The Impact of Early Amygdala Damage on Juvenile Rhesus Macaque Social Behavior

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Abstract

■ The present experiments continue a longitudinal study of rhesus macaque social behavior following bilateral neonatal ibotenic acid lesions of the amygdala or hippocampus, or sham operations. Juvenile animals (approximately 1.5–2.5 years) were tested in four different social contexts—alone, while interacting with one familiar peer, while interacting with one unfamiliar peer, and in their permanent social groups. During infancy, the amygdala-lesioned animals displayed more interest in conspecifics (indexed by increased affiliative signaling) and paradoxically demonstrated more submission or fear (Bauman, Lavenex, Mason, Capitanio, & Amaral, 2004a, this journal). When these animals were assessed as juveniles, differences were less striking. Amygdala-lesioned animals generated fewer aggressive and affiliative signals (e.g., vocalizations, facial displays) and spent less time in social interactions with familiar peers. When animals were observed alone or with an unfamiliar peer, amygdala-lesioned animals, compared with other subjects, spent more time being inactive and physically explored the environment less. Despite the subtle, lesion-based differences in the frequency and duration of specific social behaviors, there were lesion-based differences in the organization of behavior such that lesion groups could be identified based on the patterning of social behaviors in a discriminant function analysis. The findings indicate that, although overall frequencies of many of the observed behaviors do not differ between groups, the general patterning of social behavior may distinguish the amygdala-lesioned animals. ■

INTRODUCTION

Damage to the adult primate amygdala disrupts affective processing (e.g., Antoniadis, Winslow, Davis, & Amaral, 2007, 2009; Chudasama, Izquierdo, & Murray, 2009; Machado, Kazama, & Bachevalier, 2009; Mason, Capitanio, Machado, Mendoza, & Amaral, 2006; Izquierdo, Suda, & Murray, 2005; Stefanacci, Clark, & Zola, 2003; Meunier, Bachevalier, Murray, Málková, & Mishkin, 1999; Zola-Morgan, Squire, Alverez-Royo, & Clower, 1991; Aggleton & Passingham, 1981), which has consequences for social behavior when interacting with conspecifics (e.g., Machado, Emery, et al., 2008; Machado & Bachevalier, 2006; Emery et al., 2001; Kling, 1974; Mirsky, 1960; Rosvold, Mirsky, & Pribram, 1954). Changes in social behavior observed in animals with adult amygdala damage have been hypothesized to result from disruption of danger detection functions of the amygdala (Amaral, 2006) rather than to an alteration to social behavior per se. In this view, adult amygdala-lesioned animals are hypersocial (Machado, Emery, et al., 2008; Emery et al., 2001) because they fail to process the potential threat of novel conspecifics. In other words, they do not demonstrate the species-typical reluctance to engage a novel conspecific in social interaction before clear dominance relationships are established. The extent to which early damage to the amygdala results in alterations in affective and social processing is less clear. The goal of the present paper is to evaluate variation in social processing in juvenile animals that received damage to the amygdala as neonates as part of our ongoing study of variation in affect (Bliss-Moreau, Bauman, & Amaral, 2011; Bliss-Moreau, Toscano, Bauman, Mason, & Amaral, 2010, 2011) and social behavior (Bauman, Lavenex, Mason, Capitanio, & Amaral, 2004a, 2004b) following early amygdala damage.

Previous research from our laboratory (Bauman et al., 2004a; Prather et al., 2001) and others (Bachevalier, 1994; Thompson & Towfighi, 1976; Thompson, Schwartzbaum, & Harlow, 1969; Kling & Green, 1967) demonstrated that macaques with early damage to the amygdala are able to generate species-typical social behaviors. In a previous report in this journal (Bauman et al., 2004a), we reported variation in social behaviors generated by maternally reared, group-socialized macaques that received bilateral neurotoxic lesions (which spare fibers of passage) of the amygdala or hippocampus, or sham operations at approximately 2 weeks of age. During these animals' first year of life (at approximately 6, 9, and 12 months of age) social behavior was observed in a number of social conditions, when the subjects were alone, in dyadic interactions ("dyads") with familiar partners, in dyads with unfamiliar partners, or in groups of animals with which they were familiar. Although there were no overall differences

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in the amount of time that amygdala-lesioned monkeys spent interacting with their peers during the first year of life, there were lesion-based differences in the frequency of their behaviors. In two contexts, while interacting one-on-one with familiar and novel partners, amygdala-lesioned animals generated more communicative signals related to affiliation (e.g., cooing, grunting, etc.). The variety of affiliative signaling was particularly evident when interacting with novel partners; in that context, amygdala-lesioned animals also presented their bodies to be groomed and mounted more often than controls. One difference between animals with neonatal as compared with adult amygdala damage was the expression of apparent fear or submission behavior. During interactions both with familiar and novel animals, in dyads and social groups, amygdala-lesioned animals expressed more signs of fear or submission (e.g., grimacing, screaming, etc.). This latter observation stands in contrast to the typical pattern of social behaviors generated by animals that receive amygdala damage as adults (i.e., less signaling of fear; Machado, Emery, et al., 2008; Emery et al., 2001). The goal of this study was to continue the evaluation of juvenile macaque social behavior following neonatal damage to the amygdala or hippocampus, at a later developmental point during which the animals were living in a more socially enriched environment. In the time between the social behavior experiment in the previous report (Bauman et al., 2004a) and the experiments presented in this article, the subjects were housed 24 hr per day in social groups with the animals with which they had previously been socialized. Subjects lived in these permanent social groups for approximately 3 months before the start of the present experiments.

METHODS

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

Animals and Living Conditions

Subject selection and rearing history has been fully described in other publications (Bliss-Moreau, Toscano, et al., 2011; Bliss-Moreau et al., 2010; Bauman et al., 2004a, 2004b). Briefly, 24 juvenile rhesus macaque monkeys received bilateral ibotenic acid lesions of either the amygdala (five females, three males) or hippocampus (five females, three males), or sham control operations (four females, four males) at 12–16 days of age. After surgery, animals were returned to their mothers and housed alone with their mothers in standard primate caging (61 cm W × 66 cm D × 81 cm H). Following recovery, subjects and their mothers were socialized with other subjects and other mothers in large chain-link indoor enclosures (2.13 m W × 3.35 m D × 2.44 m H) for 3 hr, 5 days per week. Each social group included six subjects (two from each experimental condition) and an adult male. Subjects were weaned and separated from their mothers at 6 months of age, singly housed, but were socialized in their groups without their mothers for 3 hr each day. At this time, a novel adult female was added to each social group. Subjects were permanently housed (24 hr per day) with their social groups (i.e., peers and the adult male and female) in the large enclosures beginning at 1 year of age. The experiments described in this article occurred while subjects were living in their permanent social groups.

Indoor housing rooms were maintained on a 12-hr light/dark cycle (lights on at 6 a.m.). Animals were fed monkey chow (Lab Diet #5047, PMI Nutrition International, Inc., Brentwood, MO) twice daily, provided with fresh fruit and vegetables twice per week, and had access to water ad libitum.

One of the original amygdala-lesioned males died of causes unrelated to his lesion status at approximately 1 year of age (Bauman et al., 2004a). 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.

Surgical Procedures

The surgical procedures have been described in detail in previous publications (Bauman et al., 2004a, 2004b) and are briefly summarized here. Each subject's brain was imaged on the morning before surgery to determine the stereotaxic coordinates of the amygdala or hippocampus for subsequent ibotenic acid injections. Subjects were anesthetized with ketamine hydrochloride (15 mg/kg im) and medatomidine (30 μg/kg) before being placed in an MRI-compatible stereotaxic apparatus (Crist Instruments Co., Inc., Damascus, MD). Brain imagining occurred on a General Electric 1.5 T Gyroscan magnet with the following parameters: slice thickness = 1.0 mm, T1-weighted Inversion Recovery Pulse sequence, repetition time = 21, echo time = 7.9, NEX 3, field of view = 8 cm, matrix 256 × 256.

