aversive for latency to retrieve; neutral, aversive for all other behaviors) as the within subjects' factor with repeating measures.

For the Fear Potentiated Startle, the primary parameter was the percent fear potentiated startle (FPS) defined as: [Mean startle

amplitude on CS test trials – mean startle amplitude on startle noise alone test trials]/[mean startle amplitude on noise burst alone test trials] X 100. If during training, an animal's % FPS showed a steady decline and no improvement over an extended period, that animal was given a maximum score of 15 sessions (which was determined after testing Neo-Hibo-5 out to 15 days with no improvement). If startle values were not normally distributed (Winslow et al., 2008) then they were transformed using natural log plus constant to obtain normality. The data for Acoustic startle response and discrimination between test phase were examined using a LMM with group (Neo-Hibo, Neo-C) as fixed factors and startle amplitude (95, 100, 110, 115, & 120 dB) or test phase (A, AX, B, BX, AB) as the within subjects factor with repeating measure. Control animals learned and extinguished the aversive and safety cues in the minimum number of sessions (e.g., 2 sessions per phase) thus non-parametric Mann-Whitney U test was used to compare group (Neo-Hibo, Neo-C) differences in the animal's ability to associate and discriminate between the aversive and safety cues (A+, B-, AX+, BX-), as

well as their ability to extinguish their startle response.

For CRF and ACTH challenges, the area under the curve with respect to increase (AUC<sub>i</sub>) was used to measure the accumulative change in cortisol secretion in response to either CRF, ACTH, or vehicle injections, from baseline and over time (0, 30, and 60 min post-injection; (Pruessner et al., 2003)). LMM with drug (vehicle vs ACTH- or CRF-) and group (Neo-H, Neo-C), as fixed factors was used to analyze AUC<sub>i</sub> cortisol response. For metyrapone challenge, the difference in ACTH secretion between metyrapone and vehicle challenge was calculated. An independent *t*-test was used to compare groups (Neo-Hibo, Neo-C) with the difference in ACTH as the dependent variable.

Pearson's correlations examined the relationship between the extent of hippocampal cell loss and behavioral or pharmacological responses; however, no significant correlations were found or lesion extent. Pearson's correlations were also performed to examine the potential relationship between behavioral and pharmacological response. The animal (Neo-Hibo with the highest anxiety expression during aversive social stimuli on the Approach Avoidance task was excluded from those

correlations for being an outlier. All analyses were conducted with SPSS 26 for Windows, a  $p < 0.05$  was considered significant, and effect sizes (partial eta squared or Cohen's *d*) were calculated.

## Results

### Approach avoidance

Fig. 3 illustrates that Neo-Hibo animals took less time than controls to retrieve the grape in the presence of an innate aversive stimulus, (Group:  $F_{(1,24)} = 4.93$ ,  $p = 0.036$ ,  $\eta_p^2 = 0.17$ , Fig. 3f). Yet, this group difference did not reach significance in the presence of social or learned aversive stimuli as Neo-Hibo did not differ from controls (Group:  $F_{(1,50)} = 1.92$ ,  $p = 0.17$ ,  $\eta_p^2 = 0.48$ ;  $F_{(1,24)} = 0.41$ ,  $p = 0.53$ ,  $\eta_p^2 = 0.02$ , respectively, Fig. 3b, j). Interestingly, Neo-Hibo exhibited more anxious behaviors in response to social and learned stimuli, (Group:  $F_{(1,50)} = 5.049$ ,  $p = 0.029$ ,  $\eta_p^2 = 0.092$ ;  $F_{(1,24)} = 6.530$ ,  $p = 0.017$ ,  $\eta_p^2 = 0.214$ , respectively, Fig. 3c, k), but there was no group difference for Neo-C and Neo-

