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The effects of neonatal amygdala or hippocampus lesions on adult social behavior

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Abstract

The present report details the final phase of a longitudinal evaluation of the social behavior in a cohort of adult rhesus monkeys that received bilateral neurotoxic lesions of the amygdala or hippocampus, or sham operations at 2 weeks of age. Results were compared to previous studies in which adult animals received amygdala lesions and were tested in a similar fashion. Social testing with four novel interaction partners occurred when the animals were between 7 and 8 years of age. Experimental animals interacted with two male and two female partners in two conditions — one in which physical access was restricted (the *constrained social access condition*) and a second in which physical access was unrestricted (the *unconstrained social access condition*). Across conditions and interaction partners, there were no significant effects of lesion condition on the frequency or duration of social interactions. As a group, the hippocampus-lesioned animals generated the greatest number of communicative signals during the constrained social access condition. Amygdala-lesioned animals generated more frequent stress-related behaviors and were less exploratory. Amygdala and hippocampus-lesioned animals demonstrated greater numbers of stereotypies than control animals. Subtle, lesion-based differences in the sequencing of behaviors were observed. These findings suggest that alterations of adult social behavior are much less prominent when damage to the amygdala occurs early in life rather than in adulthood.

Keywords

amygdala; hippocampus; social behavior; Macaca mulatta; Rhesus macaque; development

1. Introduction

Classic studies of the primate amygdala point to its involvement in affective and social processing. When amygdala damage occurs in adulthood, primates fail to respond to threat and novelty in normative ways. While intact animals are wary of novel and threatening objects, animals with amygdala damage show no such wariness [1–8]. This failure to

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appropriately assess the potential threat value of stimuli likely underlies patterns of hyper-sociality following amygdala damage [9]. Without an amygdala to signal that a conspecific is potentially threatening, adult animals with amygdala damage readily approach and interact with novel conspecifics (e.g.,[10–14]). Studies of adult amygdala damage have left unanswered questions about the importance of the amygdala for the development of normal affective and social behavior. Evaluating whether or not the amygdala is required for the development of normal social behavior has been the focus of a unique, long-term longitudinal study that concludes with this report.

Accumulating evidence suggests that early amygdala damage does not disrupt the generation of species-typical primate social behaviors per se—that is, animals with damage to the amygdala can physically generate species-typical affective and social behaviors [15–24]. However, the effects of damage to the amygdala across development are largely unknown. To address these questions, we initiated a long-term, longitudinal study in 2001 in which a cohort of rhesus macaques received neurotoxic lesions of the amygdala or hippocampus at approximately two weeks of age and their affective and social behavior was accessed across their entire lives (e.g.,[15, 25–30]). Both operated peers (animals who received neonatal hippocampus lesions) and intact peers served as controls in these studies. The present report provides evidence that social behavior during adulthood, following early damage to the amygdala, is largely intact and comparable to that of control animals. Animals were tested in the same setting that was previously used to evaluate the social behavior of adult animals with adult amygdala damage [10]. To that end, subjects met four novel animals (two males and two females) from the colony at the California National Primate Research Center (*CNPRC*) in two conditions—the first in which one animal was constrained in a small cage behind a metal grille, and the second in which both animals had unlimited access to each other.

2. Methods

Experimental procedures were developed in consultation with the staff at the California National Primate Research Center and protocols were approved by the University of California Davis Institutional Animal Care and Use Committee.

2.1. Animals and Living Conditions

Subjects for the present experiment were twenty-three adult (*M*=8.61 years old, *SD*=0.23) rhesus monkeys who received bilateral ibotenic acid lesions of either the amygdala (four females, three males) or hippocampus (five females, three males), or sham control operations (four females, four males) at approximately 2 weeks of ages. These subjects have undergone extensive, longitudinal study and information about their selection and full rearing histories are available in previous publications [15, 25, 28, 29].

2.2. Surgical Procedures

Subjects underwent surgeries at between 12 and 16 days of age. On the day of surgery, each subject underwent magnetic resonance imaging (MRI) allowing for the idiographic selection of stereotaxic coordinates for injections into the hippocampus or amygdala. For the MRI

exam, subjects were anesthetized with ketamine hydrochloride (15 mg/kg i.m.) and medatomidine (30μg/kg) then placed into an MRI-compatible stereotaxic apparatus (Crist Instruments Co., Inc., Damascus, MD). Imaging was completed using a General Electric 1.5 T Gyroscan magnet (slice thickness= 1.0 mm, T1-weighted Inversion Recovery Pulse sequence, TR = 21, TE =7.9, NEX 3, FOV = 8cm, Matrix 256 × 256).

After imaging, subjects were intubated and prepared for surgery. During surgery, subjects were anesthetized with a mix of isoflurane (approximately 1.0% - but varied to maintain an adequate level of anesthesia) and intravenous infusion of fentanyl (7–10 μg/kg/hour). Subjects undergoing neurotoxic lesions received a craniotomy over both the left and right amygdala or hippocampus. Ibotenic acid (IBO, Biosearch Technologies Inc., 10 mg/ml in 0.1 M phosphate buffered saline) was then injected into either the amygdala or hippocampus using 10 μl Hamilton syringes (26 gauge beveled needles) at a rate of 0.2 μl/min. Injections into the right and left hemispheres occurred simultaneously.

Animals receiving sham-operations were prepared for surgery in the same way and received the same anesthesia. They received a midline incision to expose the skull only. They were maintained under anesthesia for the average duration of the lesion surgeries.

All animals were monitored post-operatively by a veterinarian and veterinary technical staff. They were returned to their mothers once they were awake and alert.

2.3. Rearing and Housing Conditions

Animals were returned to their mothers following surgery and housed in standard primate caging (61 cm W × 66 cm D × 81 cm H). Once all subjects had fully recovered, they were socialized with their mothers and other subject-mother pairs in social groups for three hours each of five days per week. Socialization groups occurred in large indoor enclosures made of chain-link fencing (2.13m W × 3.35m D × 2.44m H). Each social group included six subject-mother pairs with two subjects from each condition, and an adult male. Social groups continued once animals were weaned from their mothers at six months of age and a new adult female was introduced to each group. Subjects were singly housed between six and twelve months of age. At twelve months of age, subjects were permanently housed in large indoor enclosures with their peers and adults that were previously in their social groups.

Animals moved with their current social groups to large outdoor enclosures (6.10m W X 4.27m D X 2.44m H) at approximately 3 years of age and remained there for one year. At the end of that period, they were relocated into standard indoor caging and were paired with compatible social partners for at least 5 hours/day five days per week. At 4 years of age, females were moved into large outdoor enclosures (4.9 m W × 4.3 m D × 2.4 m H) into groups consisting of one female from each lesion condition and one novel adult male (see [31]). Males were moved into smaller outdoor enclosures (2.5 m W × 4.8 m D × 2.1 m H) and paired with another male from the project.

At 6.5 years of age, animals were relocated indoors and then housed in male-female pairs. The present experiment occurred while animals were living indoors in pairs. At the start of

the experiment, all but 4 animals (1 control animal, 1 amygdala-lesioned animal, and 2 hippocampus-lesioned animals) were housed in stable pairs. Each pair was allowed complete access to each other for a minimum of 6 hours per day, 5 days a week.

Indoor housing rooms were maintained on a 12-hour light/dark cycle (lights on at 6 am). Animals were fed monkey chow twice daily inside (Lab Diet #5047, PMI Nutrition International INC, Brentwood, MO) and outside (Lab Diet #5045, PMI Nutrition International INC, Brentwood, MO), and were provided with fresh fruit and vegetables twice per week; water was accessible ad libitum. While housed indoors, animals received an assortment of various sized and shaped enrichment such as pea-oat forage mixture on forage boards.

One of the original amygdala-lesioned males died of causes unrelated to his lesion at approximately 1 year of age [15]. He was replaced by another male that underwent amygdala-lesion surgery at the same time as the present cohort. That subject was reared by his mother for the first year of life and pair housed with an age-matched female after being weaned at 1 year. He was introduced to his social group at 1 year and 3 months of age. A female amygdala-lesioned animal died at approximately 5 years of age; she was not replaced as a subject. The cause of both animals' deaths was deemed unrelated to their lesion condition.

2.4. Experimental Design and Procedures

2.4.1 Test Cage—Testing occurred in a large chain link test cage (5.56 m W × 1.91 m D × 2.13 m H) with two large doors (one at each end of its front panel) with small cages attached to each end previously used in dyadic social testing in our laboratory [10, 32]—See Figure 1. The small cages were separated from the large cage by both a solid door and a door made of metal bars. The doors could be raised and lowered using a pulley system to which the experimenter had access during the experiment. Each subject entered one of the small cages from a standard transport box and then was subsequently held in or released from the small cage as indicated by the test condition (detailed below).

2.4.2 Experimental Design—In all conditions, experimental animals interacted with one of four partner animals. Two male and two female animals (the stimulus animals) were selected from the CNPRC colony based on their social rearing and social housing history. Specifically, animals were raised in the CNPRC's large outdoor field corrals (with between 50 and 200 monkeys) until approximately 4 years of age. Once relocated indoors, these animals were successfully socially housed with compatible social partners. Partner animals were an average of 7.36 years of age (SD=0.61) at the beginning of the present experiment.

Social behaviors were collected using The Observer 5.0 software package (Noldus, 1991) employing the focal sampling technique [33] to record the frequency and duration of species typical behaviors (See Table 1). We recorded two types of behaviors – states and events. States are ongoing behaviors that occur for at least 3 seconds (e.g., grooming, sitting alone) and thus have both duration and frequency. Events are momentary behaviors that have frequency only (no duration). There were 3 observers, one of which was blind to lesion

condition. Observers had an inter-observer reliability of greater than 90%. Each animals' spatial location in the cage was also recorded every 15-seconds.

All experimental animals completed testing in each of two conditions. In the *constrained social access* condition, one animal (either the experimental or stimulus) was released from the small cage to the large enclosure while the second animal remained in the opposite small cage. In this condition, the solid door between the small cage and large enclosure was raised allowing access of the animals to each other through the metal bars of the small cage. This allowed for visual, auditory, and tactile contact. It also enabled the animals to withdraw from an aggressive confrontation. Animals in the large enclosure were the focal animal for the sample. Each experimental-stimulus pair met 6 times (with the experimental animal restricted to the small cage 3 times, and the stimulus animal restricted to the small cage 3 times). Each meeting lasted 10 minutes. Only behaviors generated by the focal animal were analyzed since the observer could not clearly see the animal in the small cage at all times (because he or she moved out of the observers view).

In the *unconstrained social access condition*, both animals (i.e., an experimental animal and a stimulus animal) were released from the small cages into the large enclosure. The experimental animal was always the focal animal. Each experimental-stimulus animal pair was scheduled to meet 6 times for 20 minutes per meeting. We elected not to test one of the stimulus males with most of the experimental males after witnessing substantial aggression during the constrained condition. One of the experimental males did not meet either of the male stimulus animals because of aggression, and one of the experimental males only had 5 (rather than 6) meetings with the second stimulus male. Given these constraints, we evaluated the male-male social behavior interactions in a separate set of analyses which are included in Supplemental Materials. Only the female experimental animals consistently met both the male and female interaction partners.