Following the MRI, subjects were intubated so that they could be ventilated during surgery. Subjects were anesthetized with a combination of isoflurane (1.0%, varied as needed to maintain an adequate level of anesthesia) and intravenous infusion of fentanyl (7–10 μg/kg/hr). Each operated subject received two craniotomies over the left and right amygdala or hippocampus. Ibotenic acid (IBO, Biosearch Technologies, Inc., 10 mg/ml in 0.1 M phosphate-buffered saline) was injected simultaneously bilaterally into the amygdala or hippocampus using 10-μl Hamilton syringes (26-gauge beveled needles) at a rate of 0.2 μl/min. Sham-operated controls underwent the same presurgical preparations, received a midline

incision to expose the skull, and were maintained under anesthesia for the average duration of the lesion surgeries. Following the surgical procedure, all infants were monitored by a veterinarian and returned to their mothers once they were fully alert.

Lesion Analysis

This study is longitudinal, and therefore, the subjects for this experiment continue to be tested and have not been euthanized to complete histological analysis of their lesions. Lesion placement was confirmed via (1) T2-weighted MR images acquired 10 days after surgery, (2) T1-weighted images acquired when the animals were approximately 4 years (Machado, Snyder, Cherry, Lavenex, & Amaral, 2008), and (3) histological analysis of the one amygdala-lesioned subject who died during his first year of life. First, edema associated with the brain lesions was measured using T2-weighted MR images collected 10 days postsurgery using a General Electric 1.5 T Gyroscan magnet (slice thickness = 1.5 mm, T2 weighted Inversion Recovery Pulse sequence, repetition time = 4000, echo time = 102, NEX 3, field of view = 8 cm, matrix 256 × 256). The hyperintense T2-weighted signal for each of the 16 lesion animals (eight amygdala lesion, eight hippocampus lesion) was evaluated to confirm the general target and extent of the lesions (i.e., amygdala lesion sparing the hippocampus or hippocampus lesion sparing the amygdala). T2-weighted images of coronal sections through the middle portion of the amygdala are illustrated in previous publications (Bliss-Moreau, Bauman, et al., 2011; Bauman et al., 2004a, 2004b), indicating that the ibotenic acid was injected into and caused damage to the amygdala or hippocampus. Second, lesion extent was further characterized in T1-weight MRI images when animals were 4 years (Machado, Snyder, et al., 2008). Finally, the extent of the targeted lesion was confirmed using histological evaluation in the one amygdala-lesioned animal that died because of an unrelated illness.

Experimental Design and Procedures

Behavioral Sampling Procedure

Social and affective behaviors generated by our experimental animals were recorded in four different contexts in a large test cage (as detailed below): (1) while each animal was alone ("solo observations"), (2) while each animal interacted with a series of animals from his or her social rearing group ("familiar dyads"), (3) while each animal interacted with a series of novel animals from a different social rearing group ("novel dyads"), (4) while each animal was in his or her social group ("social group observations"). The same behavioral sampling technique was used in all four settings. Behavioral data were collected using The Observer 5.0 (Noldus, 1991) using the focal sampling technique (Altmann, 1974) to record the frequency and duration of species typical behaviors (see Table 1). There were three observers who were blind to lesion conditions and had an interobserver reliability of greater than 90%.

Experimental Test Cage

All observations occurred in one of the four large group test cages in which the animals were permanently housed (2.13 m W × 3.35 m D × 2.44 m H). The test cages were constructed of chain link on three sides (2.13 m wide front and back, as well as the top) and aluminum panels on the left and right sides (3.35 m deep). Animals entered and exited the test cages via an entry tunnel at the back of the cage made of 1 in. metal mesh. Solo observations, familiar dyads, and social group observations occurred in the test cage in which the animals lived permanently. Animals not being observed were relocated to temporary caging in the same housing room. Novel dyad observations occurred in one of the four test cages with which the animals were not familiar (i.e., a cage in which they did not live).

Behavioral Observation Timing

Figure 1 depicts the timing of the four behavioral experiments. See the figure capture for the average ages of the animals when each experiment was completed.

Solo observations. Solo observations occurred on 5 consecutive days between 8 and 11 a.m. and 1 and 4 p.m. Each subject was observed for two consecutive 5-min samples during each morning and each afternoon session, yielding a total of 20 observations per animal (note that data from one 5-min sample was not available for one subject). Solo observations occurred immediately before familiar dyad observations. Because solo observations occurred without a social interaction partner present with the focal animal, the only state behaviors that were scored correspond to a subset of those listed in the "nonsocial state" section of the behavioral ethogram (Table 1). Specifically, only instances of "nonsocial activity," "nonsocial inactivity," and "sleep" were recorded for solo observations.

Familiar dyads. Immediately following each solo observation, each subject was observed with a member of his or her social rearing group for a 20-min dyadic interaction. During the 20-min dyadic interaction, each animal was the focal animal (i.e., the focus of the observation) for 10 of the 20 min. During each dyad, the focal animal switched every 5 min, yielding a total of 20 observations per animal. Data from one 5-min sample was not available for one subject. Each focal animal met each partner animal at two time points, resulting in 10 dyadic interactions per animal. Animal testing order was counterbalanced for testing order (test day), test session time (morning or afternoon), interaction partner, and observer

Table 1. Behavioral Ethogram

Behavior[a]	*Description*
States	
Social states	
Other contact	Any physical contact between focal animal and other animal.
Proximity	Animal is within arm's reach of another animal.
Social activity	Animal is not in proximity or other contact but is actively moving among the group within arm's reach or contact of the other animal(s).
Ventral–ventral contact	Ventral surface of the focal animal contacts ventral surface of another animal.
Groom	Examination, picking, or licking of another animal's fur or body.
Extended play	Rough and tumble play or chase play.
Extended mount	Any instance of mounting.
Extended negative	Any instance of aggression or chase.
Nonsocial states	
Nonsocial activity	Animal remains out of all social states with head up, actively engaged in the environment.
Nonsocial inactivity	Animal remains out of all social states with head down, not engaged in environment.
Nonsocial avoidant	Animal remains out of all social states and is actively avoiding other animals.
Nonsocial vigilance[b]	Animal remains out of all social states and is actively tracking the other animal's position and placement.
Sleep	Animal is asleep.
Events	
Total communication	
Bark[c]	Low pitched, sharp, guttural sound.
Affiliative	
Anogenital exploration	Oral, olfactory, or manual exploration of another animal's anogenital area.
Approach	Intentional movement within arm's reach of another animal.
Coo	Clear, soft sounds, moderate in pitch and intensity; usually sounds like "whoooooo."
Follow	Intentional follow of another animal.
Grunt	Deep, muffled, low-intensity vocalization.
Lipsmack	Rapid lip movements with pursed or puckered lips, usually accompanied by smacking sounds.
Incomplete mount	Mount that includes one or two, but not all three, of the necessary components of a "Mount."
Inappropriate mount	An attempt to mount an inappropriate part of the body—head, side, or shoulder instead of perineum.
Mount	Mount that includes all of the following components: appropriate positioning of partner, hands on back, double foot clasp.
Huddle	Physical contact that involves one animal ventrally touching another animal.
Play threat	Open mouth threats, ear flaps, lunges, or head bobs that are more relaxed than typical threats. Often occurs in context of Rough and Tumble Play.
Present groom	Intentional presentation of neck, belly, or other part of body to another animal.
Present mount	Rigid posture with rump and tail elevated and oriented toward another individual.
Rough and tumble play	Contact play consisting of mounting, tumbling, and wrestling.