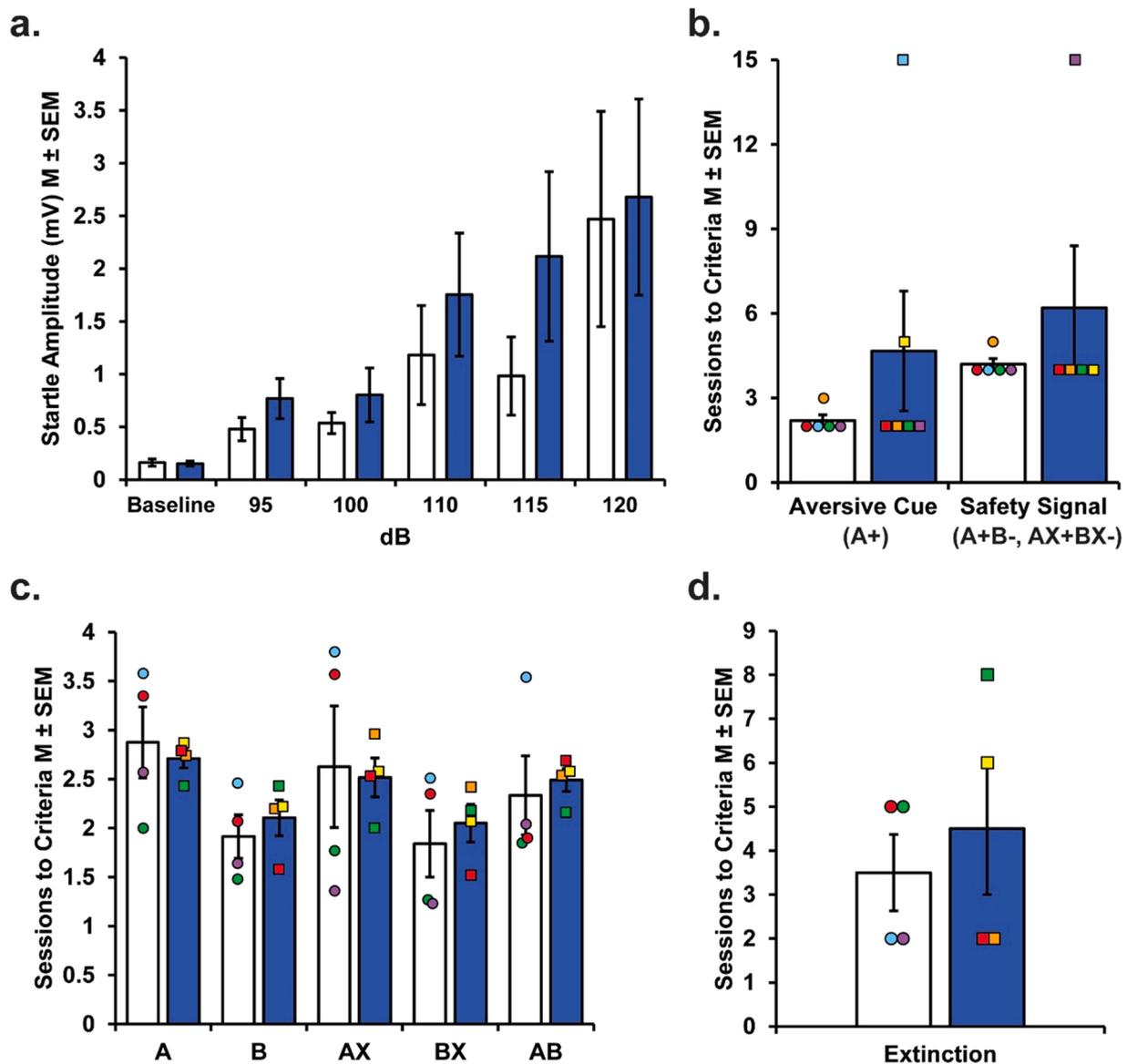


Fig. 4. Lack of impairment in Fear Potentiated Startle among Neonatal Hippocampal Lesioned monkeys. Data represent mean  $\pm$  SEM of the startle response (a), number of sessions to learn the aversive and safety cues (b), fear potentiated startle response (c) and number of sessions until extinction (d). Blue bars indicate neonatal hippocampal lesions (Neo-Hibo) and white bars indicate sham-operated controls (Neo-C). Performance of individual animals are represented by circles (Neo-C) or squares (Neo-Hibo).