2.4.3. Spatial Proximity—Spatial location was recorded every 15 seconds. The large test cage was divided into a grid with 9 rows along its length and 3 columns along its width. Grid lines were painted onto the cement floor. Vertical space in the cage was virtually divided into three rows based on metal features of the cage. Each area formed by the three dimensional grid was assigned a point in 3D space and then the distance formula was used to calculate the distances between animals in each area at the time of measurement. Spatial location scoring occurred either live (when an additional observer was available) or from video tape. Spatial scorers were students in the laboratory who reached an inter-rater reliability of greater than 90%.

2.4.4. Histological Analyses—The hippocampus-lesioned animals were perfused when they were approximately 9 years of age (range 9 years 1 month to 9 years 3.5 months). One amygdala-lesioned animal was euthanized for health reasons at 9 years and 22 days of age (after the present experiment). The remainder of the amygdala-lesioned animals continued to participate in behavioral testing for approximately 2.5 additional years. The remaining amygdala-lesioned animals and the control animals were perfused at 11.70 years of age (SD=0.09) on average.

Monkeys were deeply anesthetized with sodium pentobarbital (i.v. delivery; 50 mg/kg, Fatal-Plus, Vortech Pharmaceuticals, Dearborn, MI) and perfused transcardially with 1% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB; pH 7.4) at a rate of 250 mL per minute for 2 minutes. Perfusion solution was then switched to 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB; pH 7.4) at a rate of 250 mL per minutes for 10 minutes with a subsequent reduction of the rate to 100 mL per minute for an additional 50 minutes. Perfusion, cryoprotection and freezing protocols used by the laboratory are as previously described in detail in [34]. Frozen brains were sectioned coronally using a freezing, sliding microtome (Microm HM 440, Microm International, Germany). Hippocampus-lesioned brains were sectioned in eight series at 30μm; amygdala-lesioned brains were sectioned in six series at 30μm and one series at 60μm.

Sections were stored in 10% formaldehyde solution in 0.1 M PB (pH 7.4) for two weeks at 4°C. Sections were removed from the formaldehyde solution, washed, and mounted on gelatin-coated slides. They were air-dried for 12–15 hours at 37°C, then defatted 2 × 2 hours (1:1 chloroform:ethanol, vol). Sections were rehydrated and stained 35 seconds in a 0.25% thionin solution (Fisher Scientific, Waltham, MA, cat. no. T-409), dehydrated through a series of graded ethanol solutions and coverslipped with DPX (BDH Laboratories, Poole, UK).

Volumetric analyses were performed using StereoInvestigator 10.50 (MBF Biosciences, Williston, VT). One male control animal's brain was not available for analysis due to a freezer malfunction. The volumes of both the amygdala and hippocampus in both hemispheres were computed from the sham operated controls. The volume of remaining amygdala tissue was computed for the amygdala-lesioned animals and the volume of remaining hippocampus tissue was computed for hippocampus-lesioned animals. We adopted a conservative approach to computing the volume of tissue remaining (allowing us to compute the percent atrophy) in the lesioned cases by outlining all areas of remaining neurons in each structure, even if the morphology of the tissue was not normal. Average volumes for the left and right amygdala and hippocampus were computed separately for male and female subjects. The average volumes from the controls were then used to calculate the extent of the lesion using the remaining volumes of amygdala and hippocampus from the respective groups

2.6 Data Analysis Strategy

2.6.1 Frequency and Duration of Behavioral Analysis—Behaviors that were initiated by focal animals were grouped into broad behavioral categories as indicated in Table 1. Frequencies and durations were summed across each category for interaction partner and then averaged across the number of observations to create a mean per observation. We used analysis of variance (ANOVA) to evaluate the impact of lesion on social behavior by using animal lesion condition as the between subjects factor and we report *p*-values associated with LSD post hoc tests. For some analyses, visual inspection of the marginal means suggested significant group differences, despite the omnibus test not reaching $p<0.05$. In those cases, effects were further evaluated using *t*-tests and Cohen's *d* effect size to evaluate the magnitude of the lesion effects. We recognize that this is not the

traditional way in which analyses are conducted - where typically, the omnibus effect must be significant to warrant further exploration. But, we have elected to proceed in this fashion because nonhuman primate studies of this sort are rare, utilize small sample sizes, and are unlikely to be repeated. In proceeding this way, we aim for statistical transparency and to create a complete scientific record from which readers can draw their own conclusions.

Non-normal data were log10(x+1) transformed as indicated throughout the results section. For ease of interpretation, raw data (means and variance indices) are presented here. Log-transformed means and variances are available upon request. Mauchly's test of sphericity was used to assess whether the data violated the assumption of sphericity. Degrees of freedom were Greenhouse-Geisser corrected when necessary. Cases that required correction are noted in the tables; the corrected degrees of freedom are available upon request.

Time effects in the unconstrained condition were assessed by creating three periods each including two meetings between the same partners (e.g., meetings 1 and 2 constitute period 1) allowing us to reduce the number of repeated time points which was required because of the limited sample size.

For the *constrained social access* condition, all experimental animals (male and female) met all of the interaction partners (both male and female). This allowed evaluation of the impact of experimental animal sex and interaction partner sex in the same model. In this case, experimental animal sex was entered into the analysis as the between subjects effect and interaction partner sex as the repeated measure. To allow for comparison to the unconstrained social access condition data, a set of analyses was performed on only the mixed sex interactions—that is where the female experimental animals met male interaction partners and where the male experimental animals met female interaction partners. These analyses were highly consistent with the full analyses and so are presented in the Supplemental Materials.

Given that only the female experimental animals met both male and female interaction partners in the *unconstrained social access* condition, we performed two different analyses on those data. First, we evaluated interactions during the mixed sex condition as in the constrained social access condition (presented in the main text). Second, the female experimental animals' behavior was evaluated with female interaction partners and male interaction partners, using interaction partner sex as the repeated factor. Given that these results were consistent the analyses described below, we present them in Supplemental Materials.

2.6.2. Behavioral Sequence Analysis—A modified lag sequential analysis was conducted on the mixed-sex dyad data files to evaluate whether lesion condition might influence the sequencing of behaviors. For this analysis, we specified two types of behaviors to create "if, then" sequences. "If" behaviors were those that initiated the sequence. "Then" behaviors were counted if they occurred within a 10 second window an "if" behavior. "If" behaviors were grouped into two categories: 1) affiliative, and 2) aggressive/avoidant). "Then" behaviors were grouped into four categories: 1) aggressive/avoidant, 2) engaging, 3) stereotypic, and 4) other – see Table 2.

We conducted two types of behavioral sequence analyses. The first set of analyses evaluated "if, then" sequencing within each subject (i.e., *subject-initiated*). This analysis therefore captured sequencing of each individual's behavior. For example, if our focal subject is Monkey A, this analysis captured sequences such as if monkey A approached monkey B (an affiliative "if" behavior) and then monkey A groomed monkey B (an engaging "then" behavior). Given that we had two categories of "if" behaviors and four categories of "then" behaviors, this analysis produced eight *behavioral sequence sums* for each testing condition (constrained and unconstrained) reflecting all possible combinations of "if" and "then" behaviors. The second set of analyses evaluated "if, then" sequencing between the interaction partner and subject in order to determine if there were lesion-based differences in how subjects responded to the social behaviors initiated by their interaction partners. For this analysis, sequences were initiated by "if" behaviors generated by the interaction partner (i.e., *interaction partner-initiated*). We then counted "then" behaviors generated by the subject. Like the first set of analyses, the second set generated eight *behavioral sequence sums* reflecting all possible combinations of "if" and "then" behaviors.

Because different animals generated different numbers of behaviors, we used the behavioral sequence sums to compute proportions that reflected the total number of behaviors generated by a given animal. To that end, for each "if" category, we computed the sum of all behaviors that occurred across all "then" categories. This resulted in four totals for each animal, reflecting the total number of behaviors that occurred within 10 seconds of subject-initiated "if" behaviors and interaction partner-initiated "if" behaviors. Each of the 16 behavioral sequence sums were then divided by these totals resulting 12 values that were standardized within individual to control for the total number of behaviors that individual generated. Note that by creating ratios, the "other" category is inherently represented in these analyses. The sequence data were subjected to a series of repeated measures ANOVAs with three repeated measures reflecting whether an animals' response was avoidant, engaging, or stereotypic. Values were log-transformed to account for non-normality. Together, these analyses paint a picture of how subjects follow through on their own initiation of affiliation or aggression, as well as how subjects respond to affiliation or aggression initiated by their social partners.

3. Results

3.1. Histological findings

The histological analyses demonstrated that the lesions were largely as intended. Lesions for each of the animals analyzed in this paper are illustrated in Figure 2 (amygdala cases) and Figure 3 (hippocampal cases) and volumetric analyses of the amount of tissue loss is presented in Tables 3 and 4. These lesions generally resulted in extensive bilateral removal of the dentate gyrus, hippocampus and subicular complex. On average 85% of the neurons in those areas were removed in the experimental group. Most cases also had damage to the entorhinal cortex and the parahippocampal gyrus. The hippocampal lesions were designed to not encroach upon the amygdala and, as a result, there was some sparing of the most rostral extent of the hippocampal formation in all cases. Conversely, there was little or no direct damage of the amygdala in any of the hippocampal cases.

The amygdala cases also resulted in near complete (94%) removal of the substance of the amygdaloid complex. As indicated in Table 3, the amount of amygdala loss was no less than 88%. Amygdala tissue damage was fairly symmetrical bilaterally, with slightly smaller lesions on the right side. When amygdala tissue was spared, it was typically located superficially including the periamygdaloid cortex and cortical nuclei. All major nuclei of the amygdala (lateral, basal, accessory basal and central) were nearly completely removed by the ibotenic acid injections. Other than the superficial nuclei, no other areas were consistently spared. The lesions were designed to produce as complete a lesion of the amygdala as possible. Therefore, the lesion extended to the rostral and caudal poles of the amygdala and involved tissue located both in front of (the temporal pole) and behind (the hippocampal formation) the amygdala.

Across cases, there was substantial spatial distortion or the remaining healthy tissue and expansion of the temporal horn of the lateral ventricle. Nonetheless, it was possible to carry out a qualitative assessment of the extent of unintended damage to surrounding brain regions. The extraneous damage was consistent across cases and varied only in the amount of damage that was sustained. The lesion and extraneous damage was most extensive in Case A_4. Case A_4 had unintended damage to the cortex at the fundus of the superior temporal gyrus. This was consistent across most cases and typically occurred throughout the rostrocaudal extent of the amygdala. There was also damage of the fundus of the rhinal sulcus leading to cell loss in the perirhinal and rostral entorhinal cortex. Additionally, this case had direct damage to the inferotemporal cortex located lateral to the perirhinal cortex and surrounding the anterior medial temporal sulcus. The ventral portion of the claustrum was also damaged. The rostral portion of the hippocampal formation was heavily damaged in this case with all fields showing cell loss. The damage began to resolve at the uncal flexure although cell loss in the CA1 field continued caudally to the level of the LGN. Case A_1 had the most selective lesion of the amygdala. While there was also some cortical damage, it was confined to the levels adjacent to the amygdala and it was much less extensive than in the other cases. Cell loss in the hippocampal formation was primarily confined to the CA fields and it occurred mainly in the rostral extreme of the hippocampus.

