Development/Plasticity/Repair

The Development of Mother–Infant Interactions after Neonatal Amygdala Lesions in Rhesus Monkeys

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As part of ongoing studies on the neurobiology of socioemotional behavior in the nonhuman primate, we examined the development of mother–infant interactions in 24 macaque monkeys who received either bilateral amygdala or hippocampus ibotenic acid lesions, or a sham surgical procedure at 2 weeks of age. After surgery, the infants were returned to their mothers and reared with daily access to small social groups. Behavioral observations of the infants in dyads (mother–infant pairs alone), tetrads (two mother–infant pairs), and social groups (six mother–infant pairs and one adult male) revealed species-typical mother–infant interactions for all lesion conditions, with the exception of increased physical contact time between the amygdala-lesioned infants and their mothers. Immediately after permanent separation from their mothers at 6 months of age, the infants were tested in a mother preference test that allowed the infants to choose between their mother and another familiar adult female. Unlike control and hippocampus-lesioned infants, the amygdala-lesioned infants did not preferentially seek proximity to their mother, nor did they produce distress vocalizations. Given the normal development of mother–infant interactions observed before weaning, we attribute the behavior of the amygdala-lesioned infants during the preference test to an impaired ability to perceive potential danger (i.e., separation from their mother in a novel environment), rather than to a disruption of the mother–infant relationship. These results are consistent with the view that the amygdala is not essential for fundamental aspects of social behavior but is necessary to evaluate potentially dangerous situations and to coordinate appropriate behavioral responses.

Key words: **amygdaloid complex; social behavior; filial bond; fear; hippocampus; macaque monkey**

Introduction

The amygdala has been implicated in mediating fear responses (Davis, 1992; Whalen, 1998; LeDoux, 2000) and contributing to socioemotional behaviors (Adolphs, 1999; Meunier et al., 1999; Bachevalier, 2000; Emery and Amaral, 2000). However, the relationship between fear and social interactions, and the contribution of the amygdala to these different behaviors, remains unclear. Although previous studies have suggested that the amygdala is essential for the production of social behavior (Brothers, 1990; Kling, 1992; Bachevalier, 1994), we recently demonstrated that both mature and immature rhesus monkeys with selective bilateral amygdala lesions are able to generate species-typical social behaviors (Emery et al., 2001; Prather et al., 2001; Bauman et al., 2004). These findings call into question the notion that the amygdala is an essential component of the neural circuitry underlying social behavior.

Nonetheless, the amygdala may indirectly influence social interactions by mediating danger detection and fear responses within a social context (Amaral, 2002; Amaral et al., 2003). This hypothesis is consistent with the well established role of the amygdala in rodent fear conditioning (LeDoux, 1998), the involvement of the human amygdala in evaluating trustworthiness in faces (Adolphs et al., 1998; Winston et al., 2002), as well as the behavioral profile we have observed in amygdala-lesioned monkeys (Emery et al., 2001; Prather et al., 2001). Despite this progress in understanding amygdala function, few studies have comprehensively evaluated amygdala function at the earliest developmental stages, thereby leaving unanswered questions regarding the role of the amygdala at critical developmental time points.

We have established a program of research to evaluate the role of the amygdala in the development of macaque social behavior. In the current study, we focused on one aspect of early social development by characterizing which facets of mother–infant behavior develop normally and which are altered after neonatal amygdala lesions. Whereas the literature of amygdala-lesioned subjects reared by their mothers is modest, previous studies indicate that nursing, contact time, and general physical development are apparently normal in monkeys that received neonatal amygdala lesions (Kling and Green, 1967; Prather et al., 2001). Al-

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though these basic aspects of mother–infant interactions may not require a functional amygdala, it is not clear whether other aspects of early development might be affected by neonatal amygdala lesions.

The current study includes a comprehensive analysis of mother–infant interactions and an evaluation of the infants' preference for their mothers immediately after weaning. We have extended our original study (Prather et al., 2001) by increasing the number of subjects, including a hippocampus lesion group, and raising the infants in a social environment. The infants were reared by their mothers and given daily access to other monkeys to simulate features of the social organization of free-ranging macaques (Berman, 1980), which appear necessary to facilitate species-typical social and hormonal development (Mason, 1960; Mason and Sponholz, 1963; Shannon et al., 1998; Bastian et al., 2003; Winslow et al., 2003). Thus, any observed alterations in social behavior can be more reasonably ascribed to the effects of the amygdala damage, rather than to atypical rearing conditions.

Materials and 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 Institutional Animal Care and Use Committee at the University of California at Davis.

Subjects and living conditions

Twenty-four infant rhesus monkeys (*Macaca mulatta*) naturally born of multiparous mothers were assigned randomly to one of three lesion conditions: bilateral amygdala lesions (five females and three males), bilateral hippocampus lesions (five females and three males), or sham-operated controls (four females and four males). All surgeries were performed at 12–16 d after birth. The infants were returned to their mothers after surgery and housed in standard home cages (61 × 66 × 81 cm). After a brief recovery period, each mother–infant pair was assigned to a socialization group consisting of six mother–infant pairs and one adult male. After a supervised 5 d acclimation period, each socialization group met for a minimum of 3 hr per day, 5 d per week in a large group cage (Fig. 1*a*). The four socialization groups were each composed of two amygdala-lesioned infants and their mothers, two hippocampus-lesioned infants and their mothers, and two sham-operated infants and their mothers. The age range between the youngest and oldest infant within each group was ~2 months. Three of the socialization groups comprised one male and one female per lesion condition, and the fourth cohort consisted of two female amygdala-lesioned infants, two female hippocampus-lesioned infants, and one male and one female sham-operated infant. When the youngest subject within a socialization group reached 6 months of age, the infants were permanently separated from their mothers but otherwise continued to experience the same housing and group socialization in the absence of their mothers. At this time, a new adult female was added to each socialization cohort to provide continued exemplars of adult female social behavior.

Presurgical preparations

Precautions were taken to insure that the infants would be reaccepted by their mothers after surgery. On postnatal days 4, 8, and 11, each infant was removed temporarily for progressively longer periods of time to prepare the mother–infant pair for separation on the day of surgery (day 4 = 30 min, day 8 = 1 hr, day 11 = 1.5 hr). During these separations, the infant's head was shaved and scrubbed with Betadine and 70% ethanol to mimic the appearance and odor of presurgical preparations, and to familiarize the mother with these conditions. These procedures have resulted in a 100% successful reunion rate for all neonatal surgeries conducted by our laboratory.

Presurgical magnetic resonance imaging

Because of the variability in size and shape of the rhesus monkey head and brain, accurate lesions were facilitated by producing an individualized magnetic resonance imaging (MRI) stereotaxic atlas for each infant.

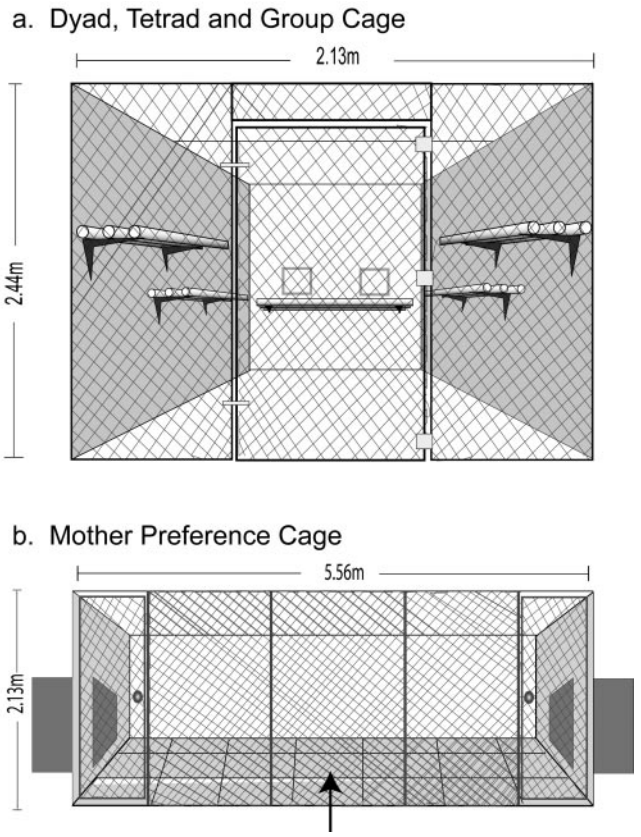

Figure 1. Schematic representations of the experimental test cages. *a*, Test cage used for dyadic, tetradic, and group observations (2.13 × 3.35 × 2.44 m). *b*, Novel test cage used for the mother preference test (5.56 × 1.91 × 2.13 m). Holding cages for the mother and the stimulus female are shown in solid gray. The arrow indicates the position of the portable infant release box placed in the center of the cage at the onset of each trial.