Hibo responses to innate stimuli (Group:  $F_{(1,24)} = 0.28$ ,  $p = 0.60$ ,  $\eta_p^2 = 0.01$ , Fig. 3g). The expression of stereotypies differed between groups in response to social stimuli (Group:  $F_{(1,50)} = 4.288$ ,  $p = 0.044$ ,  $\eta_p^2 = 0.079$ , Fig. 3d), such that Neo-Hibo animals exhibited fewer stereotypies than controls. No differences were detected for stereotypy expression in response to innate or learned stimuli ( $F_{(1,24)} = 1.53$ ,  $p = 0.23$ ,  $\eta_p^2 = 0.06$ ;  $F_{(1,24)} = 1.46$ ,  $p = 0.24$ ,  $\eta_p^2 = 0.06$ , respectively Fig. 3h, l). There were no group differences in the amount of time spent manipulating objects, duration engaged in self-directed behaviors, number of expressions of fearful, affiliative, or hostile behaviors (see Supplemental Table 3).

#### Fear-potentiated startle response

As previously reported by Heuer and colleagues (Heuer et al., 2010), the baseline startle response of two animals in Group C (cases Neo-C-2 and Neo-C-6) was greater than the maximum amplitude of the load cell. Thus, these two animals were dropped from the study. As illustrated in Fig. 4a, the magnitude of the startle responses increased with progressive rises in the amplitude of the startle noise (Startle amplitude:  $F_{(5, 40)} = 14.76$ ,  $p < 0.01$ ,  $\eta_p^2 = 0.65$ ), and Neo-Hibo did not differ from controls (Group:  $F_{(1,8)} = 0.44$ ,  $p = 0.53$ ,  $\eta_p^2 = 0.05$ ). There were no group differences in their ability to learn to associate Cue A+ with the air blast ( $U = 12.00$ ,  $p = 0.49$ ) or in their ability to learn the safety cues (A+B, AX+BX) within the minimum number of sessions ( $U = 8.00$ ,  $p = 0.37$ , Fig. 4b). Both groups were able to discriminate the aversive and safety cues, as evidence by greater startle response to the aversive (A, AX) cues compared to either the safety (B, BX) or transfer (AB) cues (Test phase:  $F_{(4,24)} = 7.33$ ,  $p = 0.001$ ,  $\eta_p^2 = 0.55$ ). Fig. 4c illustrates the similar discrimination ability in acoustic startle response (Group:  $F_{(1,6)} = 0.023$ ,  $p = 0.88$ ,  $\eta_p^2 = 0.004$ ). The ability to extinguish the startle response of the aversive cues (A-, AX-) were also similar between groups ( $U = 6.00$ ,  $p = 0.54$ ).

#### Pharmacological challenge

Pituitary response to CRF administration was similar between Neo-Hibo and Neo-C with both groups mounting a similar cortisol AUCi response to CRF administration (Drug:  $F_{(1,23)} = 257.05$ ,  $p < 0.001$ ,  $\eta^2 = 0.78$ ), and no group differences (Group:  $F_{(1,23)} = 2.97$ ,  $p = 0.1$ ,  $\eta^2 = 0.13$ ; Fig. 5a). In contrast, adrenal response to ACTH administration revealed a Drug X Group interaction, indicating that Neo-Hibo animals had a blunted cortisol AUCi response compared to controls ( $F_{(1,23)} = 6.97$ ,  $p = 0.016$ ,  $\eta^2 = 0.26$ ; Fig. 5b). When released from

glucocorticoid negative feedback with a metyrapone challenge, both groups responded with increased ACTH secretion, although this increase was less in Neo-Hibo animals than controls ( $T_{(10)} = 2.03$ ,  $p = 0.035$ , Cohen's  $d = 1.17$ ; Fig. 5c).

