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Behavioral and Neuroendocrine Effects of Neonatal Amygdala Lesion on Rhesus Monkeys Living in a Semi-Naturalistic Environment.

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An abstract of
A thesis submitted to the Faculty of the Graduate
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Abstract

Behavioral and Neuroendocrine Effects of Neonatal Amygdala Lesion on Rhesus Monkeys Living in a Semi-Naturalistic Environment.

By Jessica R. Raper

Neonatal amygdala (AMY) lesions yield changes in social and emotional behavior. Previous studies have indicated that neonatal AMY lesions produced decreased social and increased fear behavior in presence of peers (Thompson et al, Phys Behav, 1969: 4; Bauman et al., J. Cogn Neurosci, 2004:16). Because in the earlier developmental studies, fear behavior was measured while the animals were placed in small social groups, here we assessed whether similar social and emotional changes will emerge after neonatal AMY lesions in male rhesus monkeys (*Macaca mulatta)* reared in a rich and complex semi-naturalistic social environment composed of 98 adult females and 76 offspring organized in 12 matrilines. Results show that neonatal AMY lesions produce behavioral changes, which become more pronounced as subjects mature. Yet, in contrast to previous findings, neonatal AMY damage does not result in increased fear behavior when animals are navigating in a rich and complex social environment, but instead yields increased maternal independence, play, dominance, and aggressive behaviors. Additionally, neonatal AMY lesions produce blunted emotional reactivity to stressful and potentially dangerous situations. These changes are indicative of reduced fear behaviors, presumably resulting from an inability to recognize potential social risks. Thus, behavioral outcomes of neonatal AMY lesions are critically dependent on the complexity of the social context.

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INTRODUCTION

Many primate species live in large and complex social groups characterized by hierarchical relationships among individual group members. The ability to appropriately communicate, through dynamic patterns of social interactions, is essential for survival and stability of this hierarchical society. Individual group members utilize these social interactions to establish and maintain numerous long-term relationships with other group members. Maintenance of these relationships requires perception and recognition of sensory cues from other individuals in the group and selecting an appropriate behavioral response in order to appropriately function within the social group. This ability to recognize and respond with respect to socially relevant behavior requires neural systems that process social cues.

Papez (1939) and MacLean (1949) were the first to suggest a complex neural network involved in social and emotional behavior based on anatomical connectivity. The original circuit included the parahippocampal gyrus as an affective receiving area, which sends information to the hippocampal formation and amygdala. The hippocampal formation and amygdala then generate output to the hypothalamus, thalamus and cingulate cortex where social and emotional responses are initiated (MacLean, 1949). Papez and MacLean's views have now been revised and expanded by recent neuroanatomical research indicating that the amygdala, hippocampal formation, temporal cortex, perirhinal cortex, orbital frontal cortex and cingulate cortex, which are heavily interconnected, are all receiving polysensory information (Amaral, et al. 1992; Barbas, 1995; Suzuki, 1996; Cavada, et al. 2000; McGaugh, Ferry, Vazdajanova, & Roozendaal, 2000). Evidence from diverse laboratories have implicated two brain structures, the amygdala and the orbital frontal cortex, in the normal regulation of emotional and social

behaviors in primates (Rolls, 2000; Adolphs, 2001; Machado & Bachevalier, 2003; Bachevalier & Meunier, 2005). For the purpose of this paper, we are focusing on the role of the amygdala in social cognition in primates.

Structure and Electrical Properties of the Primate Amygdala

The amygdala is located in the anterior portion of the medial temporal lobe. It is comprised of 13 interconnected nuclei and receives numerous projections from the overlying temporal lobe cortex (Amaral, 1992). The lateral nucleus of the amygdala receives information from all sensory systems, including visual information, such as faces and facial expressions, gaze direction, body postures, and movements, as well as auditory information such as specific vocal sounds and intonations. The amygdala can then modulate the cortical processing of sensory stimuli through its projections to the basal nucleus which feeds back to the sensory cortical areas (McGaugh, Ferry, Vazdarjanova, & Roozendaal, 2000). Additionally the basal nucleus provides sensory-specific cortical inputs to the central nucleus, which constitutes a relay to the brainstem and hypothalamus, a pathway thought to influence the autonomic and endocrine manifestations of emotion. The amygdala influences endocrine response of emotions, such as fear, through both direct and indirect projects to the paraventricular nucleus (PVN) of the hypothalamus. The central nucleus of the amygdala not only projects directly to the PVN, it projects to the bed nucleus of the stria terminals and preoptic area which in turn project to the PVN creating an indirect pathway by which the amygdala can also influence endocrine response (Feldman, Conforti, & Saphier, 1990; Feldman, Conforti, & Weidenfeld, 1995; Davis 2000). The basal and basal accessory nuclei project