In summary, all cases reported here had extensive bilateral elimination of the neurons of the amygdaloid complex. Given that the lesions were carried out at 2 weeks of age, it is not surprising that some unintended damage occurred. However, this was restricted to relatively small regions located directly adjacent to the amygdala.

3.2. Behavioral findings

There were not significant interactions between sex of the partner animal and lesion status of the experimental animals. Therefore, sex of partner X lesion status data are not presented here for the sake of brevity but are available upon request. The analyses below examined experimental animals' lesion condition and sex as between-subject effects and time effects (whether testing occurred in the first, second, or third period) as a repeated measure in a series of ANOVAs.

3.2.1 Constrained Social Access Condition—In the constrained social access condition, all experimental animals met all interaction partners allowing us to evaluate the effect of the experimental animal's lesion condition with all partners.

3.2.1.1 Social states: Lesion groups did not differ in the duration of time spent in close social interactions, $F(2,17)=1.081$, $p=0.361$, $\eta_p^2=0.113$ (analyses on log-transformed data—Figure 4a). All animals spent more time in close social interactions during their first meeting, as compared to the second and third meeting; $F(2,34)=5.887$, $p=0.006$, $\eta_p^2=0.257$ (analyses on log-transformed data) ($M_1=128.86$, $SD_1=88.02$; $M_2=90.63$, $SD_2=77.58$; $M_3=92.62$, $SD_3=85.96$).

3.2.1.2 Communicative Signaling: Frequencies of communicative signaling differed significantly between lesion groups; $F(2,17)=5.208$, $p=0.017$, $\eta_p^2=0.380$—Figure 4b. Hippocampus-lesioned animals signaled toward their interaction partners more frequently than control and amygdala-lesioned animals (H>C, $p=0.015$; H>A, $p=0.028$). All animals displayed higher frequencies of total communicative signaling during their first meeting as compared to the second and third meetings, $F(2,17)=14.712$, $p=0.00003$, $\eta_p^2=0.464$ ($M_1=21.69$, $SD_1=9.45$; $M_2=14.96$, $SD_2=8.78$; $M_3=15.00$, $SD_3=9.07$).

Further investigation of specific types of communicative signaling indicated lesion group differences in frequencies of affiliative signaling, $F(2,17)=6.485$, $p=0.008$, $\eta_p^2=0.433$. Hippocampus-lesioned animals generated more frequent affiliative signals ($M=18.13$; $SD=5.28$) toward their interaction partners than control animals ($p=0.006$; $M=9.95$; $SD=5.06$) or amygdala-lesioned animals ($p=0.008$; M=9.96; SD=5.55). There were no lesion related differences in the frequencies of submission-related signaling, $F(2,17)=0.534$, $p=0.596$, $\eta_p^2=0.059$ (analyses on log-transformed data), or agonistic signaling, $F(2,17)=0.663$, $p=0.543$, $\eta_p^2=0.069$ (analyses on log-transformed data). Means are presented in Supplementary Materials Table 1. Lesion groups also did not differ in the frequency of facial behaviors generated, $F(2,17)=2.082$, $p=0.155$, $\eta_p^2=0.197$ (analyses on log-transformed data) (non-transformed descriptives: $M_{amygdala}=6.39$, $SD_{amygdala}=3.37$; $M_{control}=7.41$, $SD_{control}=6.15$; $M_{hippocampus}=12.63$, $SD_{hippocampus}=8.16$).

3.2.1.3 Exploratory behaviors: Lesion groups did not differ in the frequency of exploration, $F(2,17)=2.023$, $p=0.163$, $\eta_p^2=0.192$ (analyses on log-transformed data) (non-transformed descriptives: $M_{amygdala}=1.14$, $SD_{amygdala}=1.06$; $M_{control}=2.30$, $SD_{control}=1.39$; $M_{hippocampus}=3.35$, $SD_{hippocampus}=2.76$).

3.2.1.4 Stress-related behavior: Amygdala-lesioned animals generated more frequent stress-related behaviors than control and hippocampus-lesioned animals; $F(2,17)=4.791$, $p=0.022$, $\eta_p^2=0.360$ (A>C, $p=0.017$; A>H, $p=0.014$) (analyses on log-transformed data—Figure 4c). Control and hippocampus-lesioned animals did not differ in the frequency of their stress-related behaviors ($p=0.916$).

3.2.1.5 Stereotypic behavior: As compared to control animals, amygdala- and hippocampus-lesioned animals had a tendency to generate more frequent stereotypic behavior, $F(2,17)=3.030$, $p=0.075$, $\eta_p^2=0.263$ (analyses on log-transformed data). Despite

the omnibus test not reaching conventional levels of significance, we evaluated between-group differences with *t*-tests because the marginal means suggested that the controls might differ significantly from the lesioned animals. When compared directly, control animals (M=1.39; SD=2.45) did not differ significantly from amygdala-lesioned animals (M=8.41; SD=14.16) ($t(13)$=1.806, p=0.094, d=0.691; analyses on log-transformed data) but did differ from hippocampus-lesioned animals (M=7.85; SD=6.45) ($t(14)$=3.545, p=0.003, d=1.326; analyses on log-transformed data). Amygdala- and hippocampus-lesioned animals produced the same numbers of stereotypic behaviors, $t(13)$=0.994, p=0.338, d=0.050 (analyses on log-transformed data).

Lesion groups also did not differ in terms of the number of specific types of stereotypic behaviors that they generated. There were no lesion group differences in frequency of whole-body stereotypies, $F(2,17)$=1.211, p=0.322, η_p^2=0.125 (analyses on log-transformed data), or self-directed stereotypies ($F(2,17)$=2.298, p=0.131, η_p^2=0.213; analyses on log-transformed data). There were no lesion group differences in the frequency of head-twists, $F(2,17)$=0.779, p=0.474, η_p^2=0.084; (analyses on log-transformed data) ($M_{amygdala}$=4.02, $SD_{amygdala}$=10.36; $M_{control}$=0.16, $SD_{control}$=0.32; $M_{hippocampus}$=4.34, $SD_{hippocampus}$=4.80). See Supplemental Materials Table 1 for means for each stereotypy type.

3.2.1.6. Behavioral sequences: There were no significant lesion group differences in subject-initiated behavioral sequences. That is, lesion groups did not differ with regards to the behaviors subjects generated ("then" behaviors) following their own initiation of either aggression or affiliation ("if behaviors"), $F(2,20)$=0.028, p=0.972, η_p^2=0.003 (Figure 5a), and F(2,20)=1.778, p=0.195, η_p^2=0.151 (Figure 5b), respectively (data analyses on log-transformed data). Irrespective of lesion condition and irrespective of interaction partner-initiated "if" behaviors, subjects were most likely to respond by generating socially engaging behaviors and least likely to respond by generating stereotypies. The main effect for behavioral response to aggressive behaviors was $F(1.45,29.09)$=49.146, p<0.0001, η_p^2=0.711. The main effect for behavioral response to affiliative behaviors was $F(2,40)$=101.227, p<0.0001, η_p^2=0.835.

3.2.1.7. Spatial locations: There were no lesion group differences in the physical space between experimental and constrained animals across all interactions $F(2, 17)$=0.52, p=0.605, η_p^2=0.057 ($M_{amygdala}$=5.34; $SD_{amygdala}$=1.07; $M_{control}$=5.30; $SD_{hippocampus}$=0.72; $M_{hippocampus}$=4.95; $SD_{hippocampus}$=0.96). This analysis accounted for whether the experimental and constrained animals were of the same or opposite sex (i.e., same sex or mixed sex interactions) as a repeated factor, and sex and lesion status of the experimental animals as a between-subjects factor. Neither sex of experimental animal or whether the constrained animal was the same or the opposite sex were significant. When only mixed sex dyads were considered, there was a main effect of subject sex, $F(2,23)$=6.440, p=0.021, η_p^2=0.275, such that female experimental animals were in closer proximity to interaction partners than were male subjects (M_{male}=5.57; SD_{male}=0.98; M_{female}=4.63; SD_{female}=1.02). No other effects were significant.

3.2.2. Unconstrained Social Access Condition—In the analyses below, we evaluate experimental animals' lesion condition and sex as between subject effects and time effects

(whether testing occurred in period one - the first and second unconstrained meetings, period two – the third and fourth meetings, or period three – the fifth and sixth meetings) as a repeated measure in a series of ANOVAs. Intense aggression was observed initially across more than half of male-male pairs (11/20, 3 amygdala-lesion, 4 hippocampus-lesion, and 4 control). As a result, we elected to not test one of the stimulus males with the experimental males after initial meetings. One experimental male never met either stimulus male in this condition. Given this data structure, we therefore evaluated the behavior of the experimental animals in a series of analyses to best accommodate the available data. We evaluated all animals in mixed sex pairs, presented here. As a secondary set of analyses, we then evaluated the behavior of the experimental females in same sex pairs and the behavior of the experimental males with one of the male interaction partners (a separate analysis since one experimental male never met the stimulus males in this condition). The results of these analyses were very consistent with the mix-sexed pair data suggesting that the experimental animals' sexes and stimulus animals' sexes did not radically influence the patterns of behavior above and beyond lesion condition. Secondary analyses are presented in Supplemental Materials. Data from mixed sex interactions (female experimental animals meeting male social partners; male experimental animals meeting female social partners) were evaluated in the analyses below. Notably, mixed sex interactions were the only interactions in which all of the male experimental animals were tested.

3.2.2.1. Social states: There were no lesion group differences in the duration of time spent in close social interactions that were initiated by the experimental animals, $F(2,17)=1.039$, $p=0.375$, $\eta_p^2=0.109$ (analyses on log-transformed data—Figure 6a). Experimental animals spent more time in close social interactions that they initiated during the second and third period as compared to the first period, $F(1.409,23.947)=7.227$, $p=0.007$, $\eta_p^2=0.298$ (analyses on log-transformed data) ($M_1=85.37$, $SD_1=143.08$; $M_2=115.84$, $SD_2=141.95$; $M_3=124.20$, $SD_3=136.45$).

Testing period also influenced the frequency with which interaction partners initiated close social states, $F(1.377,23.402)=9.186$, $p=0.003$, $\eta_p^2=0.351$ (analyses on log-transformed data). Interaction partners initiated these states more frequently in the second and third periods as compared to the first period, ($M_1=12.83$, $SD_1=14.98$; $M_2=21.17$, $SD_2=19.84$; $M_3=21.39$, $SD_3=18.70$). Testing period effects were observed in the duration of close social interactions in which the interaction partners initiated as well, $F(2,34)=4.012$, $p=0.027$, $\eta_p^2=0.191$. Interaction partner animals also spent more time in close social states that they initiated during the third period, as compared to the first period (analyses on log-transformed data) ($M_1=74.31$, $SD_1=88.58$; $M_2=113.10$, $SD_2=117.59$; $M_3=105.64$, $SD_3=95.90$).

