

NEONATAL AMYGDALA LESIONS ALTER RESPONSIVENESS TO OBJECTS IN JUVENILE MACAQUES

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Abstract—The amygdala is widely recognized to play a central role in emotional processing. In nonhuman primates, the amygdala appears to be critical for generating appropriate behavioral responses in emotionally salient contexts. One common finding is that macaque monkeys that receive amygdala lesions as adults are behaviorally uninhibited in the presence of potentially dangerous objects. While control animals avoid these objects, amygdala-lesioned animals readily interact with them. Despite a large literature documenting the role of the amygdala in emotional processing in adult rhesus macaques, little research has assessed the role of the amygdala across the macaque neurodevelopmental trajectory. We assessed the behavioral responses of 3-year-old (juvenile) rhesus macaques that received bilateral ibotenic acid lesions of the amygdala or hippocampus at 2 weeks of age. Animals were presented with salient objects known to produce robust fear-related responses in macaques (e.g., snakes and reptile-like objects), mammal-like objects that included animal-like features (e.g., eyes and mouths) but not reptile-like features (e.g., scales), and non-animal objects. The visual complexity of objects was scaled to vary the objects' salience. In contrast to control and hippocampus-lesioned animals, amygdala-lesioned animals were uninhibited in the presence of potentially dangerous objects. They readily retrieved food rewards placed near these objects and physically explored the objects. Furthermore, while control and hippocampus-lesioned animals differentiated between levels of object complexity, amygdala-lesioned animals did not. Taken together, these findings suggest that early damage to the amygdala, like damage sustained during adulthood, permanently compromises emotional processing. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

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Abbreviations: A-IBO, subjects with amygdala-lesions created with ibotenic acid; ANOVA, analysis of variance; CON, neurologically intact control subjects; D, depth; *F*, *F* ratio statistic; FOV, field of view; H, height; H-IBO, subjects with hippocampus-lesions created with ibotenic acid; IBO, ibotenic acid; i.m., intermuscular; ITI, inter-trial interval; MR, magnetic resonance; MRI, magnetic resonance imaging; NEX, number of excitations; *P*, observed significance level; T, Tesla; TE, echo time; TR, repetition time; *W*, width; η_p^2 , partial eta-squared.

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The amygdala has long been recognized as important for the perception, generation and regulation of emotion. Research in both humans and nonhuman animals attests to the amygdala's broad role in emotion (for reviews: LeDoux, 2000; Phelps, 2006; Murray, 2007; Seymour and Dolan, 2008; Pessoa and Adolphs, 2010; Price and Drevets, 2010; Salzman and Fusi, 2010). In adult nonhuman primates, the amygdala appears critical for learning the emotional significance of new stimuli (e.g., Antoniadis et al., 2007, 2009), and for generating context-appropriate emotion-related behaviors during social interactions (e.g., Kling and Brothers, 1992; Emery et al., 2001; Machado and Bachevalier, 2006) and in the presence of provocative objects (e.g., Stefanacci et al., 2003; Mason et al., 2006; Machado et al., 2009). Despite a large body of research documenting the amygdala's role in emotion in mature animals, many questions remain about its role in emotional development. To investigate such questions, we have followed the emotional and social development of a cohort of rhesus macaque monkeys that includes animals that received neurotoxic lesions to the amygdala at 2 weeks of age, age-matched sham-operated control animals, and age-matched animals that received neurotoxic lesions to the hippocampus at 2 weeks of age. In the present experiment, we investigated the responsiveness of these animals to salient objects when the animals were juveniles (36 months of age; with sexual maturity occurring between 42 and 48 months of age and a life span of nearly three decades; Rowe, 1996).

A consistent finding in adult monkeys with amygdala lesions is that they are uninhibited in the presence of emotionally provocative or potentially dangerous objects (e.g., Aggleton and Passingham, 1981; Zola-Morgan et al., 1991; Meunier et al., 1999; Stefanacci et al., 2003; Izquierdo et al., 2005; Mason et al., 2006; Machado et al., 2009; Chudasama et al., 2008). A variety of objects are typically used in such testing to engender threat-based responding (the most common object used is a snake—usually a “life-like” toy); monkeys respond robustly to snakes even in the absence of prior experience with them (Nelson et al., 2003) leading many to believe that “fear” of snakes is biologically prepotent (Isbell, 2009; Öhman and Mineka, 2001). Researchers have long capitalized on this robust responding to explore the role of the amygdala and other neural structures in the generation of appropriate threat-based responses. Neurologically intact animals avoid emotionally provocative objects and refuse to take a de-

sired food reward placed near such objects (Nelson et al., 2003), but amygdala-lesioned animals readily interact with the same objects and retrieve proximate food rewards (Aggleton and Passingham, 1981; Mason et al., 2006; Machado et al., 2009). This lack of behavioral inhibition has been attributed to a reduction or absence of “fear” (e.g., Meunier et al., 1999; Kalin et al., 2001) or defensive behavior in general (e.g., Izquierdo et al., 2005; Chudasama et al., 2009), a lack of “avoidance” (e.g., Machado et al., 2009), or an increase in “tameness” (e.g., Zola-Morgan et al., 1991; Mason et al., 2006). Regardless of the mechanism underlying amygdala-lesioned animals’ lack of behavioral inhibition, it seems clear that the amygdala is necessary for generating an appropriate behavioral response to emotionally relevant stimuli in mature animals.