to the ventral striatum providing access to subcortical elements of the motor system, including modulation of facial and vocal expressions, body postures, and movements. The amygdala has also a high degree of intrinsic connectivity to the hippocampal formation, which implies that these structures are capable of building highly integrated representations of sensory stimuli and their context (Amaral, 1992; Suzuki, 1996; McGaugh, Ferry, Vazdarjanova, & Roozendaal, 2000). The hippocampal formation and amygdala send and receive extensive projections to the prefrontal and temporal cortices, such as the orbital frontal, entorhinal, and perirhinal cortices, which may be involved in the consolidation and retrieval of emotional and social information (Suzuki, 1996).

As indicated above, the amygdala influences the neuroendocrine response of emotions through its projections to the hypothalamic-pituitary-adrenal (HPA) axis through both direct and indirect projections from the central nucleus of the amygdala to the paraventricular nucleus (PVN) of the hypothalamus. Research conducted in rats showed that electrical stimulation of the central nucleus of the amygdala significantly increased plasma adrenocorticotropic hormone (ACTH) and corticosterone. This elevated ACTH can in turn be (a) diminished by bilateral lesions of the central nucleus of the amygdala (Feldman, Conforti, Itzik, & Weidenfeld,, 1994), (b) inhibited by negative feedback of glucocorticoids or exogenous dexamethasone administration (Weidenfeld, Itzik, & Feldman, 1997), or (c) inhibited when hypothalamic norepinephrine or serotonin was depleted by catecholamine or serotonin neurotoxins (Feldman & Weidenfeld, 1998). Therefore, either the direct or indirect connections of the amygdala to the PVN may influence the neuroendocrine responses to fearful or stressful stimuli.

Research measuring neuronal activity in the amygdala shows that there are four

main groups of neurons in the primate amygdala. The first group of neurons responds to stimuli that are primary (unlearned) reinforcers, such as taste. The second group responds to conditioned visual stimuli that have previously been paired with a primary reinforcer. This group of neurons also responds to relatively novel stimuli, which are found to be reinforcing because they are approached and inspected. Activity in these neurons seems to be linked to the positively reinforcing effects of produced by novel stimuli. The third group of neurons responds to completely novel stimuli but not familiar stimuli. The fourth group of neurons responds to faces indicating their possible involvement in social and emotional responses to faces (Rolls, 2000).

Overall neuroanatomical and electrophysiological data provides evidence indicating that the amygdala may be responsible for adaptive social behavior and emotional expression. This proposal has also been strengthened by neuropsychological studies of the amygdala functions.

Aspiration Lesion of Adult Primates:

Emotional Behavior

Aspiration removal of the medial temporal lobe, which included the amygdala and surrounding cortical areas, yields profound alterations of emotional and behavioral responses in adult monkeys as discovered by the early work of Klüver and Bucy in the year 1939. The behavioral changes that make up the Klüver Bucy Syndrome include hyperactivity, fearless or unusually tame nature, hypersexuality, “psychic blindness”, and excessive examination of objects, typically with the mouth. The loss of fear was especially striking because the monkeys selected for the study were intentionally “wild”

and aggressive.

While Klüver and Bucy's observations were detailed, they were also only qualitative and anecdotal in nature, lacking the direct quantitative measures of behavior. The first systematic study of behavior after aspiration temporal lobectomy, which included the amygdala, rhinal cortex, and temporal pole but spared the hippocampus, was conducted by Rosvold, Mirsky, and Pribram (1954). Rosvold and colleagues quantitatively collected data on 8 male rhesus monkeys observing not only their emotional reactions in individual cages but also monitoring their dominance hierarchy before and after the three top ranking males received their surgery. Temporal lobectomy surgeries began with the top ranking male proceeding down the hierarchy to the third ranking male, there was a 2 month interval between each surgery. Post surgery all three operated animals were found to be more aggressive toward experimenters during individual cage tests. Yet, in the group cage situations, the first and second ranked males became submissive upon reintroduction after surgery. While the third ranked male became hyperaggressive after surgery. This result suggests that the context of the situation with which an animal is confronted affects the outcome of emotional expression after temporal lobe damage.