3.2.2.2. Communicative Signaling: There were no lesion-based differences in the frequencies of communicative signaling, $F(2,17)=1.644$, $p=0.222$, $\eta_p^2=0.162$ (analyses on log-transformed data—Figure 6b. A closer look at particular types of communicative signaling yielded the same conclusions. Lesion groups did not differ in the frequencies with which they generated affiliative, $F(2,17)=2.300$, $p=0.131$, $\eta_p^2=0.213$ (analyses on log-transformed data); submissive, $F(2,17)=0.598$, $p=0.561$, $\eta_p^2=0.066$ (analyses on log-transformed data); or agonistic $F(2,17)=0.509$, $p=0.610$ $\eta_p^2=0.057$ (analyses on log-

transformed data) signals. Means from these behavior-by-behavior analyses are presented in Supplemental Materials—Table 3. Amygdala-lesioned animals generated fewer facial behaviors during dyadic interactions than hippocampus or control animals although the effect did not reach conventional levels of significance, $F(2,17)=3.063$, $p=0.073$, $\eta_p^2=0.265$ (analyses on log-transformed data) ($M_{amygdala}=8.43$, $SD_{amygdala}=7.96$; $M_{control}=19.00$, $SD_{control}=12.67$; $M_{hippocampus}=26.17$, $SD_{hippocampus}=18.17$). Although the omnibus test did not reach conventional levels of significance, we further evaluated the data using *t*-tests because the marginal means indicated that amygdala-lesioned animals may significantly differ from controls animals. When compared directly, the difference in the number of facial behaviors generated by the amygdala-lesioned animals in comparison to controls still did not reach conventional levels of significance although the effect size was large, $t(13)=1.79$, $p=0.097$, $d=1.00$.

3.2.2.3. Exploratory behaviors: Amygdala-lesioned animals explored the environment less frequently than both controls and hippocampus-lesioned animals although the effect did not reach conventional levels of significance, $F(2,17)=3.523$, $p=0.052$ $\eta_p^2=0.293$ (analyses on log-transformed data) ($M_{amygdala}=2.29$, $SD_{amygdala}=2.35$; $M_{control}=4.94$, $SD_{control}=2.67$; $M_{hippocampus}=5.32$, $SD_{hippocampus}=4.43$). Although the omnibus test did not reach conventional levels of significance, we further evaluated the data using *t*-tests because the marginal means indicated that amygdala-lesioned animals may significantly differ from controls animals. When compared directly, the difference in the number of explorations generated by the amygdala-lesioned animals in comparison to controls still did not reach conventional levels of significance, although the effect size was large, $t(13)=2.059$, $p=0.060$, $d=1.054$.

3.2.2.4. Stress-related behavior: Lesion status influenced the generation of stress-related behaviors, $F(2,17)=5.313$, $p=0.016$, $\eta_p^2=0.385$ (analyses on log-transformed data) ($M_{amygdala}=6.33$, $SD_{amygdala}=5.22$; $M_{control}=4.62$, $SD_{control}=4.72$; $M_{hippocampus}=1.64$, $SD_{hippocampus}=1.18$). Amygdala-lesioned animals generated more frequent stress-related behaviors than hippocampus-lesioned animals ($p=0.010$) but did not differ from control animals ($p=0.235$). Hippocampus-lesioned animals did not differ significantly from control animals either ($p=0.098$). All experimental animals generated more stress-related behaviors during the third as compared to first period of testing, $F(2,34)=4.949$, $p=0.013$, $\eta_p^2=0.225$ (analyses on log-transformed data) (non-transformed descriptives: $M_1=3.53$, $SD_1=4.50$; $M_2=4.12$, $SD_2=4.26$; $M_3=4.65$, $SD_3=4.94$).

3.2.2.5. Stereotypic behavior: Hippocampus-lesioned animals generated the most frequent stereotypic behaviors and control animals generated the least frequent stereotypic behaviors, $F(2,17)=4.866$, $p=0.021$, $\eta_p^2=0.364$ (H>C, $p=0.008$; A>C, $p=0.024$; H v. A, $p=0.689$) (analyses on log-transformed data—Figure 7). There were no lesion based differences in specific types of stereotypic behaviors: whole-body, $F(2,17)=1.233$, $p=0.316$, $\eta_p^2=0.127$ (analyses on log-transformed data); self-directed, $F(2,17)=2.008$, $p=0.155$, $\eta_p^2=0.197$ (analyses on log-transformed data). Means for behavior-by-behavior analyses are presented in Supplemental Materials—Table 3. The overall lesion group difference was likely driven by variation in one particular stereotypy—the head twist, $F(2,17)=4.031$, $p=0.037$,

η_p^2=0.322 (analyses on log-transformed data), ($M_{amygdala}$=0.67, $SD_{amygdala}$=1.69; $M_{control}$=0.03, $SD_{control}$=0.06; $M_{hippocampus}$=3.48, $SD_{hippocampus}$=4.26). Hippocampus-lesioned animals generated more frequent head twists than both control (p=0.003) and amygdala-lesioned (p=0.019) animals. Amygdala-lesioned and control animals did not differ significantly (p=0.482).

3.2.2.6. Behavioral sequences: Subject-initiated behavioral patterns varied by lesion condition. The behaviors generated by subjects immediately after they initiated aggressive interactions varied by lesion condition, F(2, 20)=5.51, p=0.012, η_p^2=0.355 (analysis on log-transformed data). Hippocampus-lesioned animals generated the most behaviors and control animals the least (H>C, p=0.035) following their own initiation of aggression. Amygdala-lesioned animals did not differ significantly from hippocampus-lesioned animals (A v. H, p=0.243) and differed from controls at only at a level that did not reach conventional levels of significance (A v. C, p=0.062). A significant effect of behavior type indicated that animals were most likely to generate engaging behaviors and least likely to generate stereotypic behaviors following subject-initiated aggression, F(2, 20)=24.70, p<0.0001, η_p^2=0.553 (analysis on log-transformed data). A lesion X behavior type effect that did not reach conventional levels of significance suggested that there may be lesion-based variation in terms of which behaviors particular lesion groups generated following subject-initiated aggression, F(4, 40)=2.31, p=0.075, η_p^2=0.187 (analysis on log-transformed data—Figure 8a). Evaluation of the marginal means indicated that amygdala-lesioned animals were most likely and control animals were least likely initiate aggression and then generate avoidant behaviors and stereotypies. In contrast, hippocampus-lesioned animals were most likely and control animals were least likely to initiate aggression and then generate engaging behaviors.

When interaction partners initiated aggression with the experimental animals, experimental animals were most likely to respond by generating engaging behaviors and least likely to respond by generating stereotypic behaviors, F(2,20)=16.94, p<0.001, η_p^2=0.452 (analysis on log-transformed data—Figure 8b). Lesion groups differed in their behavioral responses as indicated by a significant lesion X behavior type interaction, F(4,40)=3.01, p=0.029, η_p^2=0.231. Only amygdala- and hippocampus-lesioned animals generated stereotypies in response to interaction partner-initiated aggression. Evaluation of the marginal means indicated that amygdala-lesioned animals were equally likely to generate avoidant, engaging, or stereotypical behaviors. In contrast, control and hippocampus-lesioned animals were significantly more likely to respond to aggressive behaviors by generating engaging as opposed to avoidant behaviors.

Experimental animals did not vary by lesion group with regards to the likelihood of generating the behaviors of a particular class after subject-initiated affiliative behavior, F(2, 20)=1.180, p=0.328, η_p^2=0.106, and lesion group did not impact behavioral sequencing, F(2.70, 26.99)=2.12, p=0.127, η_p^2=0.175 (analyses on log-transformed data—Figure 9). All animals were most likely to generate engaging behaviors following subject-initiated affiliation, F(2, 40)=103.90, p<0.0001, η_p^2=0.839. When interaction partners initiated affiliative behaviors, all subjects were most likely to generate engaging behaviors as indicated by a significant main effect of behavioral response, F(2, 40)=97.45, p<0.0001, η_p^2=0.830. Neither the effect of lesion condition, F(2, 20)=1.343, p=0.284, η_p^2=0.118, nor

the lesion condition by behavioral response $F(4, 40)=1.50$, $p=0.221$, $\eta_p^2=0.130$ were statistically significant.

3.2.2.7 Spatial Locations: As in the analyses of social behavior (above), we evaluated the spatial distance data for same-sex and mixed-sex pairs for the female subjects and mixed-sex pairs for all animals. When only female experimental animals were considered, there was a significant main effect of lesion condition $F(2,10)=6.493$, $p=0.016$, $\eta_p^2=0.565$, ($M_{amygdala}=4.45$, $SD_{amygdala}=0.21$; $M_{control}=5.25$, $SD_{control}=0.17$; $M_{hippocampus}=4.37$, $SD_{hippocampus}=0.57$). Control animals were on average further away from interaction partners than amygdala- and hippocampus-lesioned animals, regardless of the interaction partner's sex (C>A, $p=0.016$; C>H, $p=0.007$). Female experimental animals were in closer proximity to male, as compared to female, interaction partners as indicated by a significant effect of interaction partner sex $F(1,10)=84.34$, $p<0.0001$, $\eta_p^2=0.894$ ($M_{male}=3.59$, $SD_{male}=0.72$; $M_{female}=5.77$, $SD_{female}=0.66$).

We next evaluated the spatial proximity of male and female experimental animals in mixed sex interactions. A significant effect of experimental animal sex indicated that females were in closer proximity to interaction partners than were males, $F(1,17)=15.56$, $p=0.001$, $\eta_p^2=0.478$ ($M_{male}=5.29$, $SD_{male}=1.29$; $M_{female}=3.56$, $SD_{female}=0.72$). No other effects were significant.

4. Discussion

The present set of observations demonstrates that early, selective damage to either the amygdala or hippocampus does not result in a radically altered pattern of adult social behavior. While there were subtle differences in stress-related behaviors, exploratory behaviors, stereotypies, and the sequencing of behavior, social behavior per se was entirely intact. In fact, amygdala-lesioned animals were essentially indistinguishable from controls when the frequencies and durations of social behavior were examined. There were no lesion-related differences in the initiation of close social states (those that occur within arm's reach of another animal) as might have been predicted based on previous reports [27, 31, 35]). Generally, hippocampus-lesioned animals tended to be more social than the other animals, as indicated by heightened communicative signaling and their propensity to engage interaction partners in an affiliative manner after being aggressed. The only consistent differences were in stress-related behaviors and stereotypies. Amygdala-lesioned animals consistently generated the greatest number of stress-related behaviors. Lesioned animals displayed more frequent stereotypies than did control animals.

Subtle lesion differences were observed in terms of the sequencing of behaviors. When amygdala-lesioned animals instigated aggressive behaviors, they followed them with avoidant or stereotypic behaviors more frequently than controls. When aggressive behaviors were initiated by interaction partners, amygdala-lesioned animals were equally likely to respond with avoidant, engaging, or stereotypic behaviors while control and hippocampus-lesioned animals were most likely to respond with engaging behaviors. Taken together these results point to subtle, lesion-related differences in social behavior.