Table 1. (*continued*)

Behavior[a]	*Description*
Agonistic/"aggression"	
Aggression	Grabbing, slapping, and biting of another animal.
Chase	Rapid pursuit of another animal lasting more than 3 sec.
Displacement	Physical movement in which an animal "takes the place" of another animal.
Full threat	Contains at least two or more of the following components: open mouth stare, head bobbing, ear flaps, bark vocalizations, or lunges.
Mild threat	Contains only one of the following components: open mouth threats, ear flaps, lunges, or head bobs.
Toy-steal	Deliberate and intentional taking of toy from another animal.
Submission/"fear"	
Crooktail	Tail held in stiff "?" shape.
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 3 sec.
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.
Toy-play	Exploration of toy.
Stress	
Scratch	Scratches own body.
Self-groom	Examining, picking, or licking one's own fur or skin.
Tooth grind	Repetitive, audible rubbing of upper and lower teeth.
Yawn	Yawn.
Other events	
Cage shake	Vigorous shaking of cage bars or body slams against the cage.
Crouch	Animal is quadrupedal and bending down low but not exploring, eating, or drinking.
Mount refusal	Animal who is being mounted moves away or physically pushes partner away.
Self-sex	Anogenital exploration of self.
Withdraw	Animal moves out of arm's reach of another animal after being in proximity or contact.
Tantrum	Violent shaking or spasms of the body often accompanied by a high-pitched sticatto vocalization.

Stereotypies were also scored, but those data have been reported elsewhere (Bauman, Toscano, Babineau, Mason, & Amaral, 2008) and so are not reported here. To be scored in a "state" behavior must occur for 3 sec.

[a]Behavior was not scored for any monkey during the entire study.

[b]Nonsocial vigilance was only scored in novel dyads at a low frequency.

[c]In addition to the subordinate categories, Bark was included in the Total communication category.

Novel dyads. Each subject was observed with the six experimental animals from one other social rearing group during novel dyads (i.e., two unlesioned control animals, two hippocampal-lesioned animals, and two amygdala-lesioned animals). At the start of novel dyads, interaction partners had never had any contact and were unfamiliar to each other (i.e., they were not interaction partners in the novel dyads conducted in Bauman et al., 2004a, 2004b). Each focal animal met each partner animal six times. Six dyadic interactions occurred in the morning

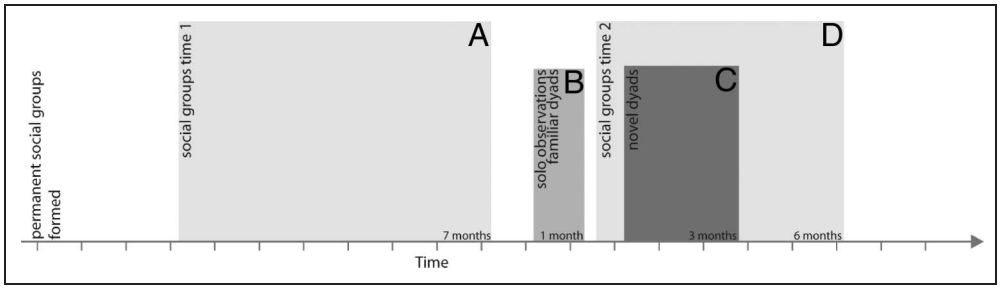

Figure 1. Experimental timeline. Note: Hashmarks on time access indicate months. (A) Mean age at the start of observations was 1.44 years (*SD* = 0.09). Mean age at the end of observations was 2.09 years (*SD* = 0.08). (B) Mean age at the start of observations was 2.17 (*SD* = 0.08). Observations were completed 4 weeks later. (C) Mean age at the start of observations was 2.34 years (*SD* = 0.10). Mean age at the end of observations was 2.46 years (*SD* = 0.10). (D) Mean age at the start of observations was 2.22 years (*SD* = 0.09). Mean age at the end of observations was 2.69 years (*SD* = 0.09).

(8–11 a.m.) and six occurred in the afternoon (1–4 p.m.) of each test day. Animal testing order was counterbalanced for weekly testing order (test day), test session time (morning or afternoon), interaction partner, and observer. As in focal dyads, observations totaled 20 min for each dyad, alternating the focal animal every 5 min, for a total of 72 observations per focal animal. Two animals were each missing data from one 5-min observation.

Social group observations. There were two sets of social group observations. Observation periods were separated by 2.5 months (see Figure 1). Each animal was observed for a 5-min sample, one or two times per week, for a total of 31–34 observations per focal animal per observation period. Social behaviors initiated by the focal animal were qualified in terms of whether they were directed at a peer (i.e., an amygdala-lesioned, hippocampus-lesioned, or sham-operated control animal), an adult (i.e., the adult male or adult female living with the social group), or the group (i.e., behavior with nonspecific or no social target). Observation order was pseudorandomized.

Data Analysis Strategy

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 each type of interaction partner (as specified above) and then averaged across the number of observations to create a mean per observation. ANOVA was performed on each broad behavioral category with focal animal lesion group as the between-subject factor. Significant subject effects were further evaluated with post hoc least significant difference tests and within-subject effects were evaluated with paired *t* tests. Data were $\log10(x + 1)$ transformed in cases where they were not normally distributed. For the purposes of interpretation, raw data (means and variance indices) are presented; log-transformed data 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.

We conducted a series of ANOVA analyses to evaluate lesion-based differences in each individual behavior at this time point to make the results more easily comparable to results found at 6 and 9 months of age (Bauman et al., 2004a) in the same animals. For the sake of brevity, only significant results and those about which there were a priori hypotheses (based on the findings of Bauman et al., 2004a) are presented here. Other analyses are available upon request.

Finally, we assessed the extent to which the organization of behaviors of the lesioned animals in the presence of the intact control animals varied by lesion condition and could be used to identify lesion groups. We also completed a series of MANOVA analyses followed by discriminant function analyses on the social behaviors generated while interacting with control animals during the dyad experiments. Beyond the lesion-based differences in the frequency and duration of behaviors during social interactions, we were interested in whether the organization of classes of behaviors (e.g., correlations across multiple behaviors) might predict lesion group membership. To assess whether lesion condition might influence the relationship between behaviors, we conducted an additional set of analyses using the data collected when focal animals interacted with control partners. Specifically, we ran MANOVAs on the dependent variables that constituted the behavioral categories above with lesion condition as a between-subjects factor and then followed those MANOVAs with discriminant function analysis to examine how the relationship between dependent variables discriminated the lesion groups. Only significant MANOVAs are discussed. We were primarily interested in whether early damage to the amygdala might alter patterns of close social interactions—those in which animals actively engage each other. As such, we conducted MANOVAs and discriminant function analyses on the social state data (frequency and duration). Given that differences in exploratory behaviors were found at earlier time points, we also conducted similar analyses on the exploration data. Those analyses are available from either

the first author (EBM: eblissmoreau@ucdavis.edu) or senior author (DGA: dgamaral@ucdavis.edu).

RESULTS

Solo Observations

There were lesion-based differences in the frequency of state changes during solo observations, $F(2, 21) = 5.31$, $p = .014$, $\eta_p^2 = .336$; A > C, H, $p = .01$ (log-transformed analyses, raw means presented below). State changes occurred more frequently for amygdala-lesioned animals ($M = 1.86$, $SE = 0.39$) who changed their state more frequently than did control and hippocampus-lesioned animals (for both groups $M = 1.00$, $SE = 0.00$). Whereas hippocampus-lesioned and control animals spent all of each 5-min sampling period moving around the cage (being "active"), amygdala-lesioned subjects also spent time in states of "inactivity" ($M = 0.46$, $SE = 0.21$) and sleep ($M = 0.05$, $SE = 0.03$). Table of all means is available by request.

State differences were reflected in the duration data as well. Amygdala-lesioned animals spent less time in the active state compared with control and hippocampus-lesioned animals, $F(2, 21) = 4.04$, $p = .033$, $\eta_p^2 = .278$ (log-transformed analyses; raw means: Amygdala-lesioned animals: $M = 284.07$, $SE = 7.83$; control and hippocampus-lesioned animals: $M = 300.00$, $SE = 0.00$). Five of the eight amygdala-lesioned animals spent time in the inactive state and/or asleep.