The second empirical evidence comes from the split-brain study performed by Downer (1961) to examine the effects of selective aspiration lesion of the amygdala. Animals were trained to perform a visual discrimination sequence, recognition of edible from non-edible objects by visual cues alone, following a midsagittal transection of the optic chiasm and cerebral commissures. After learning the visual discrimination sequence, the animals underwent unilateral removal of the amygdala of the right

hemisphere. Upon recovery from surgery, the eye contralateral (left) to the amygdalectomy was sutured shut, thus depriving the intact amygdala in the left hemisphere of visual input. The post-operative emotional changes were striking. Prior to the unilateral amygdalectomy surgery and suture of the contralateral eye, animals were "wild" and aggressive but found to be "placid" and "peaceful" following surgery. In contrast, when the ipsilateral eye was sutured, hence leaving the amygdala in the left hemisphere intact, the animal's behavior abruptly returned to the pre-operative level of aggression to visual threats.

In conclusion, aspiration amygdalectomy in adult primates cause dramatic affective changes. The data demonstrate that animals, which were previously "wild" and aggressive toward experimenters prior to amygdala damage, became "tame" and "peaceful" following amygdalectomy. However, the direction of these affective changes also depended on the context of the situation in which the animals are placed.

Social Behavior

Rosvold, Mirsky, and Pribram (1954) were not only the first to systematically study emotional reactivity after temporal lobectomy (see above); they were also the first to directly examine the social behavior changes after amygdala damage. In the group cage situations, the first and second ranked males, which were the first to receive amygdala lesions, became submissive and fell to the bottom of the dominance hierarchy upon reintroduction into the social group after surgery. In fact the first ranked male became extremely submissive such that, when attacked by other members of the group, he did not respond with aggression or retaliatory behavior even when the attack caused blood to be drawn. While the third ranked male became hyperaggressive and remained at

the top of the dominance hierarchy upon reintroduction into the social group after surgery. Although, the degree and direction of behavioral changes were not due to the extent of damage to the temporal lobe, the authors emphasized that both the first and second ranked males returned to the group with at least one or two aggressive dominant members, while the third ranked male returned to a group with no aggressive or dominant members to challenge him. Additionally, the length of time spent in the dominant positions of the social hierarchy could have affected the outcome since the third ranked male had held the dominant position for 16 weeks prior to surgery, whereas the first ranked male had held this position for only 6 weeks. These results suggest that the social environment with which an operated animal is confronted affects the outcome and social consequences of temporal lobe damage.

The initial findings of Rosvold and colleagues prompted further research into the effects of amygdalectomy on social behavior. In 1969, Dicks, Myers, and Kling directly examined the effects of amygdala damage on male rhesus monkeys in a free-ranging colony at Cayo Santiago. Seven intermediately ranked males, aged between 2 and 9 years, received aspiration lesion of either the amygdala and uncus (n = 2), amygdala only (n = 4), or sham surgery (n = 1). After the surgery, the animals were released into their original social group with grim results. Every lesion-operated animal experienced some period of social isolation after surgery. Additionally, four of the six operated animals died from wounds received in fights with other group members within 35 days after surgery. The authors noted that the operated animals did not initiate social activity but would only react when other members of the group initiated interactions with them. For example, upon reintroduction the 9-year-old operated male avoided contact with other

monkeys and when approached by the alpha male (leader) of his social group, he wrongly attacked the alpha male and was consequently chased, attacked and driven into exile. Researchers also suggested that the social consequences were due to the degree of temporal lobe damage as well as the age of operated animal. The youngest operated animals, 2 and 3 years of age, were able to reestablish their position in the social group and were not observed displaying overly apparent abnormalities in behavior. Overall, the data strengthen Rosvold's suggestion that the complexity of the social environment has an extreme impact on the effects of temporal lobe damage, but indicated that the age at which the lesion is inflicted might have a significant impact as well.