On the day of surgery, the infants were anesthetized initially with ketamine hydrochloride (15 mg/kg, i.m.) and medetomidine (25–50 μg/kg), then placed in an MRI-compatible stereotaxic apparatus (Crist Instruments, Damascus, MD). The infant's brain was imaged using a 1.5 T Gyroscan magnet (General Electric, Waukesha, WI); 1.0 mm thick sections were taken using a T1-weighted inversion recovery pulse sequence [repetition time (TR), 21; echo time (TE), 7.9; number of excitations (NEX), 3; field of view (FOV), 8 cm; matrix, 256 × 256). From these images, we determined the location of the amygdala or hippocampus and calculated the coordinates for the ibotenic acid (IBO) injections.

Surgical procedures

All surgical procedures were performed under aseptic conditions at the California National Primate Research Center. Infants were ventilated, and vital signs were monitored throughout the surgery. A stable level of anesthesia was maintained using 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). After a midline incision, the skin was displaced laterally to expose the skull, two craniotomies were made over the amygdala or the hippocampus, depending on the predetermined lesion condition, and the dura was reflected to expose the surface of the brain. We then performed electrophysiological recordings to confirm the estimated dorsoventral coordinates of the injection sites. A tungsten microelectrode was lowered into the amygdala or hippocampus at a mid-rostrocaudal, mid-mediolateral position, and recordings from salient features of the amygdala or hippocampus were documented and used to adjust the injection coordinates. IBO (10 mg/ml in 0.1 M PBS; Biosearch Technologies, Novato, CA) 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. Complete amygdala

lesions required a total of 7.0–12.0 μl of IBO per amygdala. Each amygdala lesion consisted of two rostrocaudal injection planes, each with one to two mediolateral and two dorsoventral injection sites. Complete hippocampus lesions required 5.5–7.0 μl of IBO per hippocampus. Each hippocampus lesion consisted of six to seven rostrocaudal injection planes, each with one to two mediolateral and one dorsoventral injection site. After injections, the dura was sutured, the craniotomy was filled with Gelfoam (Amersham Biosciences, Peapack NJ), and the fascia and skin were sutured in two separate layers. The bone flaps were replaced and sutured for the hippocampus-lesioned infants. Sham-operated controls underwent the same presurgical preparations, received a midline incision, and the skull was exposed. The control animals were maintained under anesthesia for the average duration of the lesion surgeries, and the fascia and skin were sutured in two separate layers. After the surgical procedure, all infants were monitored by a veterinarian and returned to their mothers once they were fully alert.

Lesion analysis

MRI-based lesion evaluation. Although the subjects in the current study are continuing behavioral testing and have not, therefore, been killed. We obtained T2-weighted MR images 10 d after surgery to examine the extent of the edema associated with the lesion. Although the exact correlation between the T2-hyperintense signal and actual lesion extent remains unclear (Malkova et al., 2001; Nemanic et al., 2002; Shelton et al., 2002), this technique provides a means of initial lesion confirmation before killing the subjects. The hyperintense T2-weighted signal for each of the 16 lesion subjects (8 amygdala lesions and eight 8 hippocampus lesions) was evaluated to confirm the general target and the lesion (i.e., amygdala lesion sparing the hippocampus or hippocampus lesion sparing the amygdala) (Fig. 2.). Their brains were imaged using a 1.5 T Gyroscan magnet (General Electric); 1.5 mm thick sections were taken using a T2-weighted inversion recovery pulse sequence (TR, 4000; TE, 102; NEX, 3; FOV, 8 cm; matrix, 256 × 256).

Behavioral observations

Home cage checklist. Each mother–infant pair was observed in their home cage on a daily basis both in the morning and the afternoon for a minimum of 220 times between 1 and 6 months of age (~10 observations per subject per week). Trained observers (M.D.B. and others) who were blind to the assigned lesion conditions conducted observations in a predetermined pseudo-random order for 10 sec periods. At the onset of each observation, the observer approached to 1 m in front of the home cage and recorded behaviors using a one–zero sampling method (Altmann, 1974). Any behavior occurring within the 10 sec observation received a score of "1" (even if the behavior was repeated), whereas behaviors that were not observed during the trial received a score of "0." All behaviors initiated by the infant or received by the infant from the mother were scored, including physical contact with mother, nursing, grooming, sleep, no contact, fear grimace, lipsmack, threat, aggressive behavior (i.e., grab, hit, bite, or slap), crouching, and tantrums.

Duration and frequency sampling overview. Behavioral data were collected with The Observer software (Noldus, Sterling, VA) (Noldus, 1991) by trained observers demonstrating an interobserver reliability of >90% [agreements/(agreements + disagreements) × 100]. Observers remained blind to the lesion condition of the infants for the duration of data collection. Focal animal samples (Altmann, 1974) were taken for each infant in a predetermined pseudo-random order using a catalog of 44 behaviors commonly used for this species, including specific infant behaviors (Table 1). In addition to frequency and duration of species-typical behaviors, observers also recorded the direction of the behavior (initiate or receive) and the identity of any other subjects directly interacting with the focal subject. Infants were observed under three distinct levels of social complexity, defined by the number of other subjects present (Table 2): (1) mother–infant dyads (one mother–infant pair alone); (2) mother–infant tetrads (two mother–infant pairs); and (3) social groups (six mother–infant pairs and one adult male).

Dyadic and tetradic interactions. Observations were conducted when the average age of the infants within a particular cohort reached 3 months and was repeated when they reached 6 months of age. Testing took place in one of four identical large chain link group cages unfamiliar to the animals (Fig. 1*a*) between 9:00 A.M. and 12:00 P.M. for 5 consecutive days. First, each infant was observed for 10 min alone with its mother (dyadic observations) to obtain a baseline of mother–infant interactions. Dyadic observations consisted of two consecutive 5 min focal samples on the first day of the test week. Then, on the same day, each mother–infant pair was observed during tetradic testing in which two familiar mother–infant pairs from the same socialization cohort were allowed to interact freely for 20 min. Each mother–infant pair was tested twice with every other mother–infant pair from the same cohort, according to a predetermined pseudo-random sequence. Behavioral data were collected for the duration of the observation period, alternating the focal infant every 5 min. Each mother–infant pair participated in two tetradic observation periods per day. On the last day of testing, each infant was again observed for 10 min alone with its mother (dyadic interactions) for two consecutive 5 min sample periods to obtain another baseline measure of mother–infant interactions.