Correlations were performed to examine the potential relationship between behavior and responses toward pharmacological challenges. There was a significant negative correlation ( $r_{(11)} = -0.66$ ,  $p = 0.014$ ) for cortisol responses to an ACTH Challenge corresponding with lower anxiety during trials of aversive social stimuli on the Approach Avoidance task (Fig. 6a). In contrast, a positive correlation ( $r_{(12)} = 0.50$ ,  $p = 0.049$ ) was revealed for ACTH challenge and latency to retrieve a reward (see Fig. 6b), such that animals with higher cortisol response to ACTH had longer latencies and were more reluctant to retrieve a reward in the presence of learned aversive stimuli. No significant correlations were found between any fear potentiated startle measure and any pharmacological challenge.

#### Discussion

The current study used conditioned and unconditioned stimuli as well as social and nonsocial stimuli to investigate the long-term impact of early hippocampal lesions on threat detection. Animals with neonatal hippocampal lesions showed more anxious behaviors than controls in response to unconditioned social and learned aversive stimuli, but reduced reactivity to innate aversive stimuli. The ability to learn and distinguish conditioned aversive or safety cues was not impacted by neonatal hippocampal lesions. The current study also demonstrated that Neo-Hibo animals had a blunted response to ACTH and metyrapone administration, but did not differ from controls after CRF administration. Together these findings add important information about the role of the hippocampus in the development of defensive behaviors and pituitary-adrenal functioning.

Monkeys with neonatal hippocampal lesions showed reduced reactivity toward innately aversive stimuli, retrieving the grape faster than controls. This result contradicts previous findings indicating that neonatal hippocampal lesioned monkeys exhibited a species typical hesitation (i.e., longer latency) to retrieve a food reward from reptile-like objects (Bliss-Moreau et al., 2011b, 2010). Discrepancies between food retrieval latencies have also been detected in monkeys with the adult-onset hippocampal lesions, with some studies reporting faster latencies (Chudasama et al., 2009, 2008), whereas others did not (Machado et al., 2009). Despite their decreased food latency, the Neo-Hibo animals exhibited little object manipulation of innately aversive objects compared to controls, similar to a previous study