No significant lesion-based differences in exploration of any kind were observed during this experiment.

Familiar Dyads

See Table 2 for frequency data and Table 3 for duration data.

Total Number of State Changes

Replicating the finding from solo observations, lesion conditions differed in the total number of state changes during familiar dyads. Amygdala-lesioned animals initiated the most state changes, and hippocampus-lesioned animals initiated the least state changes.

Social states. Amygdala-lesioned and control animals initiated social states most frequently, whereas hippocampus-lesioned animals initiated social states least frequently. Social states were initiated most frequently with control partner animals and least frequently with hippocampus-lesioned animals. Control animals initiated the longest duration of social states, although the only significant between-group difference was between control and hippocampus-lesioned animals. Social state durations were also significantly longer with control animals as compared with both amygdala-lesioned and hippocampus-lesioned animals.

Amygdala-lesioned animals initiated the highest frequency of nonsocial states, and hippocampus-lesioned animals initiated the lowest frequency, although, once again, only amygdala- and hippocampus-lesioned animals differed significantly. Control animals spent the least amount of time in nonsocial states—significantly less than both amygdala- and hippocampus-lesioned animals. Amygdala- and hippocampus-lesioned animals did not differ significantly in the duration of time spent in nonsocial states. This pattern of effects was seen in the partner lesion data as well. Nonsocial state durations were significantly shorter when focal animals interacted with control animals, as compared with both amygdala- and hippocampus-lesioned animals.

Total Communication

Amygdala-lesioned animals produced the most communicative signals. A significant partner lesion effect revealed that focal animals generated fewer communicative signals with hippocampus-lesioned animals than with animals of the other two groups. Amygdala-lesioned animals generated fewest communicative signals with control animals, whereas both control and hippocampus-lesioned animals generated fewest communicative signals with hippocampus-lesioned subjects as indicated by a significant focal lesion × partner lesion effect.

Affiliative signals. The effect of lesion condition on communicative signals was primarily driven by amygdala-lesioned animals' increased affiliative signaling. When the total frequency of affiliative signals was considered alone, amygdala-lesioned animals produced significantly more affiliative signals than did control or hippocampus-lesioned animals. Overall, affiliative signals were generated equally frequently with amygdala-lesioned and control partners and least frequently with hippocampus-lesioned partners. A significant focal lesion × partner lesion effect revealed that amygdala-lesioned animals' generation of affiliative signals was consistent across interaction partners whereas both control and hippocampus-lesioned animals produced fewer affiliative signals with hippocampus-lesioned animals.

Submission/"fear"-related signals. There were no lesion group or partner lesion effects on submission or "fear"-related behaviors.

Agonistic/"aggression"-related signals. Amygdala-lesioned animals were significantly less agonistic than both hippocampus-lesioned and control animals.

Exploratory Behaviors

Amygdala-lesioned animals explored the least, whereas hippocampus-lesioned animals explored the most. A partner lesion effect revealed that exploratory behavior was

Table 2. Mean Frequency per Familiar Dyad Observation

	Focal Lesion Condition			*Focal Lesion Effect*				*Partner Lesion Condition*			*Partner Lesion Effect*			*Partner Lesion Pairwise Comparison*		*Focal Lesion × Partner Lesion Effect*		
Behavior	*AMY*	*CON*	*HIP*	*F(2, 21)*	*p*	η_p^2	*Post hoc*	*AMY*	*CON*	*HIP*	*F(2, 42)*	*p*	η_p^2	*t(23)*	*p*	*F(4, 42)*	*p*	η_p^2
Total states	7.95 (0.48)	7.12 (0.32)	5.90 (0.53)	5.16	.015	.330	A > H p = .004 C > H p = .072	7.35 (0.46)	7.62 (0.46)	5.99 (0.50)	3.76	.031	.152	2.26 2.38	A > H p = .033 C > H p = .026	–	–	–
Social[a]	3.39 (0.28)	3.02 (0.34)	2.26 (0.28)	4.62	.022	.306	A > H p = .007 C > H p = .054	2.91 (0.28)	3.41 (0.30)	2.34 (0.26)	3.47	.040	.142	2.70	C > H p = .013	–	–	–
Nonsocial	4.56 (0.25)	4.10 (0.11)	3.64 (0.28)	4.20	.029	.286	A > H p = .009	4.44 (0.23)	4.22 (0.26)	3.65 (0.26)	2.84	.070	.119	–	–	–	–	
Total communication events[b]	18.47 (1.56)	12.62 (1.41)	12.22 (1.72)	4.98	.017	.322	A > C p = .015 A > H p = .010	15.77 (1.17)	14.81 (1.38)	12.74 (1.31)	3.28	.048	.135	2.95	A > H p = .007	2.81	.037	.211
Affiliative	17.22 (1.51)	10.10 (.84)	10.18 (1.49)	9.63	.001	.478	A > C p = .001 A > H p = .001	14.04 (1.19)	12.55 (1.13)	10.92 (1.34)	4.08	.024	.163	2.86	A > H p = .009	3.23	.021	.235
Submission/"fear"[a]	0.90 (0.22)	1.04 (0.59)	0.60 (0.16)	0.26	.776	.024	–	.58 (.18)	1.36 (.34)	.59 (.19)	–	–	–	–	–	–	–	–
Agonistic/"aggression"[a]	0.32 (0.08)	1.28 (0.19)	1.43 (0.19)	16.50	.00005	.611	C > A p = .0002 H > A p = .00002	1.05 (0.19)	0.77 (0.16)	1.22 (0.27)	–	–	–	–	–	–	–	–
Total exploration events	3.74 (0.78)	4.92 (0.53)	6.30 (0.62)	3.85	.038	.268	H > A p = .011	4.99 (0.55)	3.88 (0.45)	6.10 (0.63)	7.91	.001	.274	3.64 1.74 1.87	H > C p = .001 A > C p = .095 H > A p = .074	3.45	.016	.247

[a]Statistical analyses were performed on log-transformed data, but raw means are presented for ease of interpretation.

[b]In addition to the subordinate categories, Total communication also includes Bark.

Table 3. Mean Duration per Familiar Dyad Observation

	Focal Lesion Condition			*Focal Lesion Effect*				*Partner Lesion Condition*			*Partner Lesion Effect*			*Partner Lesion Pairwise Comparison*		*Focal Lesion × Partner Lesion Effect*		
Behavior	*AMY*	*CON*	*HIP*	*F(2, 21)*	*p*	η_p^2	*Post hoc*	*AMY*	*CON*	*HIP*	*F(2, 42)*	*p*	η_p^2	*t(23)*	*p*	*F(4, 42)*	*p*	η_p^2
Total social states[a]	37.60 (3.99)	59.14 (11.61)	32.39 (4.89)	4.53	.023	.302	C > H p = .007	38.56 (5.79)	59.89 (8.41)	30.69 (5.08)	6.37	.004	.233	2.40 3.98	C > A p = .025 C > H p = .001	–	–	–
Other contact[a]	3.48 (1.27)	3.89 (1.16)	2.90 (1.60)	–	–	–	–	2.69 (.58)	4.08 (1.82)	3.49 (1.55)	–	–	–	–	–	–	–	–
Proximity[a]	24.41 (4.45)	19.53 (1.95)	15.88 (2.54)	–	–	–	–	16.16 (2.36)	26.84 (3.99)	16.82 (1.87)	4.50	.017	.177	2.72 2.54	C > A p = .012 C > H p = .019	–	–	–
Groom[a]	.32 (.21)	28.29 (10.42)	8.30 (3.42)	6.20	.008	.371	C > A p = .002 C > H p = .042	10.50 (4.29)	19.88 (6.88)	6.54 (4.31)	2.51	.093	.107	–	–	–	–	–
Play[a]	8.12 (1.99)	5.81 (1.77)	3.88 (1.61)	–	–	–	–	7.83 (1.59)	7.36 (2.30)	2.62 (.96)	3.92	.028	.157	3.35	A > H p = .003	–	–	–
Mount[a]	1.27 (0.87)	1.18 (0.45)	1.36 (0.57)	–	–	–	–	1.04 (0.37)	1.67 (0.53)	1.11 (0.62)	–	–	–	–	–	4.37	.005	.294
Extended negative[a]	0.00 (0.00)	0.45 (0.29)	0.06 (0.06)	–	–	–	–	0.34 (0.29)	0.06 (0.06)	0.10 (0.09)	–	–	–	–	–	–	–	–
Total nonsocial states[b]	238.24 (7.54)	194.77 (7.63)	240.38 (8.34)	10.76	.001	.506	A > C p = .001 H > C p = .0005	238.33 (7.04)	193.97 (10.37)	241.09 (7.67)	14.70	.00009	.412	3.87 4.12	A > C p = .001 H > C p = .0004	–	–	–
Activity[b]	237.63 (7.65)	193.80 (7.38)	240.11 (8.26)	11.23	.0005	.517	A > C p = .001 H > C p = 0004	238.28 (7.04)	192.31 (10.48)	240.97 (7.69)	15.46	.0007	.424	3.94 4.21	A > C p = .001 H > C p = .0003	–	–	–
Inactivity[a,b]	0.31 (0.17)	0.06 (0.06)	0.00 (0.00)	–	–	–	–	0.06 (0.06)	0.27 (0.17)	0.04 (0.04)	0.89	.387	.041	–	–	–	–	–
Sleep[a,b]	0.30 (0.27)	0.91 (0.62)	0.27 (0.27)	–	–	–	–	0.00 (0.00)	1.39 (0.72)	0.08 (0.06)	3.37	.077	.138	–	–	–	–	–