Social group observations. Each cohort was assigned to one of four identical, large chain link cages (Fig. 1*a*), where daily group socialization

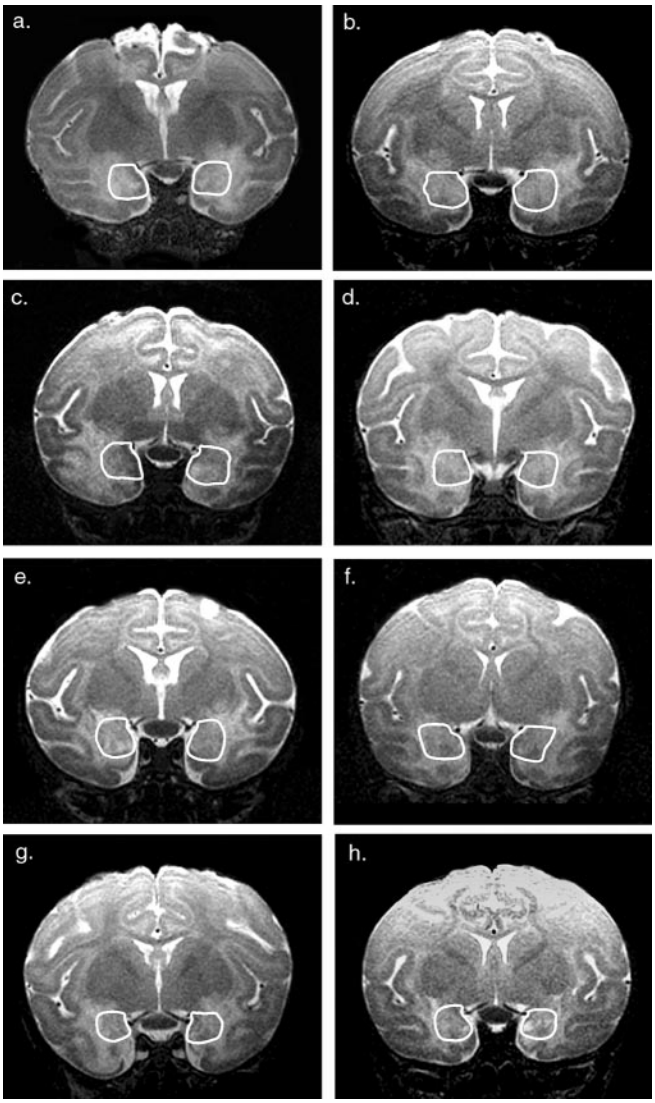

Figure 2. T2-weighted MR images of sections through the amygdala from infants that received injections of IBO 10 d earlier. Overlaid outlines represent the approximate boundary of the amygdaloid complex. The T2-hyperintense signal was used to confirm that IBO was injected and that the lesion target (amygdala or hippocampus) was in the central region of the edema. *a*, T2-weighted MR image of the same subject from which the Nissl-stained sections shown in Figure 3 is taken.

Table 1. Behavioral ethogram: definitions of species-typical behaviors

Behaviors	Description
Behaviors coded as durations	
Nursing	Breast contact for >3 sec
Extended play	Play behavior lasting for >3 sec
Extended negative	Aggressive encounters lasting >3 sec
Groom	Picking or licking another monkey's fur for >3 sec
Proximity	Within arm's reach of another subject for >3 sec
Nonsocial active	Active behavior (head up/exploring) out of proximity for >3 sec
Nonsocial inactive	Passive behavior (head down/not exploring) out of proximity for >3 sec
Other contact	Physical contact with another subject for >3 sec (not nursing or ventral)
Sleep	Eyes closed, no activity for >3 sec
Social activity	Alternating proximity and contact within a group for >3 sec
Ventral contact	Ventral surface contacting another subject's ventral surface for >3 sec
Behaviors coded as frequencies	
Aggression	Grab, hit, bite, or slap
Anogenital explore	Sniffing, touching, or licking genital area of another subject
Approach	Directed movement into arm's reach of another subject
Bark	Sharp, guttural vocalization
Cage shake	Dominance display involving shaking the cage
Chase	Quick, directed movement after another subject lasting >3 sec
Coo	High-pitched, soft vocalization
Crook tail	Tail is held in a stiff '?' formation
Fear grimace	Upper and lower lips retracted, exposing teeth
Flee	Rapid movement away from another subject
Follow	Slow, deliberate movement after another subject lasting for >3 sec
Freeze	No movement for >3 sec
Grunt	Soft, guttural sound produced in affiliative encounters
Incomplete mount	One or two of the following: double foot clasp, partner positioning, or thrusting
Lipsmack	Rhythmic lip movements, often with pursed lips
Manual explore	Use of hands to explore physical environment
Maternal rejection	Mother physically prevents the infant from obtaining contact
Maternal restrain	Mother physically prevents the infant from breaking contact
Maternal retrieve	Mother physically retrieves infant and reinstates contact
Mount	Includes double foot clasp, appropriate partner positioning, and thrusting
Oral explore	Use of mouth to explore physical environment
Social play	Rough and tumble play, grappling
Present groom	Rigid presentation of body part for grooming
Present mount	Stiff, 4-point stance, tail up, rump toward partner
Scratch	Rapid hand movements, using fingers to scratch own body
Scream	High-pitched, high-intensity vocalization indicating fear or distress
Self clasp	Grasping own body
Stereotypic movement	Abnormal motor movements, including circling, back flipping, spinning, or pacing
Tantrum	Shaking/spasms of body, often accompanied by gecker vocalization
Tooth grind	Audible rubbing of lower premolars and upper canines
Threat	One or more of the following: open mouth stare, head bob, or lunge
Withdraw	Movement out of arm's reach of another monkey
Yawn	Fully open mouth, lips retracted and teeth showing

Table 2. Behavioral observation summary: description of testing environments and behavioral sampling methods

Observations	Sampling method	Description
Dyads		
Home cage	10 sec focal samples[a]	10 observations per subject per week
3 month	5 min focal samples[b]	4 observations per subject in group cage
6 month	5 min focal samples[b]	4 observations per subject in group cage
Tetrads		
3 month	5 min focal samples[b]	20 observations per subject in group cage
6 month	5 min focal samples[b]	20 observations per subject in group cage
Social groups	5 min focal samples[b]	10 observations per subject in group cage
Other testing		
Mother preference	2 min focal samples[b]	20 observations per subject

The sampling method is by Altmann (1974).

[a]One–zero behavior scoring.

[b]Duration and frequency behavior scoring.

occurred. Infants were observed twice per week between 1 and 6 months of age during group socialization time, which took place between 12:00 and 3:00 P.M. daily. Each socialization cohort consisted of six mother–infant pairs and one adult male. Five-minute focal observations were conducted on each infant in a predetermined pseudo-random order, with no more than two observations per individual per week. Because the socialization cohorts were not formed until the youngest member had recovered from surgery and was able to join the group, there was a limited window of group observations taken while infants were all the same age. To avoid confounds related to age-related emergence of behaviors, only data collected during 10 observation periods when all animals were of a comparable developmental age (between 4.5 and 6 months) were included in the statistical analysis.

Mother preference test. Permanent separation from the mother (weaning) took place when the youngest infant of the socialization cohort reached 6 months of age. Starting on the day after weaning, each infant was observed in a test designed to evaluate one aspect of mother–infant attachment, the infant's preference for its own mother versus another familiar adult female. Five daily trials were conducted for 4 consecutive days, with each 2 min trial consisting of a choice between the infant's mother and one of the five other adult females from the infant's socialization cohort (the stimulus female). A different stimulus female was used for each trial in a predetermined pseudo-random order. Before each trial, the infant was hand-caught by a technician and placed in a plastic release box in the center of an unfamiliar chain link enclosure (Fig. 1*b*). The front of the infants' release cage was transparent, and the remaining three sides were opaque, allowing the infants to initially view only the observers. The infant's mother was placed in one of two holding cages, located at either end of the testing enclosure, and the stimulus female was placed in the opposite holding cage (right and left holding cage assignments were balanced across trials). The holding cages were separated from the testing enclosure by metal bars, and clear plastic panels prevented physical contact between the infant and the adults. Opaque plastic panels in front of the holding cages prevented the adults from seeing the infant release box before testing. At the onset of the trial, the infant's release box and the opaque plastic panels in front of the holding cages were raised simultaneously, allowing the infant to freely move around the center cage and to see both its mother and the stimulus female. The following observations were recorded during each 2 min trial: (1) the first adult approached by the infant (scored when the infant moved within a 1 m half-circle in front of the adult holding cage within the first 15 sec of the trial); (2) the spatial location of the infant every 15 sec (using a floor grid of 9 × 3 quadrants; 0.61 × 0.64 m each); and (3) focal animal samples using the behavioral ethogram (Table 1).