Preliminary findings from our laboratory indicated that infant monkeys with bilateral amygdala damage display a similar lack of inhibition when exposed to novel or potentially dangerous objects (Prather et al., 2001). Subsequent studies documented similar effects of amygdala-lesions in infant (9 month old) and young (18 month old) rhesus macaques (Bliss-Moreau et al., 2010); those animals are the subjects in the present study. Animals that received neurotoxic lesions to the amygdala at 2 weeks of age were significantly less inhibited in the presence of novel and emotionally provocative objects relative to age-matched control subjects and hippocampus-lesioned subjects. At both ages, control and hippocampus-lesioned subjects avoided objects, while amygdala-lesioned subjects physically touched them. These findings suggest that early damage to the amygdala disrupts normal emotional reactivity.

One of the strengths of this research program is that we can compare the behavior of animals that received amygdala-lesions as neonates not only to neurologically intact controls but also to animals that received hippocampus-lesions as neonates. In addition to serving as “operated controls” (thus allowing us to rule out general effects of the surgical procedures and ibotenic acid), our hippocampus-lesioned subjects allow for investigation of the role of the hippocampus in emotional responding. Little is known about the role of the hippocampus in emotional processing. The few existing studies that have tested emotional responding in hippocampus-lesioned macaques provide conflicting evidence. For example, Zola-Morgan and colleagues (1991) found that adult hippocampus-lesioned cynomolgus macaques (*M. fascicularis*) behaved like control monkeys in the presence of provocative objects insofar as they were more aggressive and fearful of objects than were amygdala-lesioned subjects. In contrast, more recent evidence indicates that in the presence of emotionally provocative objects adult hippocampus-lesioned monkeys, as compared to controls, spent more time in proximity to objects, retrieved food items placed near the objects more quickly and were less defensive and avoidant (Chudasama et al., 2008). In fact, both hippocampus-lesioned and amygdala-lesioned monkeys showed similarly reduced defensive behavior in the presence of provocative objects (Chudasama et al., 2009) suggesting that the magnitude of impact of hippocampus damage on emotional reactivity is

similar to that of amygdala-damage. When our subjects were tested at 9 months of age, hippocampus-lesioned animals behaved just like controls—they did not physically explore objects (Bliss-Moreau et al., 2010). The pattern of behavior changed at 18 months, however. In the presence of completely novel objects that were robustly provocative (moving objects), hippocampus-lesioned subjects behaved like control subjects insofar as they did not touch objects. In the presence of objects that were similar to those that had been seen before (e.g., a toy snake), hippocampus-lesioned animals touched objects like the amygdala-lesioned animals did. Clearly, further investigation of the role of the hippocampus in emotional processing is warranted.

In the present study, we investigated whether the changes that medial-temporal lobe damage produced on emotional responding persist over time. Animals previously tested at 9 and 18 months were tested for emotional responsiveness again at approximately 36 months of age. In order to further investigate our earlier finding that neonatal damage to the amygdala does not result in a failure to differentiate between stimulus salience or complexity (Bliss-Moreau et al., 2010), objects in the present study were presented at three levels of visual complexity. Finally, to investigate whether between-group behavioral variation was related specifically to the type of animal-like object displayed, we included both reptile-like and mammal-like objects.

EXPERIMENTAL PROCEDURES

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 (Bauman et al., 2004a,b; Bliss-Moreau et al., 2010). Briefly, subjects were 24 juvenile rhesus macaque monkeys (average age 34 months) that had previously 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). All surgeries were performed at 12–16 days after birth. The animals were returned to their mothers following surgery and housed in standard home cages (61 cm W×66 cm D×81 cm H). Throughout the course of infancy all subjects and their mothers participated in socialization groups. Each socialization group included six subjects (two from each lesion condition) and an adult male. Socialization groups met for 3 h, 5 days a week. An adult female was added to the group when the subjects were weaned and separated from their mothers at 6 months of age. Beginning at 1 year of age, all animals were housed 24 h per day in their socialization groups. At the time of the present experiment, all social groups were housed in large indoor chain-link enclosures (2.13 m W×3.35 m D×2.44 m H).

One male amygdala-lesioned animal died at approximately 1 year of age due to unrelated causes (Bauman et al., 2004a) and was subsequently replaced with an alternative age-matched amygdala-lesioned male. The substitute subject was reared with his mother only for the first year of life. At 1 year of age, the animal was weaned and pair housed with an age-matched female until

Fig. 1. Nissl-stained coronal section through the caudal level in an amygdala-lesioned animals' anterior temporal lobe. Cell loss is nearly complete and the ventricle is expanded on the right side. Cell damage is visible on the left side. Asterisk on left side marks cell sparing in a small portion of the parvicellular division of the basal nucleus. Adapted from Bauman et al. (2004b).

being introduced to his current cohort at approximately 1 year and 3 months of age.

Surgical procedures

The surgical procedures summarized below are described in detail in previous publications (Bauman et al., 2004a,b). Briefly, on the day of surgery, each infant was anesthetized with ketamine hydrochloride (15 mg/kg, i.m., Vedco, Inc, St Joseph, MO)¹ and medetomidine (30 μ g/kg, Orion Pharmaceuticals, Espoo, Finland), and then placed in an MRI-compatible stereotaxic apparatus (Crist Instruments Co., Inc., Damascus, MD, USA). The infant's brain was imaged using a General Electric 1.5 T Gyroscan magnet, 1.0 mm thick sections were taken using a T1-weighted Inversion Recovery Pulse sequence (repetition time (TR)=21, echo time (TE)=7.9, NEX 3, field of view (FOV)=8 cm, Matrix 256 \times 256). From these images, we determined the location of the amygdala or hippocampus and calculated the coordinates for the ibotenic acid injections. Infants were ventilated and vital signs 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, Hospira, Inc, Lake Forest, IL) and i.v. infusion of fentanyl (7–10 μ g/kg/h, Hospira, Inc, Lake Forest, IL). Following a midline incision, the skin was laterally displaced to expose the skull, two craniotomies were made over the amygdala or the hippocampus, depending on the pre-determined lesion condition, and the dura was reflected to expose the surface of the brain. Ibotenic acid (IBO, Bioscience Technologies Inc., 10 mg/ml in 0.1 M phosphate buffered saline) was injected simultaneously bilaterally into the amygdala or hippocampus using 10 μ l Hamilton syringes (26 gauge beveled needles) at a rate of 0.2 μ l/min. Amygdala lesions required 7.0 to 12.0 μ l of IBO per amygdala (modal number of injections=8; range=6–8) and hippocampus lesions required 5.5 to 7.0 μ l per hippocampus (number of injections=7). Ibotenic acid is a naturally occurring amino acid derived from the mushroom *Amanita muscaria*. It is a glutamate receptor agonist (Krogsgaard-Larsen et al., 1984) and a metabolic poison that causes highly localized damage to cell bodies while sparing the axons which pass through the targeted area and the terminals of nerves that do not originate in the targeted area (Schwarcz et al., 1979).