[a]Statistical analyses were performed on log-transformed data, but raw means are presented for ease of interpretation.

[b]Distribution violates assumptions of sphericity. Degrees of freedom were Greenhouse–Geisser corrected.

greatest during interactions with hippocampus-lesioned subjects and least during interactions with control animals. Amygdala-lesioned animals exploratory behavior was consistently low across all interaction partners, whereas control and hippocampus-lesioned animals had increased exploratory behavior with hippocampus-lesioned interaction partners.

Lesion Group Classification Based on Patterns of Behaviors with Familiar Control Animals

Social State Frequency

There was a significant effect of lesion condition on social state frequency, $V = 1.153, F(12, 34) = 3.853, p = .00094$, indicating that the organization of social states differed by lesion group. Separate univariate ANOVAs, however, on the social state variables revealed a significant effect of lesion condition only on grooming behavior, $F(2, 21) = 5.690, p = .011, \eta_p^2 = .351$ (C > A, $p = .00597$; C > H, $p = .0116$), and nonsignificant lesion group effects on mounting, extended negative, play, contact, and proximity. Discriminant function analysis revealed that the relationship between social state variables was captured by two functions, the first which explained 84.6% of the variance (canonical $R^2 = .77$) and the second which explained 15.4% of the variance (canonical $R^2 = .38$). A combination of these discriminant functions differentiated the lesion conditions, $\Lambda = 0 .141, \chi^2(12) = 36.183, p = .0003$. The correlations between the social states and discriminant functions indicated that grooming ($r = .401$) and extended negative ($r = .167$) loaded more highly onto the first factor, whereas proximity ($r = .588$), contact ($r = .435$), play ($r = .323$), and mounting ($r = -.320$) loaded most highly onto the second factor. Given that there was no extended negative initiated by the amygdala- and hippocampus-lesioned animals and that their rates of grooming compared with controls were low, Function 1 likely captured the social behavior patterns unique to control animals. Taken together, the two functions were able to correctly classify 83.3% of the animals into their correct lesion groups (7/8 controls, 7/8 amygdala-lesioned, 6/8 hippocampus-lesioned), Press's Q Statistic = 27, $p < .001$. One amygdala-lesioned subject was misclassified as a hippocampus-lesioned subject, and two hippocampus-lesioned subjects were misclassified as amygdala-lesioned subjects; one control animal was misclassified as an amygdala-lesioned subject. See Figure 2A for a visual depiction of the group classification.

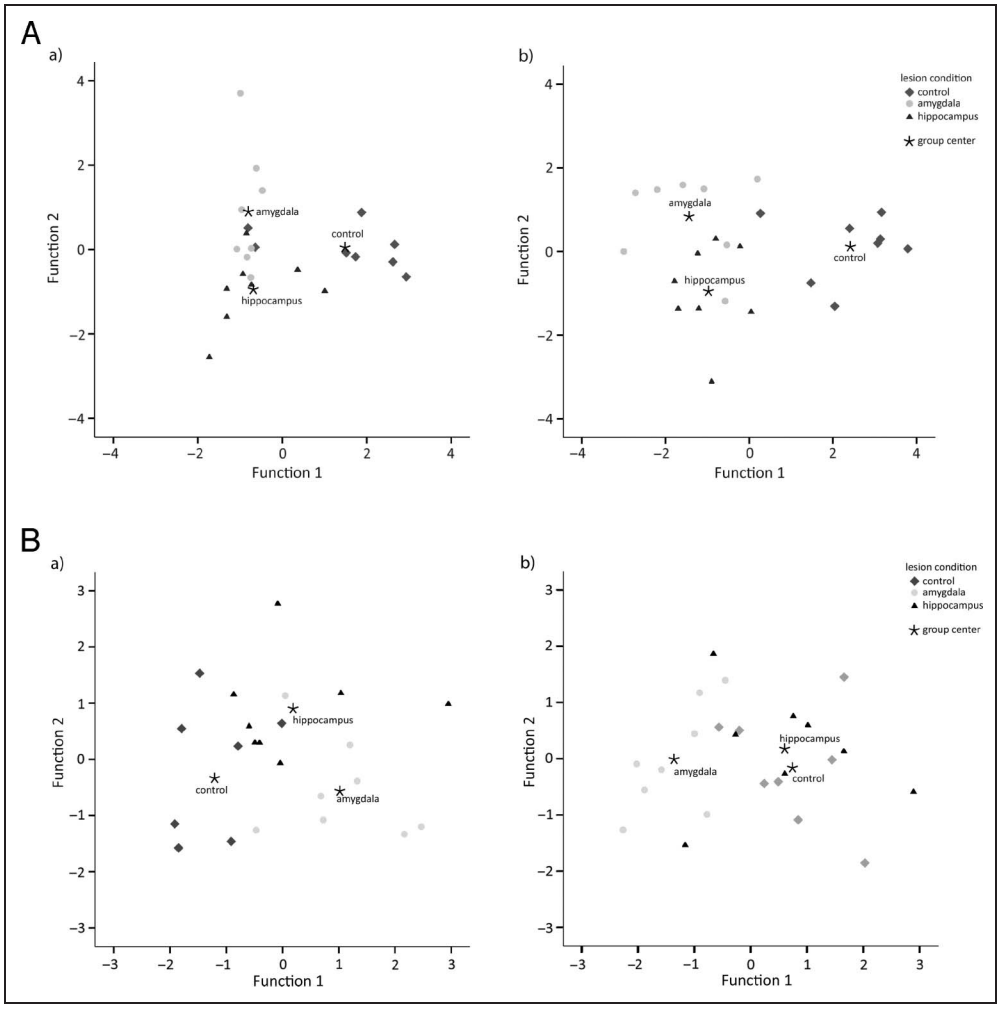

Figure 2. Classification of lesion groups based on (A) the duration of time spent in social states and (B) the frequency of social states during familiar dyads. Each individual data point represents a single animal. In both cases, Function 1 maximally separated control from lesion groups.