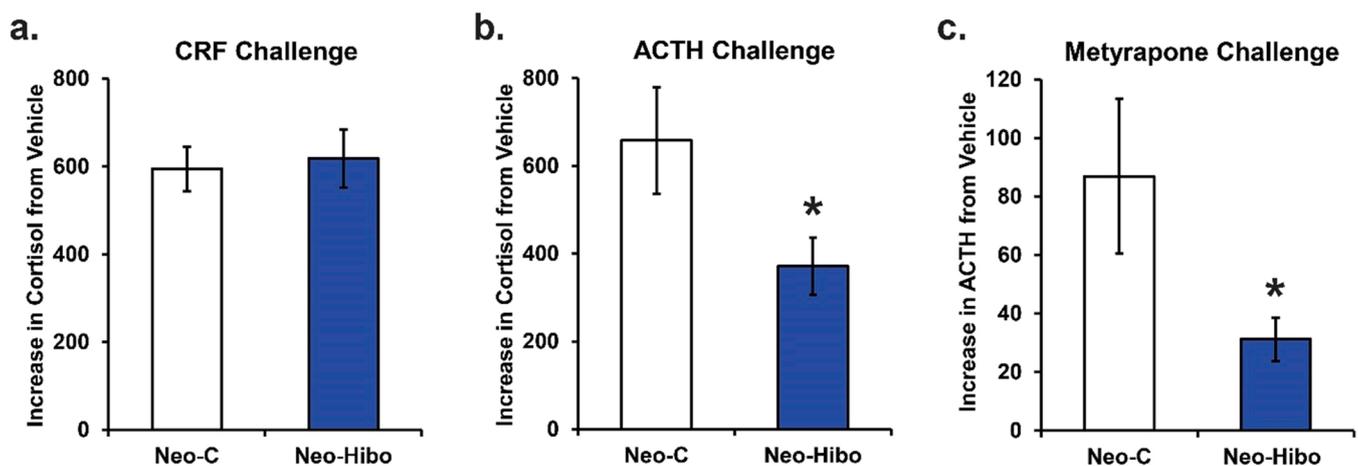
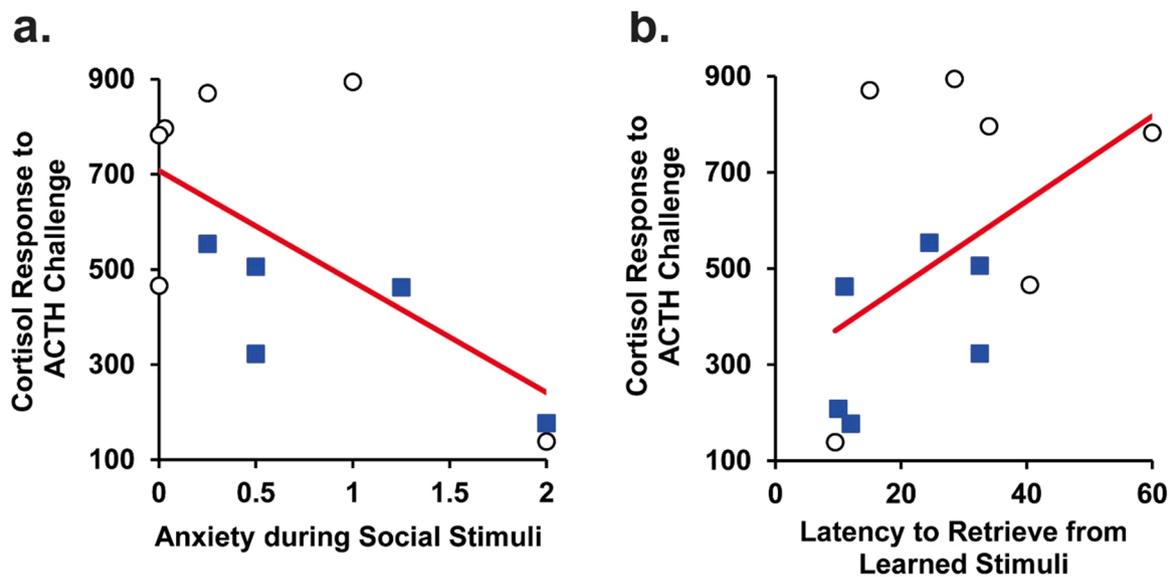


Fig. 5. Decreased adrenal responsiveness among Neonatal Hippocampal Lesioned monkeys. Data represent mean  $\pm$  SEM increase of the Cortisol AUCi from saline vehicle challenge to (a) CRF challenge or (b) ACTH Challenge. Increase in ACTH from DMSO vehicle challenge to (c) Metyrapone challenge. Blue bars indicate neonatal hippocampal lesions (Neo-Hibo) and white bars indicate sham-operated controls (Neo-C).



**Fig. 6. Adrenal responsiveness corresponds with behavioral reactivity toward objects.** Correlations between mean increase of the cortisol AUCi from saline vehicle challenge to an ACTH Challenge and (a) anxiety expressed during aversive social stimuli trials and (b) latency to retrieve a reward from learned aversive stimuli trials. Blue squares represent individual neonatal hippocampal lesions (Neo-Hibo) animals, while open circles represent individual sham-operated controls (Neo-C). Red trend line indicates the direction of the relationship between the factors.

(Bliss-Moreau et al., 2011b). Our results suggest that early hippocampal damage impacts select behavioral responses (food retrieval only) toward innately aversive stimuli.