These earlier studies suggested that social behavior is greatly impacted by damage to the temporal lobe, specifically the amygdala and that this impact is context-and age-specific. Nevertheless, the results should be viewed with caution since the use of aspiration lesion techniques to damage the amygdala necessarily do not only damage cell bodies within the amygdaloid nuclei but also damage connection fibers from the adjacent temporal cortical areas, which are passing through and nearby the amygdala (Bachevalier, 2000; Amaral, et al, 2003; Bachevalier & Meunier, 2005). Thus it is difficult from these earlier studies to ascribe the behavioral changes solely to amygdala damage. This difficulty has recently been overcome since it is possible to perform selective lesions, which destroy neural cells but spare fibers of passage, by administering neurotoxins (i.e. ibotenic acid, a glutamatergic agonists opens calcium channels causing the cell to become over excited and fire to death) directly into the amygdala using MRI-guided stereotaxic techniques.

Neurotoxic Lesion of Adult Primates

Emotional Behavior

Meunier and colleagues (1999) compared the effects of aspiration and neurotoxic amygdala lesions on emotional reactivity to threatening stimuli. Both types of lesion produced similar effects of overly hypoaggression and reduced fear. Yet, emotional changes, such as enhanced submission and decreased aggression, were more subtle after neurotoxic lesions than after the aspiration lesions. These results suggest that some affective changes after aspiration lesion of the amygdala are likely due to unintentional damage to surrounding cortical areas. This proposal has recently been confirmed in a study investigating the effects of entorhinal and perirhinal damage on emotional reactivity. The changes produced by rhinal cortical damage took mainly the form of heightened defensiveness, and attenuated submission and approach responses, that is, just the opposite of some of the most distinctive symptoms following amygdala damage. These findings raise the possibility that the rhinal cortex and amygdala have distinct, interactive, functions in normal behavioral adaptation to affective stimuli (Meunier & Bachevalier, 2002; Meunier, Cirilli, & Bachevalier, 2006).

These findings in non-human primates parallel those found in rodents using conditioned fear as a tool for studying the role of the amygdala in emotional reactivity. Studies in rodents, using either inactivation or permanent lesion of the amygdala, have shown impairments in conditioned fear response, i.e. learning that a stimulus or context predicts aversive consequences (Maren, Aharonov, & Fanselow, 1996; Muller, Corodimas, Fridel & LeDoux, 1997; Wilensky, Schafe, & LeDoux, 2000; Goosens, & Maren, 2001). Therefore, the role of the amygdala in the acquisition and expression of

fear is now well accepted. On the other hand, primate research examining the role of the amygdala in fear response and emotional reactivity has utilized unconditioned (natural) fears of primates. For example, both laboratory and wild reared primates have been found to be fearful of snakes, a natural predator (Mineka, Keir, & Price, 1980). Predatory fear response in monkeys is commonly tested by presenting the animal, placed in a Wisconsin General Testing Apparatus (WGTA), with a real or rubber snake replica, and by recording the behavioral reactions, willingness to approach, and/or latency to retrieve a food item near the snake. Several studies using neurotoxic amygdala lesions reported a blunted fear response in that the operated animals were more willing than unoperated controls to approach, even touch, and retrieve food in the presence of the snakes (Meunier, et al. 1999; Kalin, et al. 2001; Stefanacci, Clark & Zola, 2003; Kalin, Shelton, & Davidson, 2004; Izquierdo & Murray 2004; Izquierdo, Suda, & Murray, 2005; Machado & Bachevalier, In Press).

Primate fear responses to threatening social stimuli were also investigated using the Intruder paradigm in which the animal is confronted with either an unfamiliar conspecific or human intruder. Adult rhesus monkeys with lesions of the amygdala exhibit less fearful and aggressive behaviors compared to controls when presented with a taxadermic or live conspecific (Meunier, et al. 1999; Kalin, et al. 2001). However, the data on human intruders is not as clear. Several studies have found no effects of amygdala lesions (Kalin, et al. 2001; Izquierdo & Murray, 2004; Izquierdo, Suda, & Murray, 2005; Machado & Bachevalier, 2008), whereas others found reduced fear expression and increased willingness to approach an unfamiliar human in animals with aspiration or neurotoxic amygdala lesions (Meunier, et al, 1999; Kalin, Shelton, &

Davidson, 2004; Mason, et al. 2006). One possible explanation of this discrepancy may relate to a habituation effect, since some studies used pre-surgery and post-surgery tests of the intruder that could habituate the animals to the presence of the intruder and thus reduced emotional reactivity to it (Kalin, et al. 2001; Machado & Bachevalier, 2008). This effect of habituation of threatening stimuli is consistent with an fMRI study in humans demonstrating that activity in the amygdala is greater when novel than familiar faces are presented (Schwartz, et al. 2003).