Social State Duration

The analysis of social state duration paralleled the analysis of the frequency data. There was a significant effect of lesion condition on social state durations, $V = 0.952$, $F(12, 34) = 2.572$, $p = .015$, indicating that the organization of social state durations differed by lesion group. However, as in the analysis of social state frequency behaviors, separate univariate ANOVAs on the social state variables revealed a significant effect of lesion condition only on grooming behavior, $F(2, 21) = 6.982$, $p = .005$, $\eta_p^2 = .399$ (C > A, $p = .002$; C > H, $p = .009$), and nonsignificant lesion group effects on mounting, extended negative, play, contact, and proximity. Discriminant function analysis revealed that the relationship between social state duration variables was captured by two functions, the first of which explained 66.2% of the variance (canonical $R^2 = .56$) and the second of which explained 33.8% of the variance (canonical $R^2 = .39$). A combination of these discriminant functions differentiated the lesion conditions, $\Lambda = 0.268$, $\chi^2(12) = 24.369$, $p = .018$. The correlations between the social states and discriminant functions revealed that grooming ($r = .719$) and extended negative ($r = .274$) loaded more highly onto the first factor whereas proximity ($r = .580$), contact ($r = -.173$), play ($r = .296$), and mounting ($r = -.296$) loaded most highly onto the second factor. As with the duration data, Function 1 therefore likely captured the social behavior patterns unique to control animals because there were no extended negative social interactions initiated by the amygdala and hippocampus-lesioned animals and their rates of grooming were low (compared with controls). Taken together, the two functions were able to correctly classify 75.0% of the animals into their correct lesion groups (6/8 controls, 6/8 amygdala-lesioned, and 6/8 hippocampus-lesioned), Press's Q Statistic = 18.75, $p < .001$. Two controls were misclassified as amygdala-lesioned animals, two amygdala-lesioned animals were misclassified as hippocampus-lesioned animals, and one hippocampus-lesioned animal was misclassified as an amygdala-lesioned animal. See Figure 2B for a visual depiction of the group classification.

Novel Dyads

Statistics for significant analyses are presented below. All additional means and statistics are available upon request.

Total Number of State Changes

In contrast to the findings in familiar dyads, there was no effect of lesion condition on the total number of state changes or the number or duration of social or nonsocial states during novel dyads. Amygdala-lesioned animals did, however, spend more time in the inactive state ($M = 1.60$, $SE = 0.56$) than both control ($M = 0.20$, $SE = 0.20$) and hippocampus-lesioned ($M = 0.00$, $SE = 0.00$) animals, $F(2, 21) = 7.20$, $p = .044$, $\eta_p^2 = .407$; A > C, $p = .007$; A > H, $p = .002$ (log-transformed analyses, raw means presented).

Total Communication

In contrast to the findings in familiar dyads, there were no lesion-based differences in the total number of communicative signals during novel dyads.

Affiliative signals. In contrast to the findings in familiar dyads, there were no lesion-based differences observed in the total number of affiliative signals during novel dyads.

Submission/"fear"-related signals. Although there was not a significant effect of focal animal lesion on submission-related behaviors, there was a significant effect of partner lesion condition, $F(2, 42) = 4.19$, $p = .022$, $\eta_p^2 = .166$; C > A, $p = .015$; H > A, $p = .085$ (log-transformed analyses, raw means presented below). All animals were least submissive when interacting with amygdala-lesioned animals ($M = 1.65$, $SE = 0.32$) as compared with when they interacted with control animals ($M = 2.56$, $SE = 0.36$) or hippocampus-lesioned animals ($M = 2.12$, $SE = 0.37$).

Agonistic/"aggression"-related signals. Across all three partner lesion conditions, amygdala-lesioned animals initiated fewer agonistic behaviors ($M = 0.36$, $SE = 0.10$) than either control ($M = 1.20$, $SE = 0.33$) or hippocampus-lesioned animals ($M = 0.96$, $SE = 0.17$); $F(2, 21) = 4.43$, $p = .025$, $\eta_p^2 = .297$; C > A, $p = .011$; H > A, $p = .030$ (log-transformed analyses, raw means presented).

Exploratory Behaviors

There was a main effect of lesion condition on exploratory behavior, $F(2, 21) = 12.08$, $p = .0003$, $\eta_p^2 = .535$ (log-transformed analyses, raw means presented below). Rates of exploration were significantly lower for amygdala-lesioned animals ($M = 2.38$, $SE = 0.40$) than for control animals ($M = 5.60$, $SE = 0.45$) or hippocampus-lesioned animals ($M = 5.36$, $SE = 0.66$).

Lesion Group Classification Based on Patterns of Behaviors with Novel Control Animals

As in the familiar dyads, we used behaviors generated with control animals to attempt to predict lesion group membership. The MANOVAs on social state data were not significant. Only the MANOVA on the exploratory behavior yielded significant results. It is available by request.

Social Groups

See Table 4 for the duration data. The only significant effects in the frequency data were relative to time

Table 4. Mean Duration per Social Group Observation (Main Effects)

	Focal Lesion Condition			*Focal Lesion Effect*				*Partner Lesion Effect*			*Partner Lesion Pairwise Comparison*		*Time Condition*		*Test Time Effect*			
Behavior	*AMY*	*CON*	*HIP*	*F(2, 21)*	*p*	η_p^2	*Post hoc*	*F(2, 42)*	*p*	η_p^2	*t(23)*	*p*	*Time 1*	*Time 2*	*F(4, 42)*	*p*	η_p^2	*Direction of Effect*
Total social states (with all partners)[a,b]	67.37 (4.51)	106.92 (10.28)	92.74 (8.78)	7.51	.003	.417	C > A p = .001 H > A p = .016	–	–	–	–	–	80.52 (5.63)	97.50 (6.59)	12.78	.002	.378	2 > 1
Total social states (with peers)[a]	27.33 (2.06)	46.61 (6.07)	33.64 (2.77)	3.70	.042	.261	C > A p = .014	–	–	–	–	–	32.85 (2.89)	38.87 (3.08)	13.19	.002	.386	2 > 1
Other contact[a]	5.78 (1.02)	11.59 (2.00)	7.27 (0.72)	3.25	.059	.236	–	–	–	–	–	–	8.64 (1.30)	7.79 (1.16)	–	–	–	–
Proximity[a]	12.70 (1.04)	21.65 (4.13)	14.74 (1.43)	–	–	–	–	3.02	.060	.126	–	–	14.04 (1.48)	18.69 (2.07)	22.51	.0001	.517	2 > 1
Groom[a]	1.19 (.87)	7.31 (2.12)	5.92 (2.02)	4.37	.026	.294	C > A p = .011 H > A p = .038	–	–	–	–	–	3.33 (0.93)	6.28 (1.48)	9.81	.005	.318	2 > 1
Play[a]	7.47 (1.70)	5.04 (0.85)	5.57 (0.51)	–	–	–	–	2.65	.083	.112	–	–	6.29 (0.92)	5.76 (0.59)	–	–	–	–
Mount[a]	0.15 (0.08)	0.36 (0.17)	0.13 (0.05)	–	–	–	–	–	–	–	–	–	0.10 (0.05)	0.33 (0.11)	5.16	.034	.197	2 > 1
Extended negative[a]	0.01 (0.01)	0.05 (0.02)	0.01 (0.01)	2.68	.092	.203	–	–	–	–	–	–	0.03 (0.01)	0.02 (0.01)	–	–	–	–
Ventral[a]	0.02 (0.01)	0.61 (0.50)	0.00 (0.00)	–	–	–	–	–	–	–	–	–	0.42 (0.34)	0.00 (0.00)	–	–	–	–
Total nonsocial states[c]	185.44 (5.20)	138.16 (10.34)	154.01 (7.75)	8.96	.002	.460	A > C p = .0004 A > H p = .012	–	–	–	–	–	175.25 (6.74)	143.16 (6.79)	25.97	.00005	.553	1 > 2
Activity	181.26 (5.01)	132.48 (10.53)	149.59 (8.21)	9.03	.001	.462	A > C p = .0004 A > H p = .013	–	–	–	–	–	168.97 (7.02)	139.92 (6.83)	20.99	.0002	.500	1 > 2
Inactivity[a]	0.51 (0.35)	0.81 (0.65)	0.79 (0.41)	–	–	–	–	–	–	–	–	–	0.24 (0.14)	1.17 (0.49)	4.95	.037	.191	2 > 1
Sleep[a]	3.68 (1.62)	4.87 (1.45)	3.62 (1.09)	–	–	–	–	–	–	–	–	–	6.04 (1.33)	2.08 (0.47)	7.01	.015	.250	1 > 2

Data presented are behaviors that occurred in the presence of peers, except total social (with all partners). Tables representing behaviors occurring in the presence of all animals in social groups are available upon request.