Statistical analysis

ANOVAs, followed by Fisher's PLSD *post hoc* tests (with a significance level of $p < 0.05$), were used for data analyses. In appropriate cases, paired *t* tests were performed, with a significance level also set at $p < 0.05$. The frequency of vocalizations during the mother preference test was not normally distributed and contained a number of zero values. Therefore,

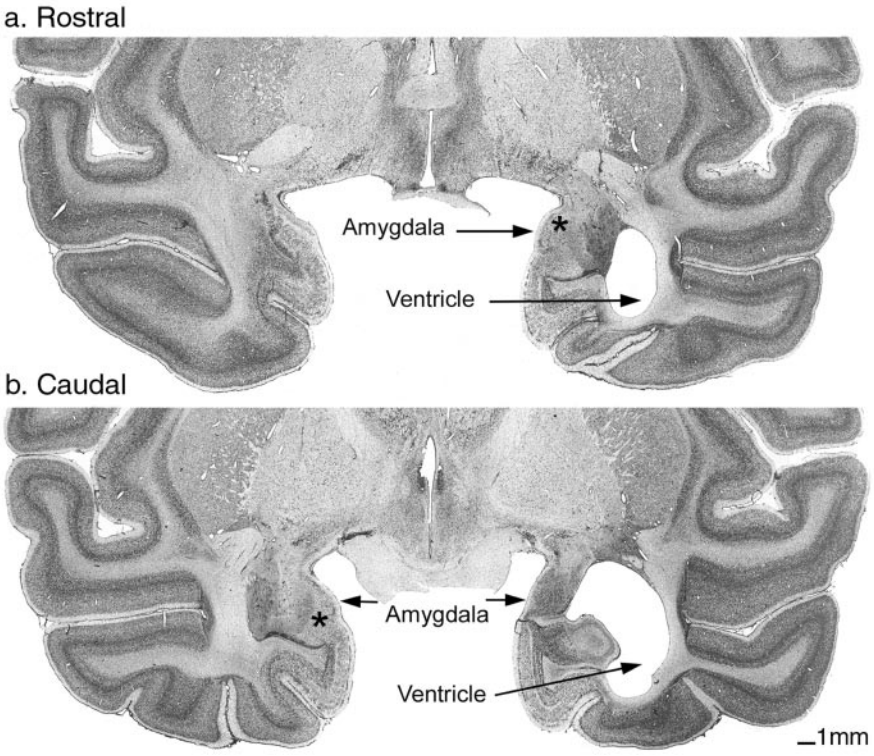

Figure 3. Nissl-stained coronal sections through two levels in an amygdala-lesioned subject's anterior temporal lobe. The amygdala in this subject was substantially damaged, whereas adjacent structures, including the entorhinal cortex, were primarily intact. *a*, Rostral level showing an expanded ventricle and substantial amygdala damage on the right side and cell damage to the rostral portion of the amygdala on the left side. The asterisk indicates sparing in the medial portion of the accessory basal nucleus and the periamygdaloid cortex. *b*, Caudal level of the amygdala showing expanded ventricle and nearly complete cell loss on the right side and substantial cell damage on the left side. The asterisk indicates sparing in a small, ventomedial medial portion of the parvicellular division of the basal nucleus on the left side.

a $\ln(X + 1)$ transformation was performed to normalize the data and respect theoretical assumptions before statistical analyses. The graph (see Fig. 5*c*) presents the nontransformed data to better illustrate the animals' actual behavior.

Results

MRI and histological evaluation of lesions

T2-weighted images of coronal sections through the mid portion of the amygdala are shown in Figure 2. The hyperintense (white) areas illustrate regions that are edematous presumably because of the neurotoxic action of the IBO. The hyperintense area involves much, if not all, of the amygdala in each of the cases. The hyperintense area also appears to extend beyond the borders of the amygdala. Given the histological analysis that we performed on one of the lesioned animals, we suspect that the signal may overestimate the actual extent of the lesion (see below). However, we do believe that this change in signal provides substantial reassurance that the IBO was injected and was focused in the amygdaloid complex (or hippocampal formation).

One amygdala-lesioned subject was killed after behavioral testing for health reasons unrelated to the lesion surgery, thus enabling us to evaluate the lesion histologically (Fig. 3). This subject sustained substantial bilateral amygdala damage, with residual cell patches limited to the medial surface of the amygdala, including the amygdalohippocampal area, the nucleus of the lateral olfactory tract, the ventromedial aspect of the parvicellular division of the basal nucleus, and the most medial portion of the accessory basal nucleus (on one side). Collateral damage was limited to focal damage in the sulcus of the superior temporal gyrus, ventral claustrum, and the most rostral portions of the hippocampal formation, primarily the subiculum. The region of actual cell damage was more confined to the amygdala than suggested by the extent of the postlesion edema visualized by the MRI hyperintense T2 signal (Fig. 2*a*). This case provides reassurance, however, that much of the amygdala (or hippocampus) is eliminated in these experimental cases.

Home cage observations

The mother–infant interactions observed in their individual home cages between 1 and 6 months of age were similar for the three experimental groups. All behaviors initiated or received by the infants were analyzed. There was no effect of the lesion on mother–infant interactions such as physical contact, nursing, or grooming, nor on other species-typical behaviors, including fear grimaces, lipsmacks, aggressive behaviors, and the frequency of sleep bouts.

Three-month dyadic and tetradic interactions

At 3 months of age, all infants spent the majority of the observation periods in contact with their mothers. Infants from the three lesion conditions spent the same amount of time in overall physical contact with their mothers, nursing, in proximity with their mothers, or exploring away from their mothers during dyadic or tetradic interactions (Table 3). However, lesion effects were found for sleep duration ($F_{(2,21)} = 3.64$; $p = 0.0439$), with amygdala-lesioned infants sleeping for longer periods of time than controls ($p = 0.0272$) or hippocampus-lesioned infants ($p = 0.0319$) during dyadic interactions (mother–infant pair alone). The difference in sleep behavior was not found during tetradic interactions at 3 months of age and was no longer present during either dyadic or tetradic interactions at the 6 month assessment (see below). No other behavior (from the 44 species-typical behaviors listed in the ethogram in Table 1) revealed any significant difference among lesion conditions at 3 months of age.

Six-month dyadic and tetradic interactions

At 6 months of age, all infants spent increasingly more time exploring away from their mothers than when they were 3 months old in both dyadic ($F_{(1,23)} = 18.639$; $p = 0.0003$) and tetradic ($F_{(1,23)} = 59.599$; $p = < 0.0001$) interactions. The lesion condition had a significant effect on mother–infant contact time during dyadic interactions (mother–infant alone; $F_{(2,21)} = 5.95$; $p = 0.009$) (Fig. 4). The amygdala-lesioned infants spent more time in contact with their mothers than hippocampus-lesioned infants ($p = 0.0024$), whereas neither the amygdala nor the hippocampus-lesioned infants differed from controls ($p = 0.1070$ and $p = 0.0920$, respectively). The lesion condition also affected the amount of time spent in nonsocial activity (out of proximity and contact with other subjects; $F_{(2,21)} = 4.73$; $p = 0.0201$) (Table 3). Amygdala-lesioned infants spent less time in nonsocial activity than hippocampus-lesioned infants ($p = 0.0059$).

