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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.

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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 imagining; NEX, number of excitations; *P*, observed significance level; T, Tesla; TE, echo time; TR, repetition time; W, width; $\eta_p 2$, partial eta-squared.

Key words: amygdala, hippocampus, responsiveness, emotion, Macaca mulatta, rhesus macaque.

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-

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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. facicularis*) 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-leisoned 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 responsivity 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