Social stimuli were objects with facial features making direct eye contact with the monkeys, which is a highly threatening gesture in macaques (Bliss-Moreau et al., 2011b, 2010; Machado et al., 2009; van Hooff, 1967; Chevalier-Skolnikoff, 1973). Neo-Hibo animals did not differ from controls in many of their responses toward aversive social stimuli, including grape retrieval latency, object manipulation, fearful, hostile, affiliative, and self-directed behaviors. However, the two groups differed in their expression of anxious-like behaviors and stereotypes. Thus, Neo-Hibo animals exhibited more anxious-like behaviors and decreased stereotypic behaviors compared to controls. These changes in anxious-like behaviors replicate our previous report of increased anxious expression in these same animals during an acute social stress test (Raper et al., 2017). However, the decreased expression of stereotypes in response to social stimuli contradicts a previous report of increased stereotypic behaviors in social settings (Bliss-Moreau et al., 2017; Bauman et al., 2008; Beauregard et al., 1995). Increased stereotypes were discovered in behavioral observations of significantly longer duration (~100 min) and with familiar conspecifics as compared to the 1-minute presentation of static social stimuli used in the current task. Thus, this shorter behavioral observation and static objects may explain the lack of a difference observed in the current study.

In response to already learned aversive stimuli, Neo-Hibo animals only differed from controls in their expression of more anxious behaviors, no group differences were found for grape retrieval latency, object manipulation, fearful, hostile, affiliative or self-directed behaviors. Thus, despite their loss of recognition memory (Zeamer and Bachevalier, 2013; Zeamer et al., 2010), Neo-Hibo animals are able to remember stimuli with which they have learned a negative association in the past. In addition, fear potentiated startle testing revealed that animals with neonatal hippocampal lesions were also able to learn to fear new neutral cues and to discriminate between aversive and safety cues. The present study complements previous findings that adult-onset hippocampal lesions in monkeys spare the ability to acquire a learned fear (Antoniadis et al., 2007). This spared ability is also consistent with the notion that the hippocampus is involved only during contextual fear conditioning and not when the predictive unconditioned stimulus is a discrete cue (Blair and Fanselow, 2014). It could be argued that learning to pair one

cue with an air-blast, and another cue with the lack of an air-blast does not require contextual learning, and thus would not be hippocampal dependent (for review, see (Holland and Bouton, 1999)). Therefore, if safety-signal learning in this task is independent of context, one might predict a lack of impairment following hippocampal damage, a prediction consistent with the current findings. In contrast, adult monkeys that received early damage to the amygdala were impaired in their ability to learn but not express conditioned fear (Kazama et al., 2012). Given the interconnectivity between the amygdala and anterior hippocampus (Weiss et al., 2021), the current findings may inform our understanding of the anterior hippocampus' role in encoding emotional stimuli and provide a complement to work done in human neuroimaging (e.g., (Clewett et al., 2022)). At the very least, these data suggest that other compensatory areas can be utilized after early damage. It has previously been suggested that the Bed Nucleus of the Stria Terminalis, with its strong connections to the hippocampus may compensate for emotional processing (Kazama et al., 2012), but it remains to be seen if a structure like the striatum may play a compensatory role to the hippocampus after early insult. Future studies may further investigate the functional contributions via temporary inactivation studies, which could rule out compensatory mechanisms and further elucidate emotional memory formation.

Responses to HPA axis stimulation revealed that Neo-Hibo animals did not differ from controls in their cortisol response to CRF administration but did show a dampened cortisol response to ACTH administration, in line with previous work (Goursaud et al., 2006). We also found that when metyrapone was used to release the HPA axis from negative feedback, Neo-Hibo animals showed a dampened increase in ACTH secretion compared to controls. The combination of a blunted response to ACTH stimulation of the adrenal cortex and blunted response to metyrapone suggests that adult animals with neonatal hippocampal lesions may be suffering from adrenal exhaustion (Kannan, 1988). This may also explain the blunted cortisol response to an acute stressor and flattened diurnal cortisol rhythm detected in these same animals previously (Raper et al., 2017). Electrical stimulation studies as well as those reporting the presence of high glucocorticoid levels in the hippocampus have implicated its key role in the negative feedback control of the HPA axis (Herman et al., 2003). This hippocampal involvement in HPA axis feedback control may be more critical during development since adult-onset hippocampal damage in monkeys results