Table 3. Percentage of time spent in defined behavioral states

Behaviors	AMY	+/−SE	CON	+/−SE	HIP	SE	Raw	Norm
3 month dyad								
Mother–infant contact	94.5	3.8	86.2	4.9	79.2	8.7		
Nursing	31.7	5.7	16.8	7.8	29.4	9.0		
Ventral/other contact	53.4	6.2	68.9	6.5	46.9	7.9		
Groom with mother	2.0	0.9	0.5	0.3	2.7	2.3		
Sleep	7.4	3.8	0	0	0.2	0.2	A>CH	
No contact with mother	5.5	3.8	13.8	4.9	20.8	8.7		
Extended play								
Proximity with mother	4.6	3.1	9.9	4.1	9.2	4.5		
Proximity with others								
Groom with others								
Contact with others								
Nonsocial activity	0.9	0.7	3.9	1.4	11.6	5.1		
3 month tetrad								
Mother–infant contact	86.4	6.9	65.9	4.9	74.1	8.8		
Nursing	33.2	5.3	17.5	4.3	27.8	6.9		
Ventral/other contact	49.7	4.5	47.2	3.3	44.9	5.4		
Groom with mother	1.4	0.4	0.9	0.4	0.9	0.4		
Sleep	2.1	0.9	0.3	0.3	0.5	0.3		
No contact with mother	13.6	6.9	34.1	4.9	25.9	8.8		
Extended play	0	0	0	0	0.1	0.1		
Proximity with mother	9.0	4.9	15.9	2.8	10.3	4.7		
Proximity with others	1.4	0.8	2.0	0.5	2.0	0.8		
Groom with others	0.1	0.1	0	0	0	0		
Contact with others	0.1	0.1	0.5	0.3	0.2	0.1		
Nonsocial activity	3.1	1.6	15.7	2.6	13.3	6.4		
6 month dyad								
Mother–infant contact	83.5	5.2	64.5	7.4	44.6	10.5	A>H	
Nursing	9.5	3.5	7.7	3.6	4.1	2.4		
Ventral/other contact	69.5	2.8	56.7	8.3	39.3	8.2		
Groom with mother	2.2	1.1	0.1	0.1	0	0		
Sleep	2.3	2.3	0	0	1.2	1.2		
No contact with mother	16.5	5.2	35.5	7.4	55.4	10.5	A<H	
Extended play								
Proximity with mother	10.3	3.5	19.3	4.0	28.0	8.1		
Proximity with others								
Groom with others								
Contact with others								
Nonsocial activity	6.0	2.2	16.2	3.9	27.4	7.7	A<H	
6 month tetrad								
Mother–infant contact	59.5	11.6	23.4	4.3	22.0	7.2	A>CH	
Nursing	13.2	6.6	3.6	1.0	5.3	3.6		
Ventral/other contact	45.5	8.2	19.7	3.5	16.5	4.2	A>CH	
Groom with mother	0.8	0.5	0.1	0.1	0.2	0.1		
Sleep	0		0		0	0		
No contact with mother	40.5	11.6	76.8	4.3	78.0	7.2	A<CH	
Extended play	0.3	0.2	1.4	0.4	1.6	0.5	A<H	
Proximity with mother	22.5	6.0	26.5	2.8	21.8	3.1		
Proximity with others	3.3	1.9	1.8	0.6	4.2	1.0		
Groom with others	0	0	0	0	0.1	0.1		
Contact with others	0.6	0.6	0.9	0.3	0.6	0.4		
Nonsocial activity	13.8	4.8	46.0	2.8	49.7	6.3	A<CH	A<CH
Social group								
Mother–infant contact	61.5	13.3	22.6	5.0	18.4	8.7	A>CH	
Nursing	16.5	5.9	5.8	2.4	1.5	0.8	A>H	
Ventral/other contact	41.3	8.7	15.5	3.3	16.3	8.2		
Groom with mother	2.7	1.5	1.1	0.9	0.2	0.2		
Sleep	1.0	0.7	0.2	0.2	0.4	0.4		
No contact with mother	38.5	13.3	77.4	5.0	81.6	8.7	A<CH	
Extended play	0.8	0.7	2.4	0.8	4.5	2.0		
Proximity with mother	14.3	4.3	23.8	4.3	15.5	2.6		
Proximity with others	9.5	5.0	5.6	0.8	12.4	4.0		
Groom with others	0.1	0.1	0	0	0	0		
Contact with others	0.4	0.3	0.1	0.1	1.1	0.7		
Nonsocial activity	13.4	5.9	45.5	4.7	48.1	5.5	A<CH	

Data are percentage of time ± SEM that amygdala-lesioned (AMY), control (CON), or hippocampus-lesioned (HIP) subjects spent in contact with the mother or out of contact with the mother across different ages and testing environments. The far right columns indicate a statistically significant difference among lesion conditions (A, amygdala lesioned; C, control; H, hippocampus lesioned). Raw data are based on actual duration measures. Normalized (Norm) data (only applied to testing conditions that revealed a significant lesion effect for mother contact time) are based on the duration of time spent in a particular behavioral state relative to the amount of time spent in contact with the mother (mother contact behaviors were divided by duration of mother contact time; non-mother contact behaviors were divided by duration of time spent away from the mother).

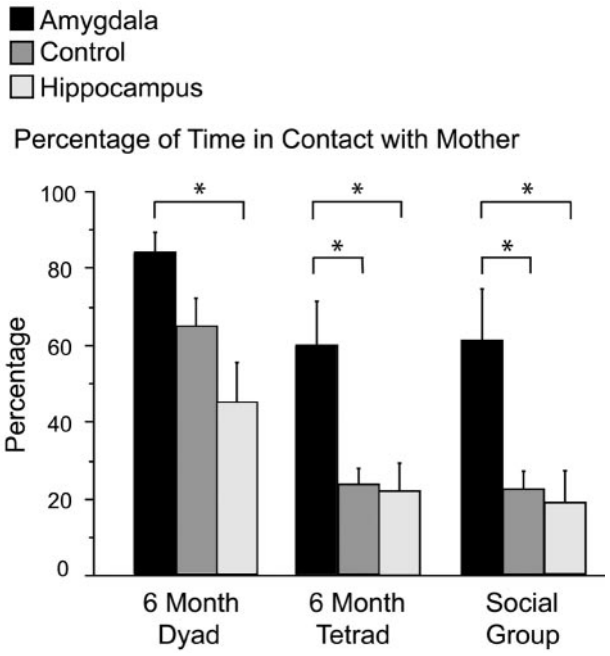

Figure 4. Amygdala-lesioned infants do not differ from controls during dyads (one mother–infant pair) but do spend significantly more time in contact with their mothers than both controls and hippocampus-lesioned infants during tetrads (two mother–infant pairs) and social group interaction (six mother–infant pairs plus one adult male). Each bar represents the average percentage of time ± SEM (per 300 sec observation period) spent in physical contact with their mothers. Asterisks denote significant *post hoc* Fisher's PLSD tests ($p < 0.05$).

Mother–infant contact time during tetradic interactions (two mother–infant pairs) also revealed a significant lesion effect ($F_{(2,21)} = 6.67$; $p = 0.0057$) (Fig. 4). In this case, however, the amygdala-lesioned infants spent more time in contact with their mothers than either the controls or the hippocampus-lesioned infants did ($p = 0.0054$ and 0.0041, respectively). This effect was essentially attributable to a difference in time spent in ventral contact with the mother ($F_{(2,21)} = 4.57$; $p = 0.0226$) (Table 3). Amygdala-lesioned infants spent more time in ventral contact with their mothers than controls or hippocampus-lesioned infants ($p = 0.0149$ and 0.0174, respectively). The lesion condition affected the amount of time spent in nonsocial activity ($F_{(2,21)} = 16.56$; $p < 0.0001$) (Table 3). Amygdala-lesioned infants spent less time in nonsocial activity than controls or hippocampus infants ($p = 0.0001$ and <0.0001, respectively). The lesion condition also affected the amount of time spent in extended play behavior ($F_{(2,21)} = 3.57$; $p = 0.0464$) (Table 3), with amygdala-lesioned infants spending less time in extended play behavior than hippocampus-lesioned infants ($p = 0.0196$); neither the amygdala-lesioned nor the hippocampus-lesioned infants differed from controls ($p = 0.0573$ and 0.6112, respectively). It is important to note that although differences were found between amygdala-lesioned and hippocampus-lesioned infants, the actual duration of play behavior during the 6 month tetrads accounted for <2% of the total observed time for all groups (Table 3). Lesion effects were also found for the frequency of play behavior ($F_{(2,21)} = 5.27$, $p = 0.0140$), with amygdala-lesioned infants playing less frequently than either control or hippocampus-lesioned infants ($p = 0.0216$ and 0.0060, respectively). There was also a lesion effect for the frequency of screams ($F_{(2,21)} = 3.87$; $p = 0.0371$). Amygdala-lesioned infants screamed more frequently than hippocampus-lesioned infants ($p = 0.0112$).