Sham-operated controls underwent the same pre-surgical 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. Following the surgical

procedure, all infants were monitored by a veterinarian and returned to their mothers once they were fully alert.

Lesion analysis

The animals are continuing behavioral testing and therefore have not been euthanized. T2-weighted MR images acquired 10 days after surgery were used to examine the extent of the edema associated with the lesion. Images were collected using a General Electric 1.5 T Gyroscan magnet; 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 \times 256). The

Table 1. Percent atrophy resulting from ibotenic acid lesion

Amygdala-lesioned animals			
Animal number	Amygdala atrophy		
	Left	Right	Average
1	67.6	70.7	69.2
2	69.1	74.7	71.9
3	81.1	80.4	80.7
4	67.3	71.8	69.5
5	73.5	61.4	67.4
6	72.5	74.2	73.4
7	76.1	73.1	71.9
Hippocampus-lesioned animals			
Animal number	Hippocampus atrophy		
	Left	Right	Average
1	83.3	74.0	78.6
2	79.0	71.4	75.2
3	79.9	81.9	80.9
4	86.0	87.7	86.8
5	81.7	88.3	85.0
6	70.6	61.4	66.0
7	71.8	69.2	70.5
8	72.2	68.1	70.2

Note: Data are the percent atrophy of the amygdaloid or hippocampal tissue for each case relative to the mean volume of those structures in the sham-operated controls ($n=8$). Left: percent tissue reduction in the left hemisphere; Right: percent tissue reduction in the right hemisphere; Average: average percent reduction across both the left and right hemisphere. Adapted from Machado et al. (2008).

¹ On average, males weighed .57 kg (\pm .024 SEM) and females weighed .54 kg (\pm .019 SEM) at the time of surgery. There were no significant differences in weight based on either sex or lesion condition.

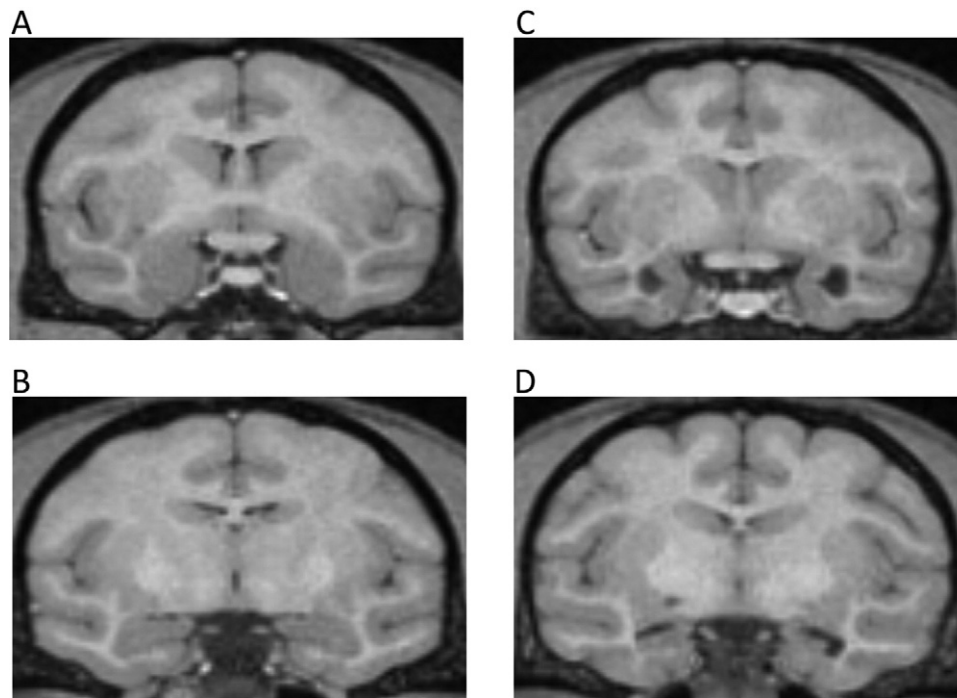


Fig. 2. T1 MRIs illustrating amygdala and hippocampus damage. Images in the left panel (A, B) are from the same control animal at (A) AP +18 and (B) AP +13. Images in the right panel illustrate (C) bilateral amygdala damage at AP +18 and (D) bilateral hippocampus damage at AP +13.

hyper-intense T2-weighted signal for each of the 16 experimental 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 mid portion of the amygdala are illustrated in previous publications (Bauman et al., 2004a,b), providing substantial reassurance that the ibotenic acid was injected and was focused in the amygdaloid complex or hippocampal formation. The extent of the targeted lesion was confirmed using histological evaluation in the one amygdala-lesioned animal that died due to an unrelated illness (see Fig. 1).