The present experiment is the final evaluation of social behavior of these experimental animals. We were thus able to quantitatively evaluate the extent of their lesions following testing. Given that we tested these animals beginning in 2001, with only MRI confirmation of lesion placement [15, 36] until now, it was comforting to determine that the lesion placement was complete and essentially as planned. Amygdala-lesioned animals had near complete damage with, on average, 94% of the amygdala tissue eliminated. Amygdala damage was fairly consistent across cases with only one hemisphere of two cases showing 90% atrophy. Given that the goal was to completely eliminate the amygdala in the lesion group, some damage was incurred by brain regions that surround the amygdala. These areas included the temporal polar cortex, the fundus of the superior temporal gyrus, the fundus of the rhinal sulcus and the anterior hippocampal formation. Thus, the subtle behavioral changes that we summarize here could be due, in part, to extraneous damage, or to plasticity resulting from the lesion. Hippocampus-lesioned animals demonstrated less damage over all – on average 85.44% of hippocampal tissue was lost. There was a greater range of atrophy in the hippocampus-lesioned animals compared to the amygdala-lesioned animals. While some animals had near total damage (e.g., Case H_2: 90.79%, Case H_6: 93.12%), others had substantial sparing (e.g., Case H_7 – right side, only 68.74% atrophy). It is important to note that in cases where tissue sparing was present, the morphology of the tissue was abnormal. These abnormalities included alterations in cell density as well as cell layer structure.

With the histology of the group complete and the lesions confirmed, we can now take a long view on the social behavior of the present cohort which was evaluated at multiple time points across their development, beginning when they were infants [15, 25, 27, 31, 35, 36]. Observations during the first year of these animal's lives suggested that social behavior following early amygdala or hippocampus damage was essentially intact [15]—operated animals could produce the facial and vocal signals used for social communication as well as approach and interact with other animals. If anything, amygdala-lesioned animals demonstrated increased positive social behavior. Amygdala-lesioned animals generated both more affiliative and submissive or fear-related signals when interacting with peers. The social behavior of hippocampal-lesioned animals was essentially the same as control animals.

In subsequent evaluations of social behavior across development, subtle differences in the execution of social behavior emerged. As juveniles (1.5–2.5 years of age), amygdala-lesioned animals generated fewer aggressive and affiliative signals (e.g., vocalizations, facial displays), spent less time in social interactions with familiar peers, and spent more time in inactivity and explored less with novel peers [27]. Again, hippocampus-lesioned animals appeared essentially identical to control animals. When the female animals were relocated into groups with an adult male at four years of age, amygdala-lesioned females spent less time interacting with the male, but comparable durations of time in social interactions with their peers [31]. In contrast, hippocampus-lesioned animals were more social than control animals with their peers [31]. When evaluated as adults in their home environments with their familiar social partner, amygdala-lesioned animals spent less time in close social interactions than did controls while hippocampus-lesioned animals spent more time in social interactions than controls [35]. This hypersociality of the hippocampus-lesioned animals was consistent in this experiment - hippocampus-lesioned animals generated more

communicative signals during the constrained social access condition. Female hippocampus-lesioned animals had similarly heightened communicative signaling with female interaction partners in the unconstrained social access condition. Finally, when aggressed against, hippocampus-lesioned animals were most likely to respond by initiating affiliative behaviors.

Across development, our subjects were evaluated in a number of social contexts – dyadic interactions (with familiar peers and novel animals), sex-mixed groups, and sex-segregated groups – and while patterns of behavior varied by developmental time point, they were fairly consistent across these contexts [15, 27, 31, 35]. Taken together, the results of previous evaluations of this cohort's social behavior [15, 27, 31, 35] also suggest that early damage to the amygdala or the hippocampus does not prevent the generation of species-typical social behaviors but may subtly impair their execution in a context-dependent way. These findings stand in contrast to the patterns of social behavior observed when animals receive amygdala damage as adults. Animals with adult amygdala damage approach conspecifics and solicit social interactions more than control animals (e.g.,[10, 12]).

Our findings stand in contrast not only to the literature on adult amygdala lesions and social behavior [10–14, 37] but also to other studies evaluating the importance of the primate amygdala for social development. Some developmental studies have documented changes to social behavior as a result of early neural damage that persisted over time (e.g.,[22, 23, 38, 39]). For example, early amygdala-lesioned animals demonstrated heighted fear or submission signals when tested as infants [22] and as young adults [23]. Animals with full, early medial temporal lobe damage that included the hippocampus and amygdala were hyposocial (reduced social initiation and reduced engagement when peers initiated social contact) as infants [38] and this reduction in sociability persisted into adulthood [39].

There are two possible explanations for the behavioral differences observed across developmental studies. First, in the experiments cited above, monkeys were peer-reared (or nursery-reared) meaning that they were separated from their mothers at birth. This sort of rearing is considered a manipulation that results in increased early life stress [40] and animals reared in this way develop behavioral abnormalities even if their brains are not manipulated (e.g.,[41–43]). We suggest that the abnormal behavioral patterns that were observed were an interaction between the neural damage and early socialization. A second possibility relates to how the lesions were created. Previous studies used aspiration lesions that remove both the target tissue (the amygdala, for example) and the fibers passing through it. As a result, it is not clear whether the differences in behavior observed in these studies were the result of damage to the amygdala per se or damage to fibers of passage. We addressed both of these issues in the present longitudinal study in which animals were reared by their mothers and focal brain damage was created using a neurotoxin that spared fibers of passage. Importantly, another cohort of animals with early amygdala damage who have been raised in large social groups also demonstrated very subtle differences in social behavior with their mothers as infants [19].

It is critical to note that in the present experiment, unlike the experiment that evaluated social behavior following damage to the adult amygdala [10], we were unable to test all

male experimental animals with both male interaction partners. We did not test all of the male experimental animals with one of the interaction partners because of the high rates of aggression—behavior that was not observed in the adult study (i.e., [10]). Critically, aggression was consistent across lesion groups indicating that the differences between the adult and neonatal study were likely not the result of the experimental manipulation of the brain. One possible explanation for the differences observed across studies is that variation in housing arrangements influenced experimental animals' expectations to be dominant in all social interactions. Animals in this study were all housed in mixed-sex pairs in which the male was dominant. Subjects in Emery et al. [10] were individually housed. It is possible that in the absence of regular social contact in their home-cages, singly housed animals were more motivated to engage in pro-social interactions or at the very least motivated to avoid aggressive interactions. Evaluating the effects of social housing and daily social experience on patterns of observed socialization in experimental dyadic interactions should be empirically evaluated in future work.

An additional point of comparison related to social interactions occurring with animals that had early versus late amygdala damage is related to how the experimental animals were treated by the interaction partners. Interaction partners of animals that received adult amygdala damage preferred them to control animals as evidenced by greater frequencies of close social states, such as grooming, spatial proximity, and physical contact [10]. Interaction partners more frequently requested sex and approached amygdala-lesioned animals as well [10]. We identified only one lesion-related difference in how interaction partners behaved with experimental animals in the present experiment—interaction partners indicated close social states with amygdala and hippocampus-lesioned animals more frequently than controls. In other words, even though there were not gross social behavior differences between lesion conditions, interaction partners did still show a preference for lesioned animals. The behavioral mechanisms subserving this preference are not clear based on the present data.

One consistent finding across their lives is that amygdala-lesioned animals physically explored their environments less than other animals during social interactions. This effect was evident when these animals were infants [15], when they were juveniles [27], and in their home cage environments as adults [35]. This pattern is particularly interesting because when faced with novel objects and objects that represent threat, amygdala-lesioned animals actually explored those objects *more* frequently than do controls [28, 29]. Taken together, these effects suggest that the decreased exploratory behavior in social settings is not related to a reduction in exploration per se, but rather related to the specific context. Given that amygdala-lesioned animals also had higher frequencies of stress-related behaviors in these contexts, one possibility is that lack of exploration is indicative of stress-related withdrawal from the environment.

Our remaining scientific challenge of this longitudinal experiment is to identify neural areas and networks that were impacted by early damage to the amygdala or hippocampus in order to understand how neural plasticity may relate to behavioral plasticity. While the social behavior of these animals was largely intact, early damage to the amygdala and hippocampus differentially influenced region-dependent processes. Animals that received

hippocampus damage during adulthood did not have intact spatial relational memory [44]. However, animals that received hippocampus damage during infancy do have intact spatial relational memory [45]. Similarly, animals that received amygdala damage during adulthood typically are hyporeactive to affective stimuli [2–8, 46] and unable to learn the affective value of novel stimuli via associative mechanisms [47, 48]. Animals with early damage to the amygdala are also hyporeactive to affective stimuli [26, 28, 29] but are able to learn affective value via associative mechanisms [49]. Together these effects point to selective plasticity. Charting that plasticity will yield insights into the amazing potential of the brain to compensate for early perturbations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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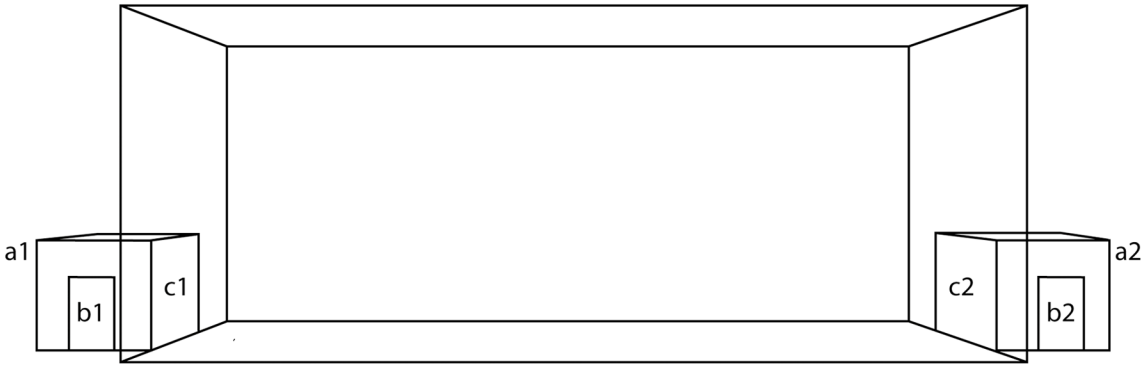
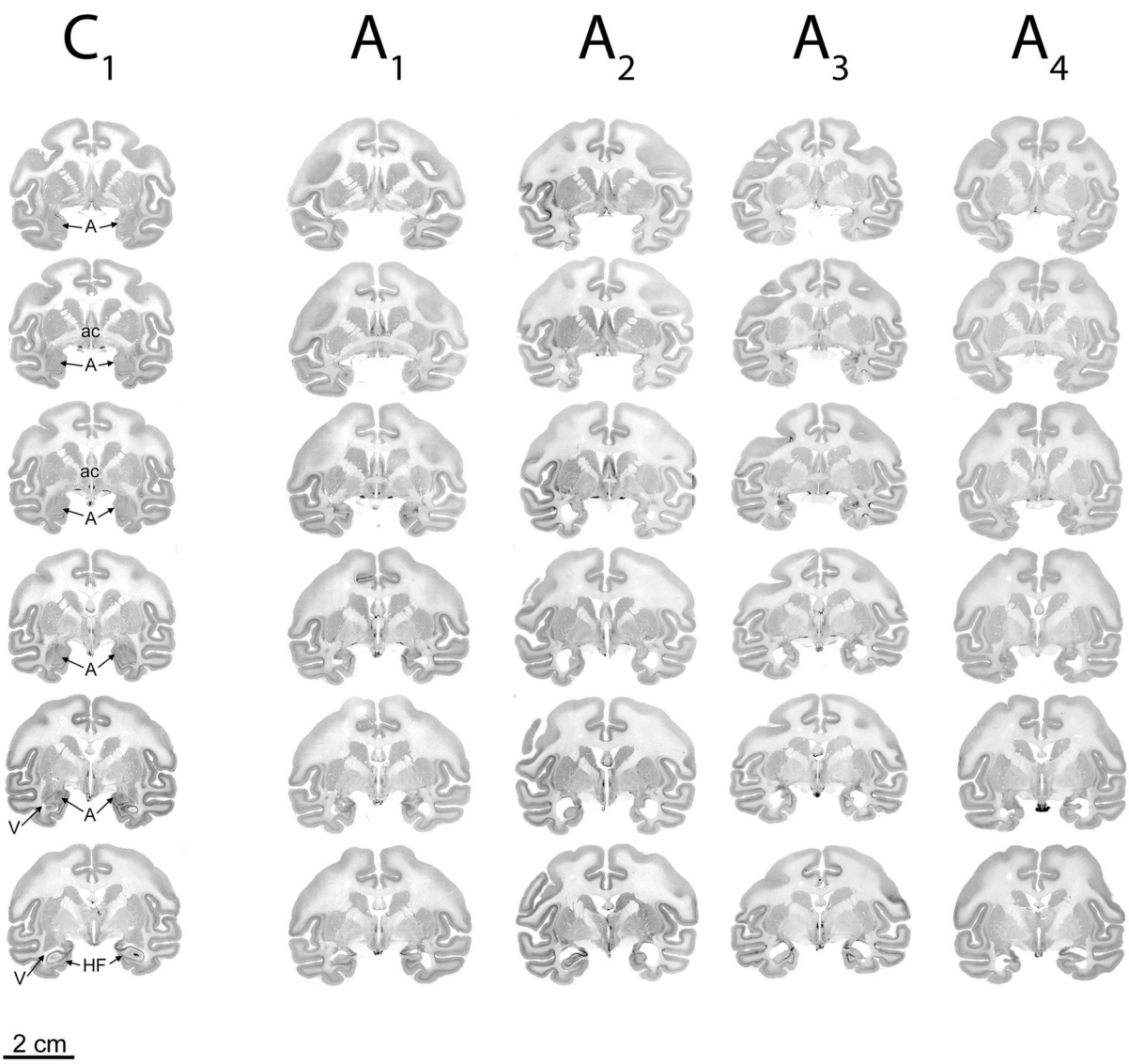