Lesion conditions clearly affected the amount of time infants spent in contact with their mothers and, thus, the amount of time available for other behaviors during the 6 month tetradic interactions. Because we were interested in discerning the relative amount of time dedicated to other behaviors when out of contact with the mother, we divided the duration and frequency of behaviors that occurred while subjects were out of contact with the mother by the time spent away from the mother, to obtain a relative measure of non-mother contact behaviors. When the infants were not in contact with their mothers, the relative amount of time spent in nonsocial activity (out of contact and proximity with other subjects) differed among the lesion conditions ($F_{(2,21)} = 24.94$; $p < 0.0001$). The amygdala-lesioned infants spent relatively less time in nonsocial activity than the controls or the hippocampus-lesioned infants (all $p < 0.0001$). Differences also remained for the frequency of play behaviors ($F_{(2,21)} = 5.42$; $p = 0.0126$), with amygdala-lesioned infants playing less frequently than either controls or hippocampus-lesioned infants ($p = 0.0218$ and 0.0052, respectively). No other behavioral measurements (Table 1) revealed any significant differences after this normalization procedure.

Social group interactions (4.5–6 months of age)

Lesion conditions affected mother–infant contact time during social group observations ($F_{(2,21)} = 6.16$; $p = 0.0078$) (Fig. 4), with amygdala-lesioned infants spending more time in contact with their mothers than either the controls or the hippocampus-lesioned infants ($p = 0.0087$ and 0.0046, respectively). Lesion effects were found for the amount of time spent in nonsocial activity ($F_{(2,21)} = 12.92$; $p = 0.0002$), with amygdala infants spending less time in nonsocial activity than either control or hippocampus-lesioned infants ($p = 0.0004$ and 0.0002, respectively). Lesion effects were also found for the amount of time spent nursing ($F_{(2,21)} = 4.29$; $p = 0.0275$), with amygdala-lesioned infants nursing for longer durations than hippocampus infants ($p = 0.0098$); neither amygdala-lesioned nor hippocampus-lesioned infants differed from controls ($p = 0.0554$ and 0.4246, respectively). After normalization of the data to account for the influence of mother–infant contact time (see above), there were no remaining group duration differences.

No lesion effects were found for the frequency of behaviors that might be indicative of abnormal mother–infant interactions, including maternal rejections ($F_{(2,21)} = 0.765$; $p = 0.4780$), maternal aggression ($F_{(2,21)} = 1.355$; $p = 0.2796$), and maternal threats ($F_{(2,21)} = 0.316$; $p = 0.7325$). Similarly, there were no occurrences of potentially maladaptive behaviors, such as motor stereotypies or tantrums, indicating that all infants developed normal patterns of species-typical interactions with their mothers. Lesion effects were again found in the frequency of play behavior ($F_{(2,21)} = 3.89$; $p = 0.0367$), with amygdala-lesioned infants playing less frequently than hippocampus-lesioned infants ($p = 0.0110$). This difference in play frequency remained significant after normalizing the data to account for the amount of time spent in mother contact (play frequency/duration of time spent away from the mother; $F_{(2,21)} = 5.12$; $p = 0.0155$) with amygdala-lesioned infants playing less frequently than hippocampus-lesioned infants ($p = 0.0043$). Neither amygdala-lesioned nor hippocampus-lesioned subjects differed from controls ($p = 0.1002$ and 0.1545, respectively). Although amygdala-lesioned infants played less frequently than hippocampus-lesioned infants, there were no lesion effects on the overall duration of play behavior ($F_{(2,21)} = 1.92$; $p = 0.1717$). No other behavioral measurements (Table 1) revealed any difference among lesion conditions.

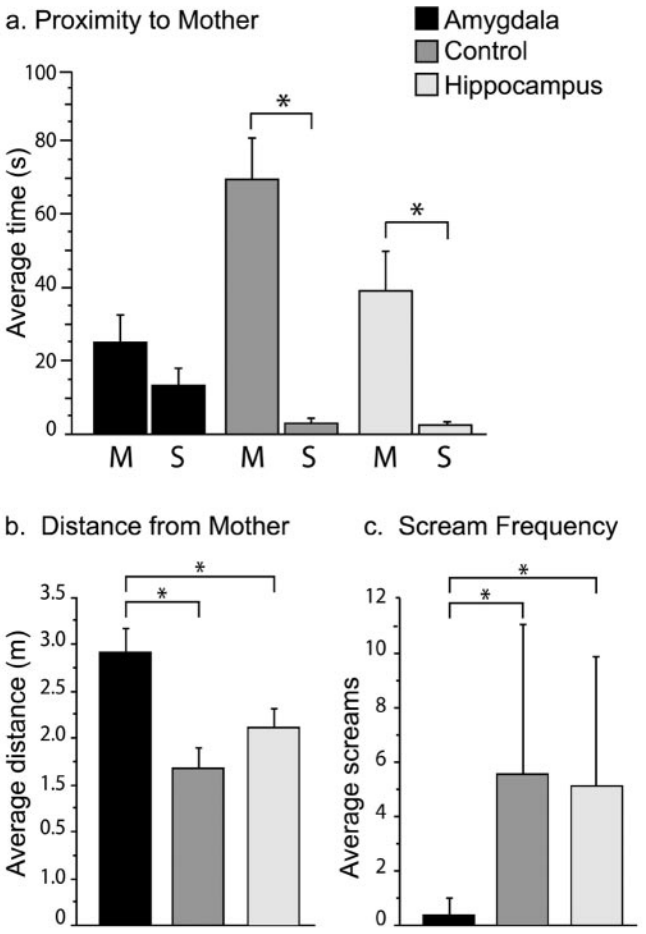

Figure 5. Amygdala-lesioned infants failed to demonstrate a species-typical preference for their mother after separation from their mother, as indicated by measure of proximity duration, distance, and distress responses. *a*, Bars represent the average time ± SEM (per 120 sec trial) spent in a 1 m semicircle "proximity zone" in front of the holding cages that contain either their mother (M) or the stimulus female (S). Proximity duration was scored only when the infant stayed within the proximity zone for at least 3 consecutive seconds. Asterisks denote significant paired *t* tests ($p < 0.05$). *b*, Bars represent the average distance from the mother (meters) ± SEM. Spacing data were collected every 15 sec during the 120 sec trial, noting the location of the infant on a 27 quadrant floor grid. Asterisks denote significant *post hoc* Fisher's PLSD tests (significance set at $p < 0.05$). *c*, Bars represent the average scream frequency ± SEM per 120 sec trial. The frequency of scream data were not normally distributed and contained a number of zero values. Therefore, a ln(scream + 1) transformation was used. For display purposes, the nontransformed scream data are shown. Asterisks denote significant *post hoc* Fisher's PLSD tests (significance set at $p < 0.05$).

Mother preference test

On the day after permanent separation from their mothers (weaning), infants were tested to assess their preference for their mother versus a familiar adult female (stimulus female) in a novel environment (Fig. 1*b*). Preference was evaluated based on measures of time spent in proximity to the mother or the stimulus female (scored only if the infant remained within 1 m of its mother or the stimulus female for at least 3 sec), the average distance from its mother or the stimulus female, and the frequency and durations of species-typical behaviors (Table 1).

There was no lesion effect on the time spent in proximity to the adults ($F_{(2,21)} = 3.31$; $p = 0.0564$), but there was a difference in the target of proximity (mother vs the stimulus female; $F_{(1,21)} = 37.66$; $p < 0.0001$) and a significant interaction between lesion conditions and the proximity target (mother vs stimulus female; $F_{(2,21)} = 6.46$; $p = 0.0065$) (Fig. 5*a*). Both the controls and hippocampus-lesioned infants showed a strong preference for their own mother versus the stimulus female [one-tailed paired *t* tests (mother vs stimulus female): controls: $t_{(7)} = 5.46$, $p = 0.0005$; hippocampus: $t_{(7)} = 3.26$, $p = 0.0070$] (Figure 5*a*). In contrast, the amygdala-lesioned infants did not demonstrate this species-typical preference for their mother ($t_{(7)} = 1.35$; $p = 0.1097$) (Fig. 5*a*). Both amygdala- and hippocampus-lesioned infants spent less time in proximity to their mothers than the controls ($p = 0.0053$ and 0.0467, respectively). However, overall activity levels, as measured by the number of times the infant crossed the midline of the cage, indicated that the hippocampus-lesioned infants displayed more locomotor behavior than the other groups ($F_{(2,21)} = 3.72$; $p = 0.0414$; hippocampus > control = amygdala; $p = 0.0450$ and 0.0191, respectively). This hyperactivity may have prevented the hippocampus-lesioned infants from maintaining the 3 sec duration required to score proximity, thereby reducing their overall proximity scores.