Additionally, lesion extent was further characterized in T1-weighted MRI images when animals were 4 years of age (shortly after the present experiment; see Table 1) (Machado et al., 2008). Example MRI images can be found in Fig. 2.

Behavioral testing procedure

Testing apparatus. Animals were tested in a modified lab care cage with which they were familiar (Experiment 2; Bliss-Moreau et al., 2010). The cage had a clear plastic front with two small openings through which animals could reach a solid platform on which the experimental objects were placed. An opaque guillotine door could be raised and lowered in front of the clear window allowing the researchers to shield the objects from view between trials. Two experimenters sat diagonally to the right of the testing cage and a camera was positioned directly in front of the cage.

Test cage acclimation. Animals were acclimated to the test cage during the 5 days preceding the object responsiveness task. During each acclimation day, animals were placed individually in the test cage and presented with a single food reward (a mini marshmallow) on the platform. Each 30-s trial began when the opaque guillotine door was lifted and ended when the opaque door was closed. Criterion to move to the task phase was retrieval of the food item eight out of 10 times on two consecutive days.

Object responsiveness task. Each animal completed nine testing days, with a 3-day break in testing between days 5 and 6. Each testing day included five trials. There were two types of trials—trials during which food reward was presented alone (called “food only trials”; trials 1 and 5) and trials during which a food reward was presented in the presence of an object (called “object trials”; trials 2–4). Therefore, there were 45 total trials—18 food only trials and 27 food plus object trials. Food rewards were always presented in the same position—centered on the platform, within arm’s reach such that it was directly in front of the object on trials that included an object. Three categories of objects were presented—reptile-like objects, mammal-like objects and non-animal objects. In each category, there were three different specific objects (e.g., the reptile-like object category included a snake, a dinosaur and an alligator), for a total of nine different specific objects. Each object was presented at three levels of complexity.

Animals had previously seen a coiled snake (Bliss-Moreau et al., 2010), that was similar to the one used in this experiment but significantly smaller in size. All other objects were novel to the animals. Additionally, animals had no previous experience with other living mammals (with the exception of their human caretakers and experimenters) or reptiles because they were born, raised and housed indoors.

On each testing day, trials 2–4 consisted of the presentation of one object type (e.g., snake, bear, etc.) at three levels of object complexity (simple, medium, and complex) (see Fig. 3 for examples of object sets).

The most complex form of the reptile-like and mammal-like objects were children’s toys (alligator, snake, dinosaur, bear, red panda, lion) that were positioned on the platform so that the face of the toy faced the test cage. For the intermediate form of each mammalian object (i.e., bear, red panda, lion) and the intermediate dinosaur object, the objects were presented backwards (facing away from the test cage) so that the color and texture of the toy were visible but the facial features were not. Because the snake and alligator toys were relatively flat and the facial features could be seen even if the object was facing backwards, the intermediate

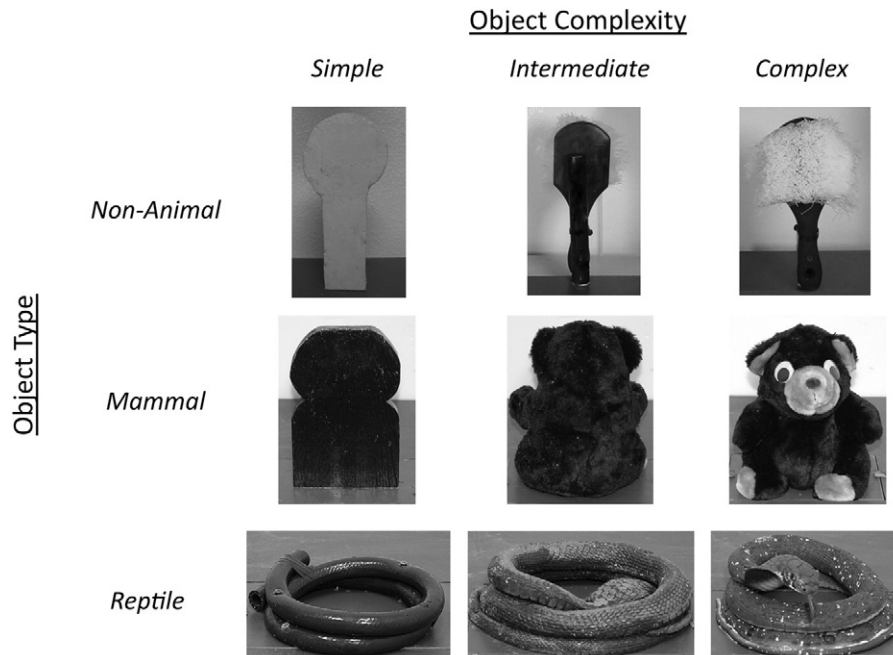


Fig. 3. Salient objects. Examples of objects used during testing from each of the three types (non-animal, mammal, reptile) and each of the three levels of visual complexity (simple, intermediate, and complex).

forms were painted a solid color to cover the eye and facial features but not their texture. The head of the snake was positioned face down between the coils. For the complex version of non-animal objects (scrub brush, ice cube tray, book), objects were placed with the most prominent features facing towards the test cage. For the intermediate version of each non-animal object, objects were placed with the prominent features facing away from the test cage. For example, the scrub brush was presented with the bristles projecting towards the animal for the complex presentation form, but presented with the bristles facing away from the animal for the intermediate presentation form. The simplest form of each object was a piece of wood cut to approximate the shape and size of the most complex object and painted a solid color similar to the most prominent color of the corresponding complex object.