in only a transient rise in cortisol, which returns to normal within a few months (Jacobson and Sapolsky, 1991). Combined with a previous report that neonatal hippocampal lesions impaired negative feedback suppression in infant monkeys (Goursaud et al., 2006), our data suggest that a transient rise in glucocorticoids during infancy leads to life-long alterations of HPA axis functioning ultimately resulting in adrenal exhaustion in adulthood.

As one might expect a blunted stress response corresponds with lower reactivity toward aversive stimuli, such that animals with lower cortisol response to an ACTH challenge were more willing to quickly retrieve a reward when a learned aversive stimulus was present. Interestingly, the opposite is true for the expression of anxiety, such that lower cortisol corresponds with higher anxiety toward social aversive stimuli. Human patients with hippocampal damage have been reported to have higher negative affect ratings after an acute stress test despite exhibiting a blunted cortisol response to the test (Buchanan et al., 2009). Taken together these data suggest an interesting and opposite relationship between cortisol and anxiety expression as compared to cortisol and fearful responses.

The current behavioral and HPA axis findings did not correlate with the extent of hippocampal cell loss. Hippocampal lesions varied from 66% in one case to only 15% in another, with all other cases between 45% and 50% range. However, in all cases (even the smallest lesion) the damage included the anterior portion of the hippocampus (see (Glavis-Bloom and Bachevalier, 2018)). The anterior hippocampus has dense projections with the amygdala and ventromedial prefrontal cortex (Aggleton, 1986; Ongür and Price, 2000; Alexander et al., 2019a, 2019b), two structures critical for emotional regulation (see review (Aggleton, 2012)). In addition, an earlier neuroimaging study on these animals reported white matter changes in ventromedial prefrontal cortex onto which the hippocampal-fornix efferent fibers terminate (Meng et al., 2014). Thus, it is likely that the behavioral changes are the result of damage to the anterior hippocampus in all six cases.

The present results have important clinical implications. Reduced hippocampal volume has been reported in patients with post-traumatic stress disorder (PTSD) (O'Doherty et al., 2015; Logue et al., 2018), decreased hippocampal activity can predict future PTSD severity (van Rooij et al., 2018), and may be a common vulnerability factor for anxiety and depression (van Tol et al., 2012). Further, evidence of abnormal interactions between the amygdala, hippocampus, and medial prefrontal cortex is a common feature of this disorder (Liberzon and Sripada, 2008; Shin et al., 2006). Lower cortisol secretions are also common in patients with PTSD or anxiety disorders from early life adversity (Yehuda et al., 2001; Daskalakis et al., 2013; Steudte-Schmiedgen et al., 2015; van der Vegt et al., 2009). Thus, the long-term changes in anxious behavior expression and HPA axis dysfunction reported here following early hippocampal insult, are strikingly similar to those reported in clinical populations. Utilizing developmental animal models to examine the neural circuitry subserving the regulation of emotions and neuroendocrine function can provide a foundation for understanding the neuroanatomical and neuropathological basis of human neuropsychiatric disorders.

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### CRediT authorship contribution statement

**Emma McKeon:** Formal analysis, Writing – original draft. **Jennifer Torres:** Data curation, Writing – review & editing. **Andrew M. Kazama:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **Jocelyne Bachevalier:** Conceptualization, Funding

acquisition, Writing - review & editing. **Jessica Raper:** Data curation, Formal analysis, Conceptualization, Writing - review & editing.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

The data that support the findings of this study are available within the article, its supplementary materials, and from the corresponding author, JR, upon reasonable request.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.dcn.2022.101165](https://doi.org/10.1016/j.dcn.2022.101165).

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