Figure 1. Testing cage

The experiment took place in a large chain-link enclosure, pictured here. Small cages adjoined each end of the larger cage (a1 and a2). Monkeys entered the small cages via a door (b1 and b2). The small cages could be separated from the large cage with either a solid door or a door made of metal bars (c1 and c2).



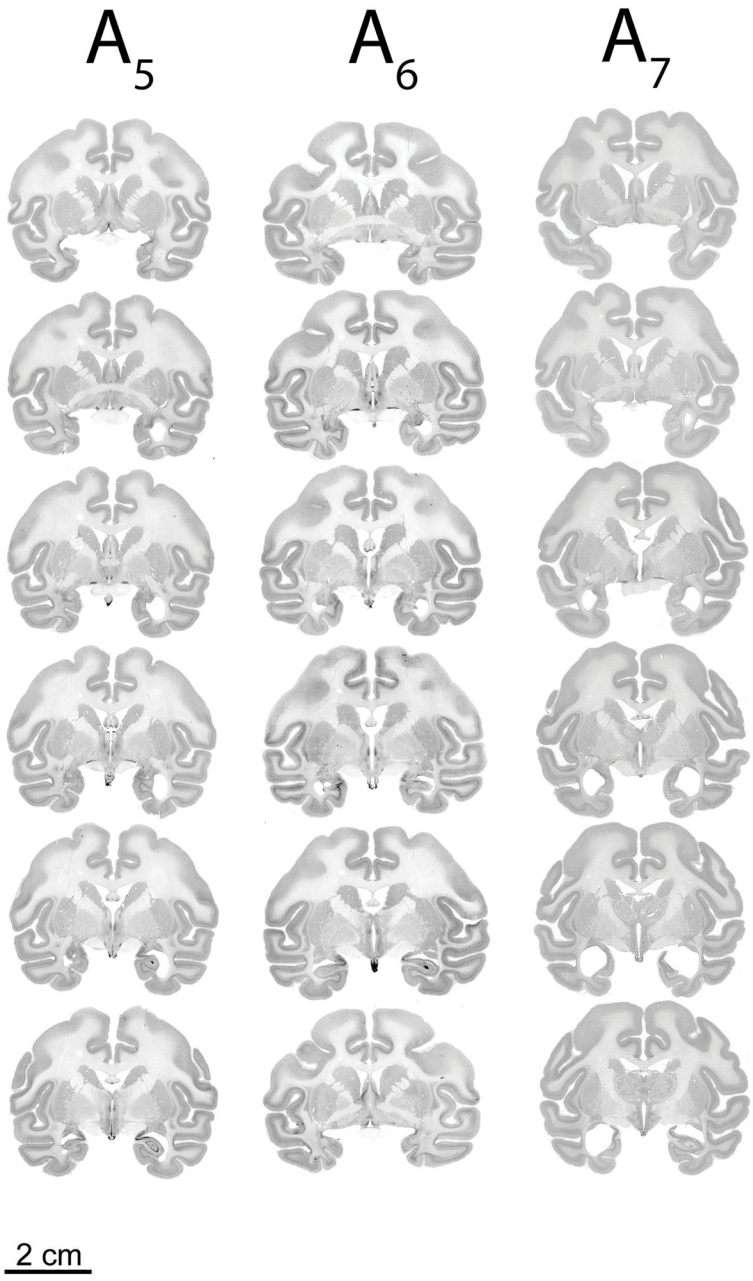
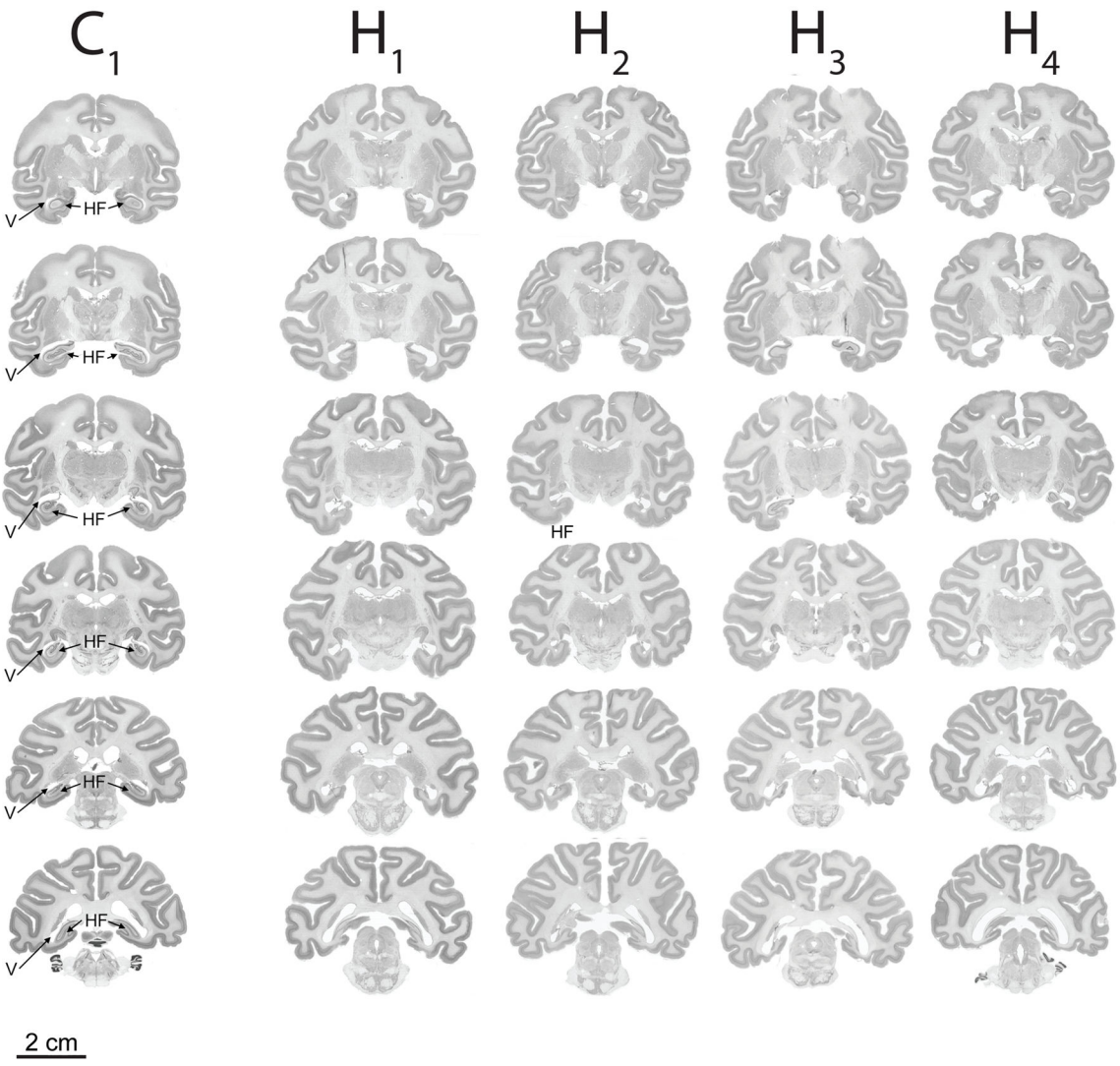


Figure 2. Histology Confirming Amygdala Lesions

Representative histological sections from one control animal (C_1) and the seven amygdala-lesioned cases (A_1–A_7) arranged rostral (top) through caudal (bottom). The amygdala (A) in C1 is labeled in the first 5 sections, followed by the hippocampal formation (HF) in the 6th section. Additionally labeled is the ventricle (V) and anterior commissure (ac). Case labels correspond with those in Table 3.