Each trial began when the opaque door was lifted and ended 30-s later when the opaque door was lowered (inter-trial interval (ITI) = ~30-s). The frequency and timing information (latency and/or duration of behavior) of the primary dependent variables (i.e., retrieval of food reward, and physical contact (exploration) of objects) as well as other species-typical behaviors (Table 2) were recorded using The Observer (Noldus Information Technology; Leesburg, VA, USA).

Data analysis strategy. Means for frequency and latency/duration of behavior were computed for each trial type. Data were $\log_{10}(x+1)$ transformed in cases where they were not normally distributed (i.e., latency to retrieve food when no object was present, frequency and duration to explore objects). For the purposes of interpretation, raw data (means and variance indices) are presented; log transformed data are available upon request. On trials during which animals did not take the food or explore the objects, missing latency data were replaced with the length of the trial (30 s). Data were subjected to ANOVA. Lesion condition was the between subjects factor for all analyses. Object complexity (simple, intermediate, complex) and object type (reptile, mammal, non-animal) were repeated measures. Following ANOVAs, we assessed pair-wise between group differences using a series of

Mann–Whitney comparisons on the raw data; *P*-values associated with the Mann–Whitney tests are presented in parentheses.

RESULTS

Test cage acclimation

With the exception of one amygdala-lesioned female, all animals met the criterion to retrieve a single food reward (a mini marshmallow) within 30 s on eight out of 10 trials over two consecutive days. The animal that did not meet the criterion was excluded from subsequent analyses.

Object responsiveness task

We assessed differences in behavior related to lesion group, object type, and object complexity (and their interactions). Significant, meaningful, differences between lesion groups were only found in food retrieval and object exploration behaviors. There were no group differences in any of the other behaviors that were recorded (see Table 2). Therefore, we focus the presentation and interpretation of the results on food retrieval and object exploration only.

Retrieval of food reward. There were no lesion group differences in food retrieval on trials when no object was present—frequency: $F(2,22)=.73$, $P<.49$, $\eta_p^2=.068$; latency: $F(2,22)=.31$, $P<.74$, $\eta_p^2=.030$ (Fig. 4).

Overall, the amygdala-lesioned animals tended to retrieve food more frequently and faster than both control and hippocampus-lesioned animals in the presence of all objects—frequency: $F(2,20)=3.097$, $P<.067$, $\eta^2=.236$; latency: $F(2,20)=2.95$, $P<.080$, $\eta_p^2=.228$. Between group comparisons indicated that amygdala-lesioned animals retrieved food significantly more frequently and faster than

Table 2. Behavioral ethogram

Behavior	Description
Position in Cage	
Front	The animal's head or body is positioned in the front half of the testing cage for at least 3 s.
Rear	The animal's head or body is positioned in the rear half of the test cage for at least 3 s.
Nonspecific activity	Animal does not remain in the front or rear of the cage. Default state in which each sample is started.
Exploratory Behavior	
Manual exploration	Animal physically touches object.
Take food	Animal takes food reward.
Emotion-related expressions & vocalizations	
Grimace	Exaggerated grin with teeth (scored as directed either to the object or the observer).
Lipsmack	Rapid lip movements with pursed or puckered lips (scored as directed either to the object or the observer).
Threat	Scored when one or more of these components are present: open mouth stare, head bobbing, ear flaps, bark vocalizations, or lunges (scored as direct either to the object or the observer).
Coo	Clear, soft sound, moderate in pitch and intensity; usually sounds like "whoooo."
Scream	High-pitched vocalization with extreme high intensity; usually sounds like "eeeeeee."
Grunt	Deep, muffled, low-intensity vocalization.
Bark	Low pitched, sharp, guttural sound.
Tooth grind	Repetitive, audible rubbing of upper and lower teeth.
Stereotypies	
Backflip	Animal flips backwards at least two times in a row.
Bounce	Repetitive hopping or bouncing in the same place (must occur more than three times in a row to score).
Head-twist	Animal twists neck in a dramatic display; often seen when turning at corners and/or in conjunction with pacing.
Other stereotypy	Repetitive motor pattern not described by any of the above definitions.
Pace	Repetitive undirected pacing with the same path repeated (must last longer than 3 s to score).
Salute	Animal covers hand over eye or holds hand over eye.
Self-bite	Biting oneself.
Self-clasp	Unusual holding of body part or limb with another body part.
Spin	Repetitive twirling or spinning for at least two rotations
Swinging	Repetitive swinging in the same place (must last at least 3 s to score).
Miscellaneous	
Cage shake	Vigorous shaking of cage bars, or animals slams body against cage.
Self-sex	Anogenital exploration of self.
Self-groom	Grooming oneself.
Yawn	Open mouth, exposing teeth.
Scratch	Animal scratches own body.

control animals (frequency: $P < .016$; latency: $P < .028$). Hippocampus-lesioned animals' food retrieval behavior did not differ significantly from amygdala-lesioned animals, nor from control animals.

Food retrieval behavior differed across object types—frequency: $F(2,40) = 5.594$, $P < .007$, $\eta_p^2 = .217$; latency: $F(2,40) = 7.56$, $P < .002$, $\eta_p^2 = .274$. Food was retrieved most frequently and fastest in the presence of non-animal objects. The effect of object type differed by lesion condition—frequency: $F(4,40) = 6.66$, $P < .0001$, $\eta_p^2 = .400$; latency: $F(4,40) = 6.69$, $P < .0001$, $\eta_p^2 = .401$. Evaluation of the marginal means indicated that both hippocampus-lesioned and control animals' rate and speed of retrieval were the same for non-animal and mammal-like objects, but food was retrieved significantly less frequently and slower for reptile-like objects. In contrast, amygdala-lesioned animals retrieved food significantly less and slower on trials with mammal-like objects compared to non-animal and reptile-like objects. Interestingly, there were no lesion

group differences when only mammal-like objects were considered—frequency: $F(2,20) = 1.414$, $P < .267$, $\eta_p^2 = .141$; latency: $F(2,20) = .665$, $P < .530$, $\eta_p^2 = .061$.