[a]Statistical analyses were performed on log-transformed data, but raw means are presented for ease of interpretation.

[b]Degrees of freedom for focal lesion effect and test time effect are 1, 21.

effects (Time 1 vs. Time 2); those data are available upon request.

Social Behaviors

Lesion-based differences in the duration of time spent in social states was first assessed across all possible interaction partners (including behaviors scored in the presence of individual peers, adults, and the entire group). There was a significant effect of time such that all animals spent more time in social states during the second as compared with the first observation period. Amygdala-lesioned animals spent less time in social states than control and hippocampus-lesioned animals. There was no time × lesion effect indicating that time effect did not vary by lesion condition nor did the lesion effect vary by time. Animals initiated more social states with all possible interaction partners at Time 2 as compared with Time 1. There were no focal lesion condition differences in the frequency of social states.

Lesion-based differences in social states were driven by interactions with peers rather than with the adults, and so only those effects are discussed further (all other analyses are available upon request). There were no significant effects in an analysis of the social state data with adults only.

All animals spent more time interacting with their age-matched peers at Time 2 as compared with Time 1. This was true for the total time spent in social states, and the effect was driven by time spent with peers in proximity, grooming, and mounting. The effect of time on proximity was qualified by a complex significant focal lesion × partner lesion × time effect, $F(3.128, 32.841) = 3.903$, $p = .016$, $\eta_p^2 = .271$ (log-transformed analyses). Control animals spent the same amount of time in proximity with amygdala-lesioned animals at both time points, yet greater lengths of time with members of the other groups at Time 2 as compared with Time 1. In contrast, amygdala- and hippocampus-lesioned animals spent more time with amygdala-lesioned animals at Time 2 than Time 1, but the same length of time with both control animals and hippocampus-lesioned animals.

Amygdala-lesioned animals spent less time grooming their peers than both controls and hippocampus-lesioned animals. Amygdala-lesioned animals also groomed less frequently than control and hippocampus-lesioned animals across both meetings, $F(1, 21) = 5.035$, $p = .016$, $\eta_p^2 = .324$ (log-transformed analyses; raw means; Amygdala-lesioned: $M = 0.057$, $SE = 0.036$; control animals: $M = 0.171$, $SE = 0.039$; hippocampus-lesioned: $M = 0.144$, $SE = 0.033$), although grooming frequency increased overall between observation period Time 1 ($M = 0.095$, $SE = 0.021$) and Time 2 ($M = 0.153$, $SE = 031$), $F(1, 21) = 6.857$, $p = .016$, $\eta_p^2 = .246$.

Although there was not a significant effect of lesion on extended negative behavior overall and the frequencies of agonistic behaviors were extremely low, the general pattern of lesion-based differences in the duration of agnostic behavior paralleled previous findings (Bauman et al., 2004a). The effect of focal lesion on the duration of all initiated extended negative was not statistically significant. However, there was a trend for control animals to have longer durations of extended negative behavior than amygdala- and hippocampus-lesioned animals. This was qualified by a significant focal lesion × partner lesion interaction, $F(4, 42) = 3.18$, $p = .023$, $\eta_p^2 = .232$ (log-transformed), in which control animals engaged in the longest duration of extended negative towards hippocampus-lesioned animals.

Significant lesioned-based differences in the frequency of both displacement and aggressive grabbing paralleled the observations with these animals earlier in their development (Bauman et al., 2004a). Specifically, amygdala-lesioned animals displaced their peers less frequently than control and hippocampus-lesioned animals, $F(2, 21) = 5.612$, $p = .011$, $\eta_p^2 = .348$ (log-transformed analyses; raw means; Amygdala-lesioned: $M = 0.034$, $SE = 0.008$; hippocampus-lesioned: $M = 0.038$, $SE = 0.011$; control: $M = 0.038$, $SE = 0.011$). Amygdala-lesioned animals also initiated fewer instances of aggression than did control and hippocampus-lesioned animals, $F(2, 21) = 4.863$, $p = .018$, $\eta_p^2 = .317$ (log-transformed analyses; raw means; Amygdala-lesioned: $M = 0.034$, $SE = 0.014$; hippocampus-lesioned: $M = 0.133$, $SE = 0.030$; control: $M = 0.129$, $SE = 0.030$). The frequency of aggression decreased from Time 1 to Time 2 for control and hippocampus-lesioned animals, but not for amygdala-lesioned animals whose frequency remained low over time, $F(2, 21) = 4.258$, $p = .028$, $\eta_p^2 = .289$.

There were a number of time-related effects that speak to the development of social behavior. Animals spent more time in social states during the second set of observations as compared with the first set of observations (including the total duration of social states, total proximity, grooming, and mounting), but the frequency of communicative behaviors was lower in the second as compared with the first set of observations. A focal lesion by time effect that did not reach conventional levels of significance, $F(2, 21) = 2.90$, $p = .077$, $\eta_p^2 = .216$ (log-transformed), indicated that whereas controls did not change in their communicative signaling from Time 1 to Time 2, both amygdala-lesioned and hippocampus-lesioned animals tended to become less communicative.

Nonsocial Behaviors

Overall, all monkeys spent more time in nonsocial states during the first set of observations as compared with the second set of observations. Amygdala-lesioned animals spent more time in nonsocial states as compared with control and hippocampus-lesioned animals. This effect was driven by time and lesion-based differences in the active state. All animals spent more time being inactive during the second as compared with the first set of

observations and more time asleep during the first as compared with second set of observations.

DISCUSSION

These experiments demonstrate that early amygdala damage results in subtle differences in juvenile behavior across a variety of social contexts. Amygdala-lesioned animals were the least aggressive animals in the present experiments. They did not show heightened submission/"fear" signaling as seen earlier in development (Bauman et al., 2004a). Social partners were least submissive when interacting with amygdala-lesioned animals. When interacting with familiar partners, amygdala-lesioned animals spent less time than the other animals in social states, particularly highly interactive social states like grooming. This difference became more pronounced over the course of the experiments. This pattern of effects is remarkable because, despite spending less time socially interacting and being least aggressive, amygdala-lesioned animals had heightened communication, were the most affiliative group, and were more likely to reciprocate play behaviors. In other words, the members of their established groups should have found the amygdala-lesioned animals to be nonthreatening and engaging social partners leading to longer social interactions, but that was not the case. As in previous reports (Bauman, Toscano, Mason, Lavenex, & Amaral, 2006; Bauman et al., 2004a, 2004b), the hippocampus-lesioned subjects behaved essentially like control animals when assessing the frequency and duration of specific behaviors.

General developmental patterns in the maturation of social behavior were observed for all animals over time. For example, all animals spent more time socially interacting and were less aggressive in social groups during the second set of observations as compared with the first set, which were separated by 2.5 months. Communicative signaling decreased from the first to second set of observations, particularly for amygdala- and hippocampus-lesioned subjects. Changes in communicative signaling were also observed across experimental test phases (i.e., infancy to present). See Table 5 for a summary comparing amygdala-lesioned and control animals as infants (Bauman et al., 2004a) and the present test period. As infants, amygdala-lesioned animals, compared with control animals, had both heightened submissive/"fear" and affiliative signaling (Bauman et al., 2004a). As juveniles, amygdala-lesioned animals had heightened affiliative signaling during familiar dyadic interactions but no evidence of heightened affiliative signaling in other contexts or submissive signaling in any context. These changes over time likely reflect general social development, stabilization of the social dynamics in the permanently house social groups, as well as experience-dependent brain plasticity.