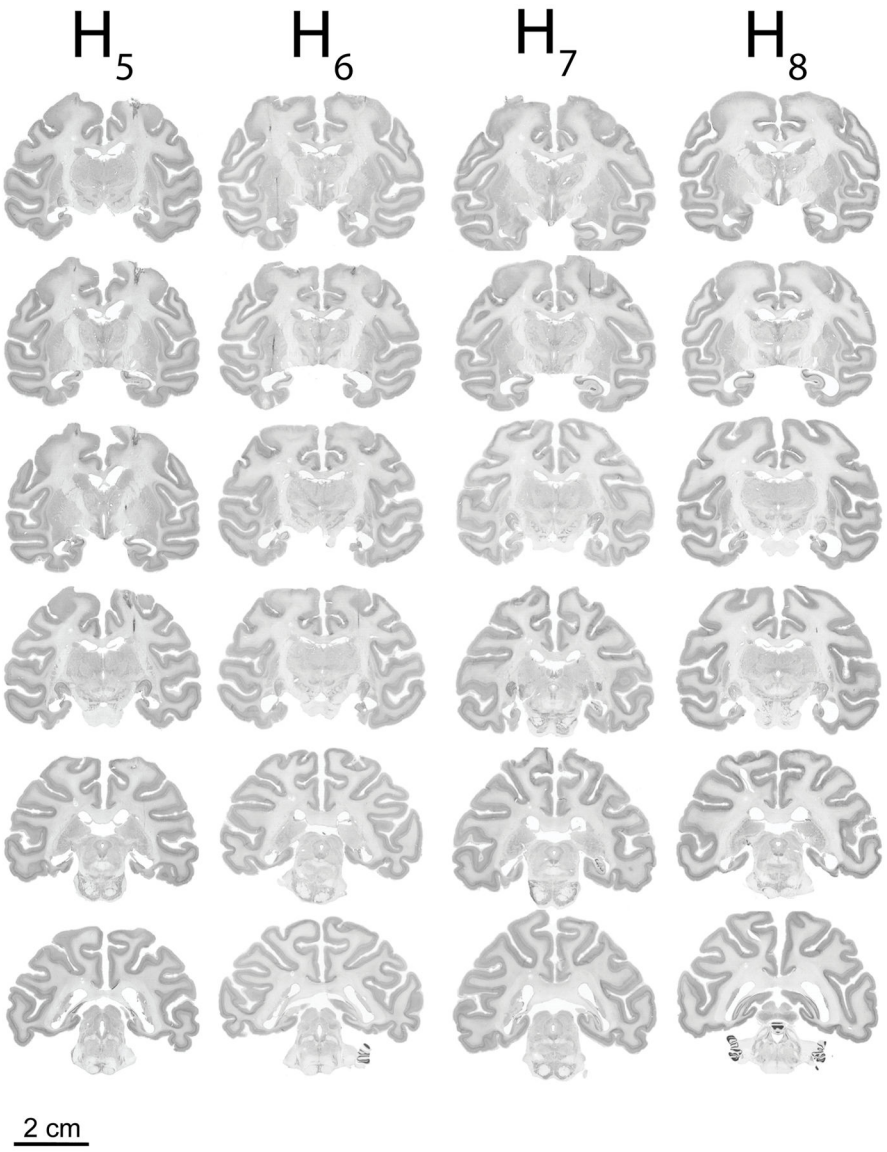


Figure 3. Histology Confirming Hippocampus Lesions

Representative histological sections from one control animal (C_1) and the seven amygdala-lesioned cases (H_1–H_8) arranged rostral (top) through caudal (bottom). The hippocampal formation (HF) and ventricle (V) are labeled in the C_1. Case labels correspond with those in Table 4.

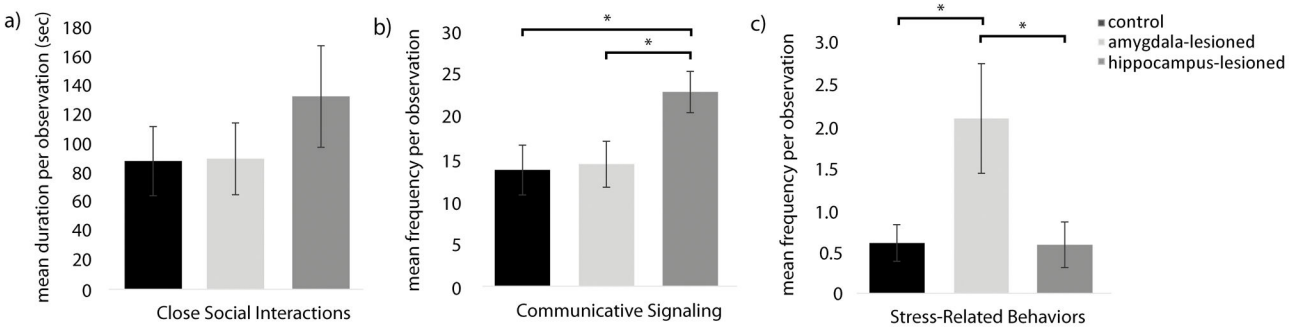


Figure 4. Behaviors during the Constrained Social Access Condition

(a) Mean durations and (b and c) frequencies of behaviors generated with all possible interaction partners (*mixed-sex* and *same-sex*).

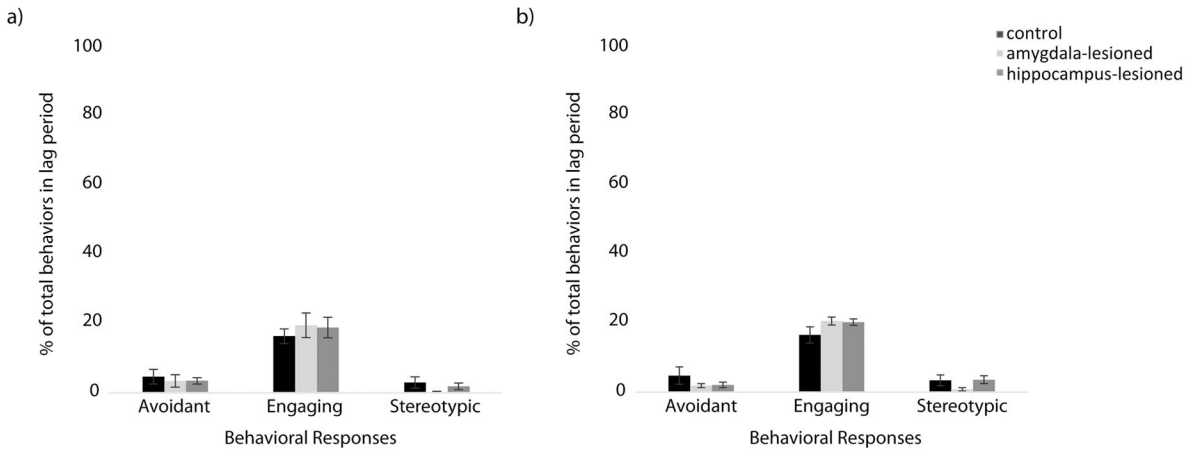


Figure 5. Behavioral Sequences during the Constrained Social Access Condition

Behaviors generated immediately following (a) subject-initiated aggressive interactions or (b) affiliative interactions.

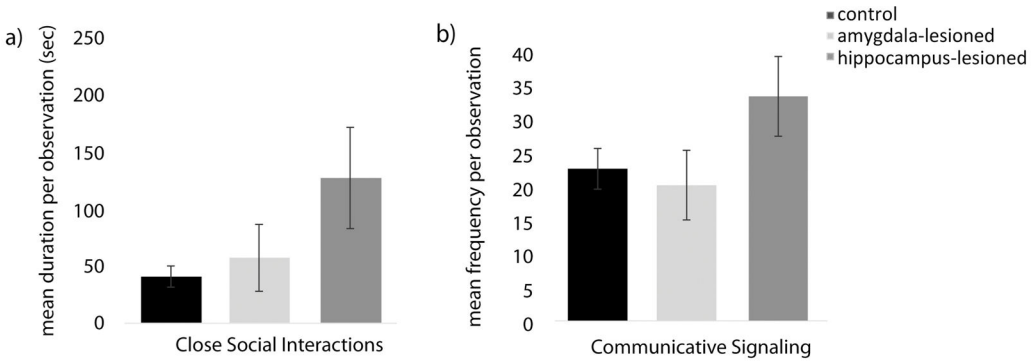


Figure 6. Behaviors during the Unconstrained Social Access Condition

(a) Mean durations of close social interactions and (b) frequencies of communicative signaling of behaviors generated during *mixed-sex interactions*.

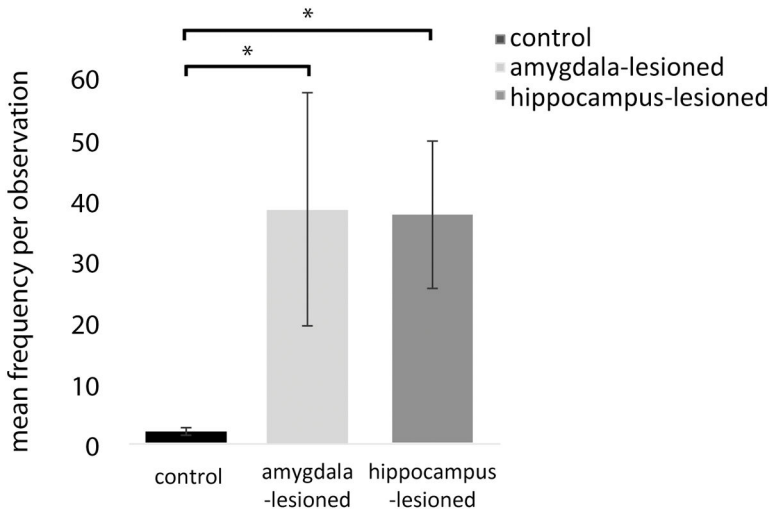


Figure 7. Stereotypic Behaviors

generated by focal animals during mixed sex pairs in the unconstrained social access condition.

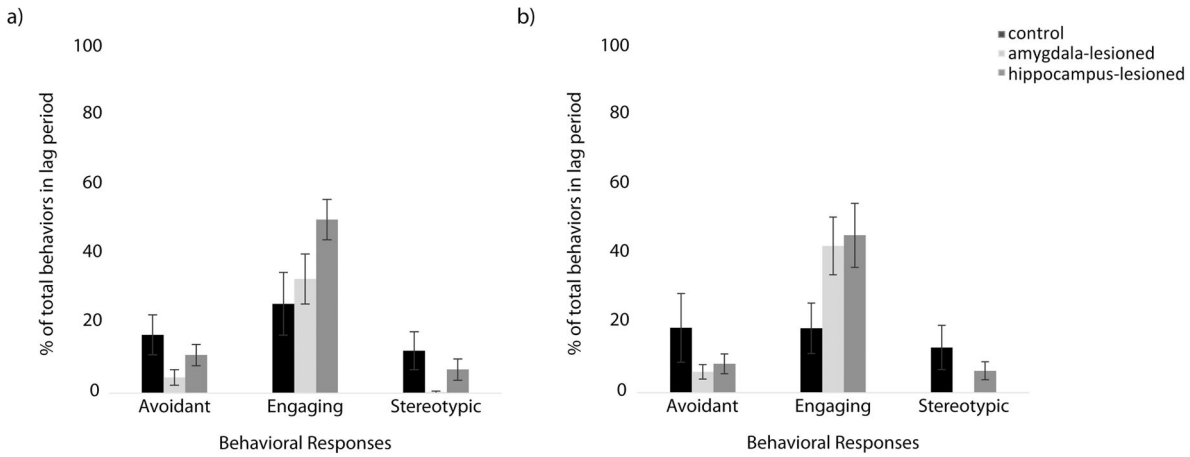


Figure 8. Behavioral Sequences during the Unconstrained Social Access Condition Following Initiation of Aggressive Interactions

Behaviors generated by the experimental animals immediately following (a) subject-initiated aggressive interactions or (b) interaction partner-initiated aggressive interactions.