Object complexity influenced food retrieval behavior. In general, animals retrieved food most frequently in the presence of the simple objects and least frequently in the presence of complex objects—frequency: $F(2,40) = 25.946$, $P < .0001$, $\eta_p^2 = .565$; latency: $F(2,40) = 43.50$, $P < .0001$, $\eta_p^2 = .685$. As with the effect of object type, the effect of object complexity was influenced by lesion condition—frequency: $F(2,40) = 3.622$, $P < .013$, $\eta_p^2 = .266$; latency: $F(4,40) = 4.85$, $P < .003$, $\eta_p^2 = .327$. Evaluation of the marginal means indicated that amygdala-lesioned animals did not modulate their behavior based on object complexity. In contrast, both hippocampus-lesioned and control animals retrieved food significantly more frequently and significantly faster on simple as compared to medium and complex trials.

Object type and object complexity interacted to influence food retrieval. Food retrieval was most frequent and fastest

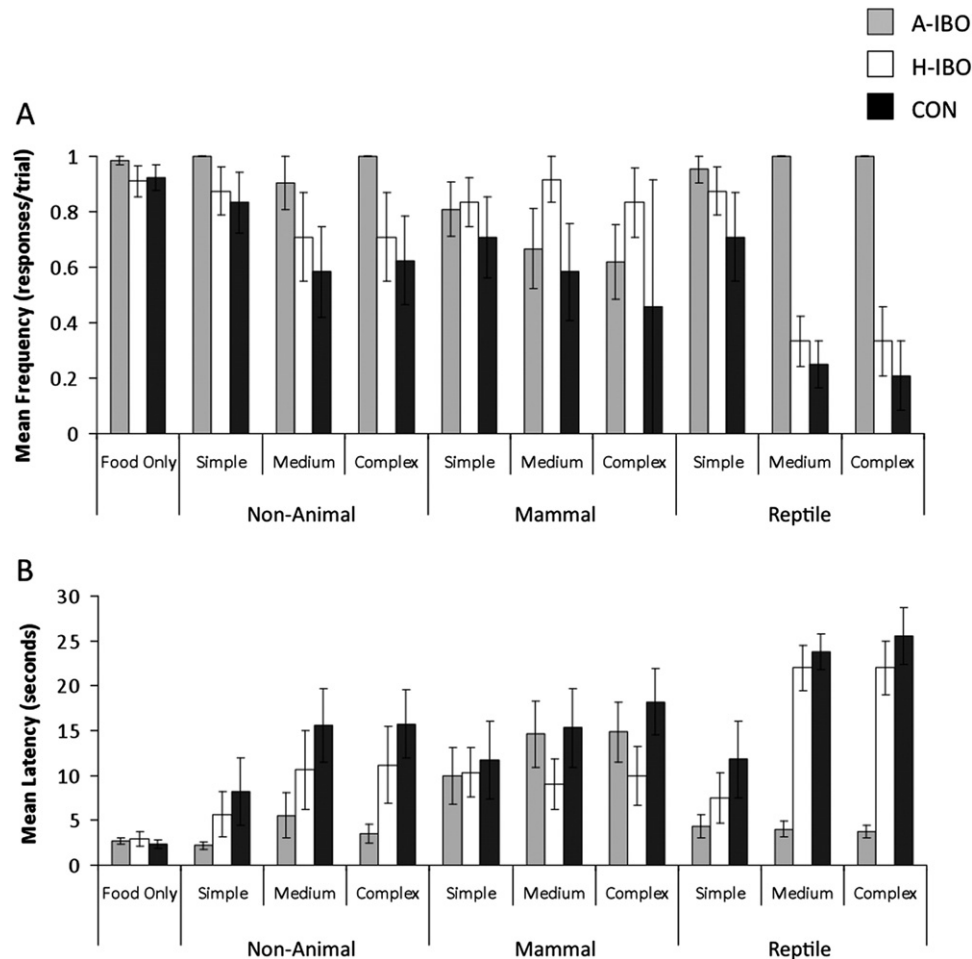


Fig. 4. Food retrieval. (A) Mean frequency of food retrieval. (B) Mean latency of food retrieval. Means \pm SEM. A-IBO, amygdala-lesioned animals; H-IBO, hippocampus-lesioned animals; CON, control.

on trials with simple non-animal objects and least frequent and slowest on trials with medium and complex reptiles—frequency: $F(4,80)=4.055$, $P<.005$, $\eta_p^2=.169$; latency: $F(4,80)=4.27$, $P<.003$, $\eta_p^2=.176$. Interestingly, this pattern of effects held only for control and hippocampus-lesioned subjects but not for amygdala-lesioned subjects, indicating that these animals did not respond to differences in the objects' features—frequency: $F(8,80)=3.279$, $P<.003$, $\eta_p^2=.247$; latency: $F(8,80)=3.90$, $P<.001$, $\eta_p^2=.281$.

Object exploration. The amygdala-lesioned animals' lack of responsiveness to specific features was also evident in data on exploration, although the frequency with which animals physically explored was low across all trial types (see Fig. 5). The amygdala-lesioned animals explored objects most frequently, for longest, and were quickest to first explore—frequency: $F(2,20)=3.98$, $P<.035$, $\eta_p^2=.285$ (amygdala-lesioned > control, $P<.082$; amygdala-lesioned > hippocampus-lesioned, $P\leq.05$); duration: $F(4,20)=4.27$, $P<.029$, $\eta_p^2=.299$ (amygdala-lesioned > control, $P<.094$; amygdala-lesioned > hippocampus-lesioned, $P<.067$); latency: $F(4,20)=3.07$, $P<.069$, $\eta_p^2=.235$ (amygdala-lesioned < hippocampus-lesioned, $P<.045$). No other effects were significant; nei-

ther object type, nor object complexity influenced animals' propensity to explore objects.