The present findings also illustrate that, although early brain damage may not cause profound variation in specific social behaviors, it does impact the patterns of expressed behavior. Typical statistical analyses used to assess variation in individual social behaviors (as in Machado, Emery, et al., 2008; Machado & Bachevalier, 2006; Bachevalier, Málková, & Mishkin, 2001; Emery et al., 2001) do not account for how brain damage may impact the covariation between social behaviors. MANOVA evaluates the covariation of behaviors across groups but is typically not suited for the small sample sizes in most nonhuman primate studies. By subjecting small subsets of behaviors to MANOVA, we were able to observe lesion-based differences in the organization of social states and exploratory behavior.

Table 5. Comparison of Amygdala-lesioned and Control Animals over Time

Behavioral Categories	*Effects during Infancy after Weaning*[a]	*Effects during Juvenile Period*[b]
Solo Observations		
Exploration	C > A	–
Activity	–	C > A
Familiar Dyads		
Affiliation	A > C	A > C
Submission/"Fear"	A > C	–
Agonistic/"Aggression"	–	C > A
Exploration	C > A	–
Novel Dyads		
Affiliation	A > C	–
Submission/"Fear"	A > C	–
Agonistic/"Aggression"	C > A	C > A
Exploration	C > A	C > A
Social Groups		
Submission/"Fear"	A > C	–
Agonistic/"Aggression"[c]	C > A	C > A
Exploration	C > A	–
Social Duration	–	C > A

For ease of interpretation, only the differences between the amygdala-lesioned and control groups are presented here. The hippocampus-lesioned group did not differ consistently from the control group in either period of evaluation. Note that Bauman et al. (2004a) evaluated each behavior in individual analyses rather than grouping behaviors into broad categories. Lesion group variation in the broad categories of behavior is presented here for consistency with the present report.

[a]Previously reported in Bauman et al. (2004a).

[b]Reported in this article.

[c]Lesion group difference only in displacement behavior, rather than broad category.

Furthermore, in cases where there were significant lesion-based behavioral organization differences, we were able to use those data to classify, with high fidelity, the subjects into groups that reflected their experimental status. Notably, despite the fact that hippocampus-lesioned animals appeared to behave like controls when the average frequency and duration of behaviors were considered, when patterning of behavior is considered, the hippocampus-animals appear to be unique. The classifier analyses were accurate at classifying all three groups. This finding suggests that, whereas the overall rates of behavior may be comparable between control and hippocampus-lesioned animals, the organization of behavior is not. Overall, group classification based on the social state data was more accurate than classification based on the exploration data, suggesting the importance of social interactions in the daily life of the rhesus macaque.

The relatively minor impact of early amygdala damage on the frequency and duration of specific social behaviors is remarkable given the impact of damage to the amygdala in adulthood and how these same animals behave in tests of nonsocial threat responding at the same time point. When interacting with novel objects and objects thought to engender threat responding at 18 months of age, amygdala-lesioned animals physically explored objects whereas control animals did not (Bliss-Moreau et al., 2010, Experiment 2), indicating that amygdala-lesioned animals' affective processing was perturbed in nonsocial contexts. This supports the view that the amygdala is not necessary for social processing per se, but rather serves a broader function related to evaluating threat.

One notable finding in the present experiments is that the amygdala-lesioned animals had periods of time during which they disengaged from their environments as evidenced by long durations of inactivity and low frequencies of environmental exploration. This disengagement occurred while they were in contexts where social engagement was nonexistent (solo observations) or low by design (during the introduction of novel animals, where the base rates of behaviors were extremely low for all animals). One possible explanation for these results is that socially engaging contexts provide amygdala-lesioned animals with signals that they use to regulate their own behavior. This possibility is consistent with findings that in the presence of "mammal-like" objects (e.g., stuffed animals with eyes and fur), neonatally amygdala -lesioned animals were as slow as control animals to retrieve concurrently presented food rewards (Bliss-Moreau, Toscano, et al., 2011). In this view, features of social stimuli (e.g., mammalian eyes) might provide animals with early amygdala damage cues that they need to regulate their behavior in a fashion more comparable to control animals.

The idea that social context allows amygdala-lesioned animals to regulate their behavior is consistent with the idea that a rich early social environment may be important for ameliorating the impact of early damage on social behavior. Other laboratories that have conducted similar experiments, but have isolate-reared or peer-reared (rather than mother-reared) their subjects, have found that early perturbations in social behavior persist across the developmental trajectory (e.g., Málková, Mishkin, Suomi, & Bachevalier, 2010; Bachevalier et al., 2001; Thompson & Towfighi, 1976; Thompson et al., 1969). Similarly, social isolation exacerbates the impact of neonatal damage to the rat amygdala, such that amygdala-lesioned animals that spend time socially isolated when they were young spent less time interacting with peers than amygdala-lesioned animals raised socially later in life (Diergaarde, Gerrits, Stuy, Spruijt, & van Ree, 2004). In this view, mother-rearing in combination with early group socialization and then permanent group housing may have potentiated brain plasticity resulting in varied patterns of socioaffective behavior across the developmental trajectory. Investigating the impact of social environment on neural and behavioral plasticity is an important area for further research.

The present findings, in concert with experimental evidence collected in other laboratories and from the current experimental group, illustrate that the impact of neonatal brain damage may differ from that of brain damage during adulthood and that such impact may be brain region specific. For example, neonatal damage to the medial-temporal lobe (including the amygdala, entorhinal cortex, hippocampal formation, and parhippocampal gyrus) prevents prefrontal down-regulation of striatal dopamine release (Saunders, Kolachana, Bachevalier, & Weinberger, 1998) and reduces the binding of a dopamine antagonist to D2 receptors in the striatum (Heinz et al., 1999), although damage to the same structures in adulthood does not alter dopamine regulation. In some cases, putative functions of a structure can be completely accommodated for during neural development. For example, animals that receive damage to the hippocampus as adults are unable to use spatial relational cues to locate food rewards (Banta Lavenex, Amaral, & Lavenex, 2006), yet subjects that received bilateral hippocampus lesions as neonates, like sham-operated control animals, are able to use these cues to locate food rewards (Lavenex, Banta Lavenex, & Amaral, 2007). Further evidence for neural reorganization following early damage in these animals comes from a functional neuroimagining study conducted approximately 2 years after the present experiments (Machado, Snyder, et al., 2008). Resting state glucose metabolism was indexed using PET imagining. Compared with control animals, amygdala-lesioned animals had greater glucose metabolism in the cerebellum, but lower glucose metabolism in the orbital, ventromedial, and dorsolateral frontal cortices and in the ACC as well as in the caudate nucleus and hippocampus. Hippocampus-lesioned animals only differed from controls in terms of lower glucose metabolism in the retrosplenial cortex. Taken together, these findings suggest that the behavioral and neural consequences of early brain damage may vary based on the area damaged.

Our findings speak to the longstanding debate about whether phenotypic recovery is better following brain

damage that occurs early as compared with late in life (see Kolb, 2010, for a review). Although our amygdala-lesioned subjects' social behavior did differ from control animals, their heightened affiliative signaling and mildly reduced propensity for social interaction is not particularly remarkable given the robust social behavior deficits observed in macaques that receive amygdala lesions as adults. Damage-related variation in social behavior appears to have been partially ameliorated with age and potentially as a result of housing our subjects in fulltime social groups before the start of these experiments. The question remains whether neurodevelopment that occurs after this time point will continue to accommodate early damage and therefore further ameliorate variation in social behavior and threat responding, rendering the amygdala-lesioned animals more "normal" (i.e., like controls) at later points in development. Future experiments will explore whether early damage-related variation in social behavior may emerge at various critical points in development such as puberty and sexual maturity.

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