There was no lesion effect on the average distance from the adults ($F_{(2,21)} = 1.09$; $p = 0.3554$), but there was a difference between distance to the mother versus distance to the stimulus female ($F_{(1,21)} = 84.34$; $p < 0.0001$) and a significant interaction between lesion condition and distance from adult females ($F_{(2,21)} = 7.60$; $p = 0.0033$) (Fig. 5*b*). Amygdala-lesioned infants maintained a greater average distance from their mothers compared with controls ($p = 0.0011$) and hippocampus-lesioned infants ($p = 0.0223$), while maintaining a closer average distance to the stimulus female than the controls ($p = 0.0011$) and the hippocampus-lesioned infants ($p = 0.0240$). Similarly, there was no lesion effect on the shortest distance from the adults ($F_{(2,21)} = 0.27$; $p = 0.7695$), but there was a difference between the shortest distance to the mother versus the shortest distance to the stimulus female ($F_{(1,21)} = 48.17$; $p < 0.0001$) and a significant interaction between lesion condition and the shortest distance from the adults ($F_{(2,21)} = 3.84$; $p = 0.0379$). The shortest distance recorded between the amygdala-lesioned infants and their mothers was greater than the shortest distance between controls or hippocampus-lesioned infants and their mothers. Amygdala-lesioned infants approached within 1.6 m, on average, whereas both controls and hippocampus-lesioned infants approached within 1 m, on average ($p = 0.0164$ and $p = 0.0484$, respectively). There was no lesion effect on the shortest distance recorded between the infants and the stimulus female ($F_{(2,21)} = 1.534$; $p = 0.2389$). Overall, amygdala-lesioned infants remained farther away from their own mother than controls or hippocampus-lesioned infants.

The frequency and duration of all species-typical behaviors described in the social behavior ethogram (Table 1) were also recorded during the mother preference tests. Among all these behaviors, the frequency of screams was the only one to reveal a lesion effect ($F_{(2,21)} = 3.69$; $p = 0.0424$) (Fig. 5*c*), with the amygdala-lesioned infants producing fewer screams than either controls ($p = 0.0275$) or hippocampus-lesioned infants ($p = 0.0295$). Importantly, affiliative vocalizations did not reveal any lesion effect (coos: $F_{(2,21)} = 0.699$, $p = 0.5082$; grunts: $F_{(2,21)} = 0.823$, $p = 0.4529$; barks: $F_{(2,21)} = 1.028$, $p = 0.3750$).

Thus, unlike controls or hippocampus-lesioned infants, the amygdala-lesioned infants did not show a significant preference for their own mothers, as indicated by measures of proximity or distance to their mother. Moreover, the amygdala-lesioned animals placed in an unfamiliar environment did not demonstrate distress behaviors that were observed in the control and hippocampus-lesioned animals.

Additional analysis of their behavior, however, revealed that the amygdala-lesioned infants did recognize their own mothers.

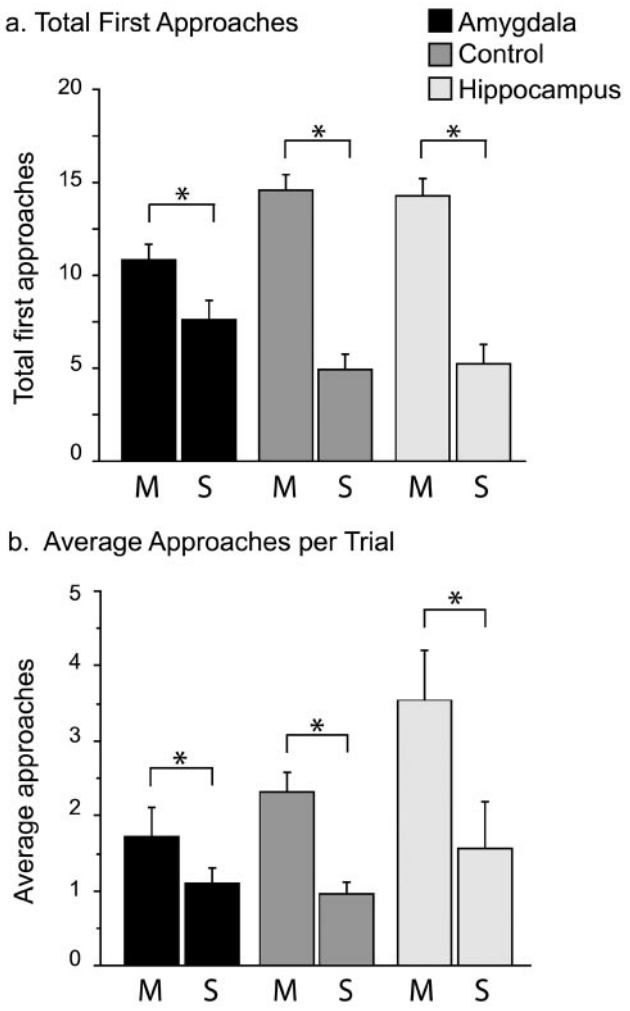

Figure 6. Amygdala-lesioned infants are capable of visually identifying their mother, as indicated by their preference to approach their mother (M) as opposed to the stimulus female (S). *a*, Bars represent the group average for the number of first approaches ± SEM (scored when an infant moves into the proximity zone within the first 15 sec of the trial) of twenty trials. Asterisks denote significant one-tailed paired *t* tests ($p < 0.05$). *b*, Bars represent the group average frequency of approaches ± SEM per 120 sec trial. Asterisks denote significant one-tailed paired *t* tests ($p < 0.05$)

Indeed, infants from all three lesion conditions approached their mothers first (Fig. 6*a*) [lesion: $F_{(2,21)} = 1.120$, $p = 0.3450$; target (mother vs stimulus female): $F_{(1,21)} = 55.202$, $p < 0.0001$; interaction: $F_{(2,21)} = 4.327$, $p = 0.0267$; one-tailed paired *t* tests (mother vs stimulus female): control: $t_{(7)} = 6.049$, $p = 0.0025$; hippocampus: $t_{(7)} = 4.692$, $p < 0.0011$; amygdala: $t_{(7)} = 2.060$, $p = 0.0392$]. Similarly, infants from all three lesion conditions approached their mother more frequently than the stimulus female (Fig. 6*b*) [lesion: $F_{(2,21)} = 2.171$, $p = 0.1389$; target (mother vs stimulus female): $F_{(1,21)} = 71.680$, $p < 0.0001$; interaction: $F_{(2,21)} = 6.399$, $p = 0.0068$; one-tailed paired *t* tests (mother vs stimulus female): controls: $t_{(7)} = 8.855$, $p = < 0.0001$; hippocampus: $t_{(7)} = 6.125$, $p = 0.0025$; amygdala: $t_{(7)} = 1.990$, $p = 0.0435$]. Thus, all infants could clearly identify their own mother versus the stimulus female, although the tendency to approach their own mothers was not as pronounced in the amygdala-lesioned infants compared with controls and hippocampus-lesioned infants. Altogether, these data indicate that although the amygdala-lesioned infants did not show a strong species-typical preference for their mother or display distress behaviors in response to physical separation from their mother in an unfamiliar environment, they did recognize their mother.

The behavior of the mothers was only scored when clearly directed toward the infant. Because it was unclear whether the vocalizations produced by the mothers were directed to the infants, other subjects in the testing room, or to the observers, these data were not included in the final analysis. Only facial expressions clearly directed at the infants were included in the analysis. Lipsmacks were the most common behavior produced by their mothers and directed toward the infants, and there were no lesion group differences in the frequencies of lipsmacks received from the mothers ($F_{(2,21)} = 0.648$, $p = 0.5333$).