DISCUSSION

Consistent with previous assessments of other monkeys with neonatal amygdala lesions (Prather et al., 2001), with previous assessments of this cohort (Bliss-Moreau et al., 2010) and studies of responsiveness in adult amygdala-lesioned monkeys (e.g., Mason et al., 2006; Machado et al., 2009, etc.), juvenile monkeys that received lesions to the amygdala as neonates showed a lack of species-typical behavioral inhibition in the presence of emotionally provocative objects. Compared to neurologically intact control and hippocampus-lesioned animals, amygdala-lesioned animals had a greater propensity to physically touch objects and more readily retrieved foods presented in proximity to objects.

The amygdala-lesioned animals' pattern of physical exploration has been remarkably stable across time. These same animals were more likely to physically explore objects when tested at 9 and 18 months (Bliss-Moreau et al., 2010). In the present study we also found lesion group effects for physical exploration of objects, though it is

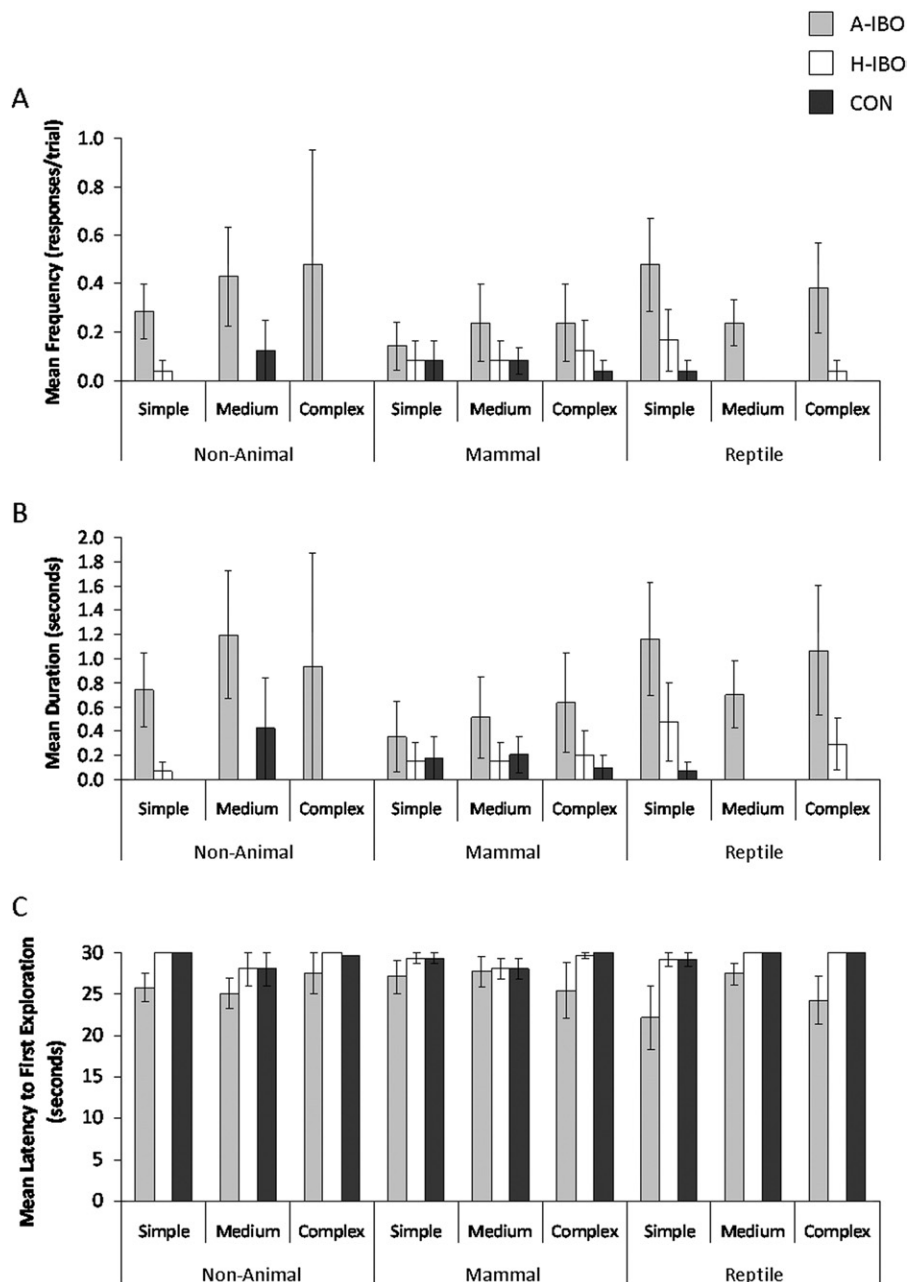


Fig. 5. Object exploration. (A) Mean frequency of exploration. (B) Mean duration of exploration. (C) Mean latency to first exploration. Means \pm SEM. A-IBO, amygdala-lesioned animals; H-IBO, hippocampus-lesioned animals; CON, control.

notable that the rates and durations of manual exploration were extremely low. This is consistent with findings that neurologically intact rhesus macaques become less likely to physically explore their environments as they mature (Reinhardt, 1990). Despite low frequencies of exploration in the current study, amygdala-lesioned animals still explored more frequently and for longer periods of time than control and hippocampus-lesioned animals.