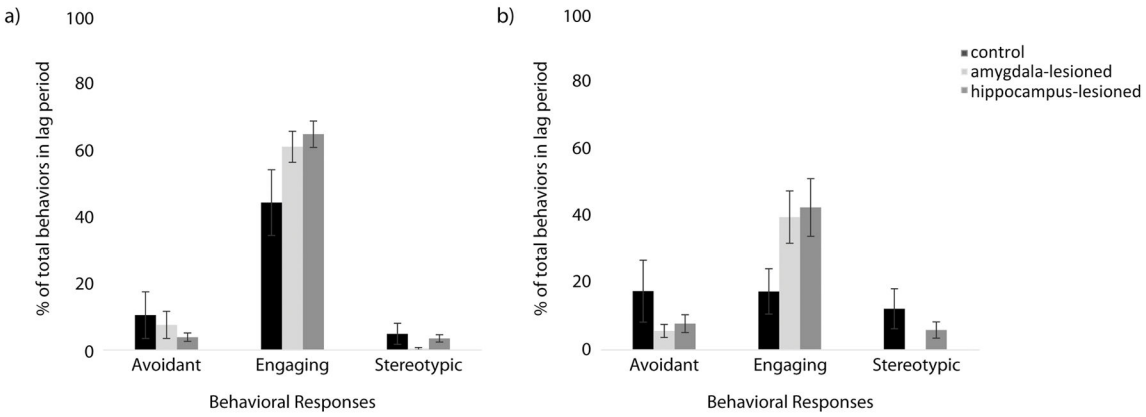


Figure 9. Behavioral Sequences during the Unconstrained Social Access Condition Following Initiation of Affiliative Interactions

Behaviors generated by the experimental animals immediately following (a) subject-initiated affiliation or (b) interaction partner-initiated affiliation.

Table 1

Behavioral Ethogram

Behavior	*Description*
States	
Close Social Interactions	
Extended Aggression	Animals bite, slap, chase, or grab at each other
Extended Contact	Animals are in physical contact.
Extended Groom	Examination, picking, or licking of another animal's fur or body.
Extended Mount	Any instance of mounting.
Extended Play	Continuous rough and tumble play and/or play threats, including playful chase.
Proximity Zone†	Focal animal is within the marked rectangle in front of the stimulus cage.
Proximity	Focal animal is directly in front of the constrained animal
Non-Social States	
Nonsocial Activity	Animal remains out of all social states and Is locomotive with head up, actively engaged in the environment.
Nonsocial Stationary	Animal remains out of all social states and Is non-locomotive with head up, actively engaged in the environment.
Nonsocial Inactivity	Animal remains out of all social states with head down, not engaged in environment, often staring off into space.
Extended Stereotypy	Focal animal is engaged in repetitive self-directed or motor stereotypic behavior.
Events	
Communicative Signaling	
Affiliative	
Approach	Intentional movement within arm's reach of another animal.
Accept Approach	Animal remains within arm's reach after the other animal approaches.
Anogenital Exploration	Oral, visual, olfactory, or manual exploration of the other animals anogenital area.
Contact	Physical contact between animals that is not aggressive.
Coo	Clear, soft sounds, moderate in pitch and intensity; usually sounds like "whoooooo.."
Follow	Intentional follow of another animal.
Groom	Examination, picking, or licking of another animal's fur or body.
Grunt	Deep, muffled, low-intensity vocalization.
Girney	Quiet, nasal whine, usually emitted during affiliative encounters.
Lipsmack	Rapid lip movements with pursed or puckered lips, usually accompanied by smacking sounds.
Huddle	Physical contact that involves one animal ventrally touching another animal.
Jaw-Thrust	Rapid lipsmack and grimace and/or brow raise usually seen while the animal walks by or approaches and then leaves quickly.
Joint Threat	Both animals threaten observer in unison
Mount	Mount that includes all of the following components: appropriate positioning of partner, hands on back, double foot clasp.
Mount Attempt†	Any component of a mouth that is attempted through the metal grille.
Present Groom	Intentional, exaggerated presentation of a part of body to another animal.
Present Neck	Presentation of neck to another animal.
Present Rump	Rigid posture with rump and tail elevated and oriented toward another individual.
Threat-Solicitation	Animal recruits the other animal in threatening the observer or another animal.
Agonistic/"Aggression"	

Behavior	*Description*
Aggression	Grabbing, slapping, and biting of another animal.
Aggressive Grunt	Low-pitched guttural sound, accompanied by a threat
Alarm Bark	Short, sharp sound.
Cage Shake	Vigorous shaking of cage, or body slam against bars
Crooktail	Tail held up stiff in a "?" shape
Displacement	Physical movement in which an animal "takes the place" of another animal.
Muzzle	Contact
Threat	Contains one or more of the following components: open mouth stare, head bobbing, ear flaps, bark vocalizations, or lunges.
Submission/"Fear"	
Avoid	Animal leaves the area when the other animal comes near or is about to approach.
Grimace	Exaggerated movement of lips such that lips are pulled back with teeth showing.
Flee	Rapid, intentional movement away from another animal.
Freeze *	Stiff body posture without any movement for more than three seconds.
Scream	High-pitched vocalization, with extreme high intensity; sounds like "eeeeeeeeee.."
Exploration	
Manual	Exploration of the cage or environment with the hands.
Oral	Exploration of the cage or environment with the mouth.
Stress-Related	
Scratch	Scratches own body.
Self-Groom	Use of hands to pick through or lick a fur or non-fur body part.
Self-Shake	Vigorous shaking of the body.
Tooth Grind	Repetitive audible rubbing of upper and lower teeth.
Yawn	Yawn.
Other	
Self Sex	Anogenital exploration of self.
Stereotypic	
Self-Directed	Repetitive behavior acted on self, including: repetitive swaying back and forth, covering hand over eye or eye pokes, unusual holding of body part or limb, biting at oneself, self-strumming.
Whole-Body	Repetitive motor behavior including: back flipping, hopping, twirling, swinging, or undirected movement with the same path repeated.
Heat Twist	Animal twists neck in a dramatic display.

† Behaviors were only applicable to the Limited Social Access Condition

* Behaviors were included in the "other" category for log sequential analyses.

Table 2

Distribution of behaviors across “if” and “then” categories for behavioral sequence analysis.

“If” Categories		“Then” Categories			
Affiliative	***Aggressive/Avoidant***	***Aggressive/Avoidant***	***Engaging***	***Stereotypic***	***Other***
Approach	Aggressive Grunt	Aggressive Grunt	Approach	Self-Directed	Undirected Facial Signals *
Accept Approach	Avoid	Avoid	Accept Approach	Whole Body	Manual Exploration
Contact	Cage Shake	Cage Shake	Contact	Head Twist	Non-Social States
Coo	Crooktail	Crooktail	Coo		Oral Exploration
Anogenital Exploration	Displacement	Displacement	Anogenital Exploration		Scratch
Follow	Extended Aggression	Extended Aggression	Follow		Self-Groom
Girney	Flee	Flee	Girney		Self-Shake
Grimace	Freeze	Freeze	Grimace		Yawn
Groom	Mount Refusal	Mount Refusal	Groom		
Grunt	Muzzle	Muzzle	Grunt		
Hiptouch	Reject Approach	Reject Approach	Hiptouch		
Huddle	Scream	Scream	Huddle		
Jaw-Thrust	Tooth Grind	Tooth Grind	Jaw-Thrust		
Joint Threat	Threat	Threat	Joint Threat		
Lipsmack			Lipsmack		
Mount			Mount		
Play			Play		
Present Groom			Present Groom		
Present Rump			Present Rump		
Proximity			Proximity		
Threat Soliciation			Threat Soliciation		

* A few subjects would generate facial signals (grimace, lipsmack) while staring off in space; the behavior was not directed to a specific recipient (dyadic partner or observer).

Table 3

	Volume of Left Amygdala	Volume of Right Amygdala	Percent Atrophy Left Amygdala	Percent Atrophy Right Amygdala
Control Animals				
Males				
Case C_1	187.31	188.69	-	-
Case C_2	247.50	246.94	-	-
Case C_3	252.70	244.86	-	-
Male Control Average	*229.17*	*226.83*		
Females				
Case C_4	199.15	197.70	-	-
Case C_5	189.34	190.17	-	-
Case C_6	202.38	195.31	-	-
Case C_7	193.02	187.69	-	-
Female Control Average	*195.97*	*192.72*		
Amygdala – Lesioned Animals				
Males				
Case A_1	13.77	19.55	94.01%	91.38%
Case A_2	9.73	4.15	95.76%	98.17%
Case A_3	6.43	13.56	97.19%	94.02%
Average	*9.96*	*12.42*	*95.65%*	*94.52%*
Females				
Case A_4	17.00	22.01	91.32%	88.58%
Case A_5	7.29	13.38	96.28%	93.06%
Case A_6	14.21	4.85	92.75%	97.49%
Case A_7	4.02	21.42	97.57%	88.89%
Average	*12.84*	*13.42*	*94.57%*	*92.00%*

Note: Volumes are in cubic millimeters. Percent atrophy was calculated by sex and by hemisphere:

(Average volume for control animals – volume of each individual amygdala lesioned animal) / Average volume for control animals. Calculations were computed in cubic microns and converted to cubic millimeters, then rounded to two decimal places. One control male's brain was not available for histological analyses because of a freezer malfunction prior to cutting.

Table 4

	Volume of Left Hippocampus	Volume of Right Hippocampus	Percent Atrophy Left Hippocampus	Percent Atrophy Right Hippocampus
Control Animals				
Males				
Case C_1	373.20	383.28	-	-
Case C_2	492.96	500.46	-	-
Case C_3	508.56	506.40	-	-
Male Control Average	*458.24*	*463.38*		
Females				
Case C_4	443.64	433.02	-	-
Case C_5	411.24	414.12	-	-
Case C_6	405.24	395.52	-	-
Case C_7	489.84	487.26	-	-
Female Control Average	*437.49*	*432.48*		
Hippocampus – Lesioned Animals				
Males				
Case H_1	49.44	53.52	89.21%	88.45%
Case H_2	42.90	41.94	90.64%	90.95%
Case H_3	94.62	99.84	79.35%	78.45%
Average	*65.10*	*62.32*	*86.40%*	*85.95%*
Females				
Case H_4	25.08	90.36	94.27%	79.11%
Case H_5	19.98	69.90	95.43%	83.84%
Case H_6	50.22	9.84	88.52%	97.72%
Case H_7	73.32	135.18	83.24%	68.74%
Case H_8	94.26	95.76	78.45%	77.86%
Average	*52.57*	*80.21*	*87.98%*	*81.45%*

Note: Volumes are in cubic millimeters. Percent atrophy was calculated by sex and by hemisphere:

(Average volume for control animals – volume of each individual hippocampus lesioned animal) / Average volume for control animals. Calculations were computed in cubic microns and converted to cubic millimeters, then rounded to two decimal places.