Discussion

The present series of experiments indicates that neonatal amygdala or hippocampus lesions do not alter the development of mother–infant interactions within the first 6 months of life. Indeed, amygdala-lesioned, hippocampus-lesioned, and sham-operated control infant rhesus monkeys demonstrated normal mother–infant interactions, with the exception of increased physical contact between amygdala-lesioned infants and their mothers. However, after permanent separation from their mothers at 6 months of age, the amygdala-lesioned infants failed to show a species-typical response in a mother-preference test. These results might seem inconsistent given that amygdala-lesioned infants seemed to develop a normal attachment to their mothers and actually spent more time in physical contact with their mothers than hippocampus-lesioned or control infants. Although it is plausible that the abnormal response of the amygdala-lesioned infants during the preference test could be attributed to a failure in forming an attachment to their mothers, this explanation seems unlikely given that all other behavioral measures indicated that infants developed species-typical mother–infant interactions irrespective of their lesion condition.

After the lesion procedures at 2 weeks of age, all infants were successfully reaccepted by their mothers and showed normal patterns of weight gain and physical development, a result consistent with previous findings of mother-reared amygdala-lesioned infants (Kling and Green, 1967; Prather et al., 2001). At 3 months of age, there were no lesion effects on the amount of time subjects spent nursing, in proximity, or in contact with the mother. All infants spent the vast majority of their time in ventral contact with their mothers. All infants continued to display seemingly normal mother–infant interactions when observed in dyads, tetrads, and social groups at 6 months of age. There were no lesion effects on the frequency of mother–infant behaviors, including maternal rejection, maternal restraint, maternal retrieve, aggression, threats, or grooming. None of the infants engaged in maladaptive behaviors such as self clasping, crouching, rocking, or motor stereotypies, which are indicative of abnormal social behavior development (Capitanio, 1986). Furthermore, the amygdala-lesioned subjects did not display Kluver–Bucy symptoms previously associated with bilateral amygdala damage, such as compulsive examination of objects or hyperorality (Kluver and Bucy, 1939).

The one consistent difference demonstrated by the amygdala-lesioned infants between four and 6 months of age was an increased contact time with their mothers, compared with controls or hippocampus-lesioned infants. This difference seemed most pronounced when other monkeys were present (tetradic and social group observations), raising the possibility that the presence of other subjects influenced the mother–infant contact time of amygdala-lesioned monkeys. According to previous studies

(Thompson et al., 1969; Prather et al., 2001), neonatal amygdala lesions result in abnormal fear of conspecifics. Given that situations invoking fear or anxiety in young primates cause the subject to seek contact with its attachment figure (Mason and Capitanio, 1988), one can reasonably propose that the increased mother–infant contact time of the amygdala-lesioned infants may reflect an early mechanism of coping with the fear evoked by the presence of other conspecifics. The play interactions between amygdala-lesioned infants and conspecifics further supports this possibility, indicating that amygdala-lesioned infants played less frequently than controls or hippocampus-lesioned infants during the 6 month tetradic interactions, although the amygdala-lesioned subjects did not differ from controls in the total duration of play. Although this interpretation of early social fear is speculative, additional analysis of the postweaning development in this population of amygdala-lesioned infants is currently underway to evaluate the emergence of social fear associated with neonatal amygdala lesions. Thus, with the exception of increased physical contact time, the interactions between amygdala-lesioned infants and their mothers were indistinguishable from those of control or hippocampus-lesioned infants, suggesting that a failure to develop filial bonds is unlikely to account for the lack of mother preference displayed by the amygdala-lesioned infants in the mother preference test.

A second possible explanation for the lack of mother preference is an inability of the amygdala-lesioned infants to visually discriminate their mother from another familiar female during the preference task. This is also unlikely, because all infants, irrespective of lesion conditions, approached their mother first and returned to her more frequently during the preference test, indicating that the amygdala-lesioned animals, like the other groups, were capable of recognizing their mother.

A third plausible explanation for the absence of mother preference is that the amygdala-lesioned infants did not recognize physical separation from their mother in a novel environment as potentially dangerous and, therefore, did not seek proximity to their mother. Normal infants physically separated from their mothers respond with species-typical protest behaviors (Sackett, 1970; Bayart et al., 1990) and immediately seek proximity to their mothers (Sackett et al., 1967; Suomi et al., 1973, 1983). Furthermore, separation paradigms in which the mother is visible but not physically accessible, such as in the mother preference test used in this study, provoke severe behavioral distress responses, as indicated by increased vocalizations (Seay and Harlow, 1965; Levine et al., 1984). Among the vocalizations commonly produced in response to separation, the scream (also referred to as a screech or shriek) is typically associated with fear or extreme distress (Rowell and Hinde, 1962). In the current study, both the controls and hippocampus-lesioned infants produced numerous screams and appeared distressed when separated from their mothers. In contrast, the amygdala-lesioned infants appeared calm and produced almost no screams. This difference in fear vocalizations is all the more compelling because there were no lesion effects for other vocalizations interpreted as affiliative signals (Kalin et al., 1992). This lack of behavioral distress exhibited by the amygdala-lesioned infants during the preference test is consistent with the view that the amygdala is critical in evaluating potential danger (i.e., physical separation from their mother in a novel environment) and producing an appropriate behavioral response (i.e., scream vocalizations and seeking proximity to their mother). A subtle, but distinct, alternative explanation for their lack of reaction is that the amygdala-lesioned infants were actually so secure in their attachment to their mother that visual access to their mothers was sufficient to alleviate the distress response during physical separation in the unfamiliar environment. However, given the overall lack of fear exhibited by amygdala-lesioned infants during exposure to novel environments (Thompson et al., 1969) and unfamiliar objects (Prather et al., 2001) without their mothers present, we favor the interpretation that the amygdala-lesioned infants simply fail to identify potential danger, whether or not the mother is present.

Altogether, our recent studies indicate that the amygdala does not seem essential for fundamental aspects of social behavior. Indeed, adult macaques with amygdala lesions engage in positive social behavior more frequently than controls, thus demonstrating an ability to produce social signals and interact effectively in a social context (Emery et al., 2001). Similarly, infants with bilateral amygdala lesions develop an age-appropriate repertoire of social signals and do not display a flattened affect or disinterest in conspecifics (Prather et al., 2001). The current study extends these findings by demonstrating that infants with neonatal amygdala lesions develop remarkably normal social interactions with their mothers and seem to form a species-typical filial bond. Collectively, these studies demonstrate that a functional amygdala is not needed to form a filial bond, develop a complete repertoire of species-typical social signals, or to communicate with conspecifics in a social context.

Adult macaques without an amygdala engage in greater amounts of social interaction, particularly early in a social encounter, because they apparently lack the normal reluctance to engage another conspecific before it is determined to be safe to do so (Emery et al., 2001). In contrast, infant macaques without an amygdala respond to unthreatening social interactions with heightened fear behaviors (Thompson et al., 1969; Prather et al., 2001). Although it is unclear why amygdala lesions result in decreased social fear responses in mature subjects and increased social fear responses in immature subjects, it is clear that amygdala lesions consistently disrupt the ability to correctly evaluate potential social danger (novel adult social partner), or lack thereof (unthreatening infant social partner), and respond appropriately. A similar disruption of danger detection has also been reported in nonsocial testing paradigms, in which both mature and immature subjects with selective amygdala lesions show a blunted fear of novel and potentially fear-inducing objects (Zola-Morgan et al., 1991; Meunier et al., 1999; Kalin et al., 2001; Prather et al., 2001). These findings suggest that the amygdala plays a critical role in evaluating stimuli for potential danger and producing an appropriate behavioral response.

Rather than being essential for social cognition, we view the amygdala as playing a prominent role in evaluating environmental danger (both social and nonsocial) and marshalling appropriate behavioral responses. One of these responses could be to inhibit interactions with objects or individuals before determining their safety. One could easily imagine that the disruption of this response system through alterations in amygdala function could contribute to a variety of human behavioral pathologies such as social anxiety and social phobia (Thomas et al., 2001; Amaral, 2002).

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