Overall, results from studies utilizing predatory and social fear suggest that the amygdala plays a key role in the regulation and expression of fear response and emotional reactivity in nonhuman primates. These fear-elicited behavioral changes are also associated with significant changes in neuroendocrine responses.

Neuroendocrine Response to Stress

The stress of being restrained has often been utilized to examine neuroendocrine response. Kalin, Shelton, and Davison (2004) compared the neuroendocrine response to restraint stress in adult male rhesus macaques with bilateral lesions of the central nucleus of the amygdala to that of control males. Basal hormone levels and response to stress were assessed twice for each animal, at least 1 week apart. Blood samples were collected immediately before and after a 30-minute stressor, which consisted of animals being restrained for 10 minutes followed by 20 minutes of confinement to a transport cage. Cerebrospinal fluid was also collected following the 30-minute stressor and examined for corticotrophin-releasing factor (acts on the anterior pituitary resulting in the release of ACTH). The lesion had no effects on cortisol, but significantly decreased adrenocorticotropic hormone (ACTH) and corticotrophin-releasing factor concentrations,

as compared to controls. Machado and Bachevalier (2008) expanded on the findings of Kalin and colleagues (2004) by comparing the neuroendocrine response of adult monkeys with amygdala lesions to controls under the following conditions: pre- and post-lesion surgery basal blood samples, restraint stress, exposure to an aversive object, and exposure to an unfamiliar conspecific. Basal blood samples were taken in the animal's home cage, stressor blood samples were taken in a rolling cage similar to the home cage, and all samples were taken while the animal was under anesthesia. Results showed that amygdala lesions had no effect on basal cortisol levels between the pre- and post-surgery. There was also no effect of lesion on cortisol levels from the restraint stress condition, yet lesion of the amygdala decreased cortisol levels compared to controls in both the exposure to an aversive object and unfamiliar conspecific.

Overall results from lesion studies of adult macaques have shown that damage to the amygdala disrupts neuroendocrine response to stress causing decreased endocrine response. Presumably the decrease in neuroendocrine response to stress is caused by disruption of amygdala projections to the hypothalamus.

Social Behavior

Using ibotenic acid lesions, Emery and colleagues (2001) reinvestigated the role of the amygdala on dyadic social interactions in adult male rhesus monkeys when placed with familiar and unfamiliar monkeys. Animals with amygdala lesion displayed more positive social behaviors and lower levels of aggressive behavior compared to controls. Additionally, monkeys with amygdala lesions would solicit more social interactions from unfamiliar stimulus monkeys on first encounter than would controls. This display of behavior by operated animals likely lead to stimulus monkeys finding them more

appealing and seeking out more positive social interactions (contact, grooming, etc.) from monkeys with amygdala lesions than with controls. The authors suggested that amygdala lesion yielded to social disinhibition; a finding opposite to that described earlier by Dicks, Myers, and Kling (1969). Although the different pattern of results between the two studies could have resulted from the lesion extent, which was more extensive in the Dicks and colleagues study than in the Emery and colleagues study, an alternative explanation could be the difference in complexity of the social environment in which the animals were placed after their surgical procedure. The social dyad is a far more restricted environment than the large complex social group setting. This latter possibility has already been suggested in the earlier experiments described above.

A recent study by Machado and Bachevalier (2006) examined social behavior of adult male rhesus monkeys with neurotoxic amygdala lesions when placed in small groups of four animals both pre- and post-surgery. Compared to their presurgical assessments, animals with amygdala lesions exhibited increased levels of excitability, activity, cage exploration, aggression, anxiety and social avoidance behaviors. Additionally, amygdala-operated animals showed a decrease in affiliation and popularity with the other members of the group. While these results are contradictory of those reported by Emery and colleagues (2001), they show that the consequence of amygdala damage on social behavior is directly related to the complexity of the social environment. Lastly, unlike the earlier study by Rosvold and colleagues (1954), animals with neurotoxic amygdala lesions did lose their dominance hierarchy rank, again suggesting that the complexity of the social situation could explain the divergent results.