The amygdala-lesioned animals, as compared to control and hippocampus-lesioned animals, also retrieved a food reward placed near emotionally salient objects both more quickly and most frequently. This finding provided

another indication that they were behaviorally uninhibited in the presence of salient objects. These findings are consistent with testing performed with adult animals with amygdala lesions (e.g., Aggleton and Passingham, 1981; Zola-Morgan et al., 1991; Meunier et al., 1999; Stefanacci et al., 2003; Izquierdo et al., 2005; Mason et al., 2006; Machado et al., 2009; Chudasama et al., 2009).

Another similarity in the patterns of behavior between animals that received damage to the amygdala as neonates (the present subjects) and animals that received damage to the amygdala as adults (Mason et al., 2006) is that they did not modulate their behavior based on object

complexity. While control animals' rate and speed of food retrieval decreased as object complexity increased, amygdala-lesioned animals' behavior did not change as object complexity increased. On measures of food retrieval, amygdala-lesioned animals were the fastest and retrieved food most frequently, while control animals were the slowest and retrieved food least frequently. This pattern differs from what was observed when these animals were 9 and 18 months of age (Bliss-Moreau et al., 2010). At those time points, all animals modulated their behavior based on stimulus complexity (Bliss-Moreau et al., 2010).

Perhaps the most unexpected finding in the current study was that the amygdala-lesioned animals were sensitive to the mammal-like objects. They retrieved food more slowly and less frequently when presented with mammalian objects as compared to reptile objects. In contrast, hippocampus-lesioned animals and control animals retrieved food more quickly in the presence of non-animal and mammal-like objects compared to reptile-like objects. As a result, there were no lesion group differences in food retrieval behavior on mammal-like object trials—amygdala-lesioned, control and hippocampus-lesioned animals retrieved food at equal rates and speeds. In other words, some property of the mammal-like objects resulted in the amygdala-lesioned animals engaged in more “normal” behavioral regulation. One possible explanation is that the mammal-like objects engendered “normal” social responding because most resembled conspecifics since they had visible mammal eye-spots (as opposed to small slit eyes of the reptile-like objects), had four legs, and were furry.

Previous research with this cohort indicated that amygdala-lesioned animals' repertoire of social behaviors was largely intact (i.e., they have the capacity to generate a broad repertoire of species-typical behaviors in social contexts) and during the first year of life they actually generated more prosocial behavior towards conspecifics (Bauman et al., 2004b). Interestingly, during the course of social interactions, the amygdala-lesioned animals produced more fear-related behaviors (e.g., grimacing, screaming) than control or hippocampus-lesioned animals (Bauman et al., 2004b). Thus, despite the loss of their amygdala, they appeared to be expressing fear in social contexts. The neurobiological substrate for this unusual social fear remains elusive. This history raises the possibility, however, that the lower food retrieval in the presence of mammal-like objects may be due to a residual fear or threat-related processing generated in the presence of conspecifics; at the very least, this interfering processing may delay behaviors directed at retrieving the food. In this view, the amygdala-lesioned animals' respond “normally” only to objects that have similarity with conspecifics. Investigating what specific characteristics of the mammal-like objects elicited more normal behavioral inhibition and the specific regulatory mechanism underlying the inhibition are potentially fruitful avenues for future research. Regardless of the mechanism underlying the amygdala-lesioned animals' responding to mammal-like objects, the paradoxical finding allows us to rule out the possibility that amygdala-lesioned animals' food retrieval and exploration behav-

ior on the other trial types was driven by global impairments in visual processing or attention which would have also impaired their responding to mammal-like objects.

Notably, hippocampus-lesioned animals in the present study behaved like controls both in their low rates of physical exploration and their food retrieval behavior, suggesting that early damage to the hippocampus does not disrupt normal juvenile emotional responding to salient objects. This pattern of findings is similar to what was observed when these animals were 9 months of age (Bliss-Moreau et al., 2010). Interestingly, behavior observed in this experiment differs from what was observed when the animals were 18 months of age. At 18 months of age, hippocampus-lesioned animals touched, albeit at low rates, objects that were similar to ones they had seen previously (e.g., a toy snake) (Bliss-Moreau et al., 2010) leading us to conclude that their emotional processing was compromised to some degree, although not to the extent that amygdala-lesioned animals' emotional processing was compromised. Investigating whether that compromise maintained, became exacerbated, or resolved across their neural developmental trajectories was one purpose of the present experiment. It is possible that neural reorganization that occurred between the 18 month and 36 month test periods subserved the development of normative emotional responding for the hippocampus-lesioned (but not amygdala-lesioned) animals since their impairments at 18 months of age were relatively mild in magnitude. This is feasible because we know that the hippocampus-lesioned animals' brains, in particular, underwent neural reorganization allowing for intact spatial memory in the absence of hippocampi (Lavenex et al., 2007). Importantly, the present findings with our neonatally hippocampus-lesioned animals stand in contrast to the findings of Chudasama et al. (2009) who found that adult animals that sustained hippocampus damage as adults, as compared to controls, retrieved food faster and were less defensive in the presence of provocative objects. It is possible, even plausible, that our hippocampus-lesioned animals behaved normally as juveniles because their brains were able to undergo neural reorganization in order to accommodate for their deficits because the damage to their hippocampi occurred so early in their neurodevelopmental trajectory. Understanding the specific patterns of neural reorganization in these animals is a future goal.

In summary, the present study demonstrated that damage to the amygdala, but not the hippocampus, at 2 weeks of age perturbs emotional behavior observed at 3 years of age. These findings, in concert with previous assessments of this cohort's emotional responding at 9 and 18 months of age (Bliss-Moreau et al., 2010), suggest that early damage to the amygdala impedes emotional responding across the developmental trajectory.

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