To summarize, studies examining the effects of neurotoxic (selective) lesions of

the amygdala together with the early research in nonhuman primates demonstrates that the amygdala represents a critical component of a neural circuit involved in processing appropriate social behavior and emotional reactivity. These results also stress the role of the environment in the emergence of disturbed behavioral responses after amygdala damage. Thus, it is becoming well accepted that in adult monkeys as well as in humans, who have had normal development of social behavior and emotional regulation, damage to the amygdala yields significant changes in emotional reactivity and social interactions. But, what will be the consequences of amygdala damage if this damage occurred at a time when subjects have not yet acquired the social skills necessary to navigate their complex social environment, namely in infancy? There is in fact little information available to directly answer this question.

In humans, cases with neonatal damage to the amygdala are exceedingly rare and the damage extends to surrounding structures (Chutorian & Antues, 1981; Tonsgard, Harwicke, and Levine, 1987; Rossitch & Oakes, 1989; Lanska & Lanska, 1993; Caparros-Lefebvre et al, 1996; Adolphs, Tranel, & Damasio, 1998; Adolphs, 2001) and there exist only a handful of developmental studies in monkeys, (described below), which have reported alterations in social and emotional development after neonatal amygdala lesion. Information about early onset condition is vital to the elucidation of how social competencies develop from a neurobiological standpoint and particularly the role of the amygdala in normal social development.

Behavioral Developmental and Amygdala Maturation

There are critical periods in primate development when significant refinements in behavioral repertoire appear to coincide with neural development of the amygdala.

Immediately following birth and until approximately three months of age, infant macaques lack fear and defensive behaviors and do not seem to understand the meaning of social signals (Mendelson, 1982a; Mendelson, Haith, & Golman-Rakic, 1982b). Projections from higher-order visual cortices (areas TE and TEO) to the amygdala develop between one week and three months of age giving the amygdala more accurate visual information regarding social signals (Rodman, 1994). In addition, at approximately eight weeks of age the stria terminalis, which connects the amygdala to the hypothalamus, basal ganglia, basal forebrain, and areas of the brainstem, has developed moderate myelination (Amaral, et al, 1992; Gibson, 1991). Refinements of the cortical-amygdala projections and increased myelination of amygdala connections correlate with the emergence of appropriate responses to social signals in infant macaques. Mendelson and colleagues (1982a, 1982b) found that one-week-old infant macaques looked equally at pictures of monkey faces that are looking away or directly staring at them. Yet, by three and seven weeks of age, they responded to the monkey faces with the appropriate adult responses, that is lipsmacking and gaze aversion, to direct stare. In addition, it is also around three months of age that infant primates begin to enter into social interactions with peers (Hinde, Rowell, & Spencer-Booth, 1964). Considering the correlations between neuroanatomical and behavioral development, several important questions emerge. Would early-onset lesions lead to the appearance of persistent defects comparable to those seen in adult-onset lesions? Or would further development and brain plasticity reduce or even prevent the appearance of the defect? Finally, would early-onset lesions be dormant in early infancy but result in a cascading effect causing stark behavioral changes later in life? The following sections briefly review the little

information we have on the effects of amygdala damage incurred in infancy.

Neonatal Aspiration Lesion of Primates

Emotional Behavior

Kling and Green (1967) conducted the first study to examine the effects of neonatal amygdalectomy in maternally reared and maternally deprived infant primates. Growth patterns, mother-infant interaction, visual exploratory behavior, and quantitative measures of affective changes were recorded. No affective behavioral differences were found between operated animals and controls. The maternally reared operated animals reacted to human observers with species typical responses, such as grimace, withdrawal, threat postures and barking. Likewise, maternally deprived operated animals reacted to human observers in the same way as unoperated maternally deprived animals, displaying withdrawal, cowering, and rocking. However, these operated animals were only observed through the first two years of life, thus before the animals had reached puberty. Kling and Green suggested that behavioral changes following early amygdala damage may require some degree of sexual maturation to manifest.

Thompson and colleagues conducted a longitudinal study to examine the effects of neonatal amygdalectomy on 12 female rhesus monkeys: 6 females received an aspiration lesion of the amygdala at 3 months of age and the other 6 remained as sham operated controls (Thompson, Schwartzbaum, & Harlow, 1969). At 6.2 months of age, the authors examined emotional reactivity by placing the animals alone in a novel cage, and after a 6 day period of habituation, they presented five different pictures: threatening monkey, frightened monkey, relaxed monkey, infant monkey, and a control picture of

inanimate objects to the animals. During the habituation phase the amygdala-operated animals were less disturbed and engaged in more activity than control subjects. When the pictures were presented, the amygdala-operated animals demonstrated less fear than the controls. This result seems to contradict Kling and Green's (1967) finding of no difference in emotional reactivity between amygdala-operated animals and controls. This difference may reflect a difference in the measurement of emotional reactivity. Kling and Green merely measured subject's reactivity to human observers, whereas Thompson and colleagues specifically designed a test based on knowledge of normal rhesus monkey behavioral reactivity to novel situations; by 3 months of age infants will respond to strange situations by freezing, screaming, and exploratory behavior will only occur after the animal is accustomed to the new environment (Harlow, 1966). Yet, this still leaves the question of whether the behavioral changes after neonatal amygdalectomy may emerge after further maturation of the animals.

To directly address this issue Thompson and colleague's (1977) reexamined emotional reactivity at 6 years of age after the animals had reached sexual maturity. Again animals were placed alone in a novel cage for six days of habituation before being left alone for a 24 hour period to examine their behavioral reaction. Neonatal amygdalectomy yielded an increased activity in a novel environment. The adult control animals participating in this study received amygdala lesion as adults and received the same tests to allow comparisons between the behavioral effects of neonatal and adult amygdala lesions. There was no difference in the emotional reactions of neonatal and adult amygdala lesions. These results suggest that neonatal and adult lesions have similar effects of blunting the emotional reactivity of rhesus monkeys.

Studies examining the effects of neonatal amygdala lesions on affective behavior have had mixed results. Kling and Green (1967) found not difference between amygdala-operated and control animals during, passive observation of animals' responses to a human observer. By contrast, using an emotional reactivity test Thompson and colleagues (1969; 1977) found blunted fear response and increased activity as early as 6 months, and these emotional changes remained when subjects had reached sexual maturity. Additionally, Thompson found that adult animals with neonatal amygdala damage had the same changes in affective response as animals that received amygdala damage as adults. Therefore, concerning emotional reactivity, damage to the amygdala early in infancy causes affective blunting similar to that found after adult amygdala lesions.

Social Behavior

Dicks, Myers and Kling (1969) found that younger males (2 or 3 years of age) that received amygdalectomy were able to reestablish themselves in their social group and appeared to show less behavioral changes than the older males (4 to 10 years of age) with the same lesion. In addition, the first study examining the effects of neonatal amygdalectomy on maternally reared and maternally deprived monkeys found that the neonatal amygdala lesion had no effect on mother-infant behavior (Kling & Green 1967). This result suggests that early-onset amygdala lesions have less negative effects on social behavior than adult-onset lesions. However, in the case of Kling and Green's study the operated animals were observed only through the first two years of life and left the question of whether changes in social behavior effects would occur after puberty open.

Thompson and colleagues specifically examined the long-term effects of neonatal

aspiration lesion of the amygdala on social behavior in 12 female rhesus monkeys (Thompson, Schwartzbaum, & Harlow, 1969; Thompson & Towfighi, 1976, Thompson, Bergland, & Towfighi, 1977). Systematic observations of the animals revealed that, unlike operated-controls, amygdalectomized animals exhibited increased fear response and hyperactivity during social testing as early as 6 months of age (Thompson, Schwartzbaum, & Harlow, 1969). When retested at 3 and 6 years of age, the amygdala-operated animals continued to display hyperactivity and reached lower ranking status being subordinate to controls (Thompson & Towfighi, 1976; Thompson, Berland, & Towfighi, 1977). Yet, when tested with an unfamiliar conspecific, amygdala-operated animals displayed less fear even after being attacked by an older more dominant animal (Thompson, Bergland, & Towfighi, 1977). Finally, when comparing the effects of early-onset versus adult-onset amygdala damage, Thompson and colleagues (1977) found no behavioral differences between the neonatally-operated and adult-operated animals. Overall, the results demonstrate that neonatal damage causes similar behavioral changes in social behavior than adult damage. In fact changes after neonatal amygdala damage become increasingly more evident with age.

The findings by Thompson were confirmed and expanded by the studies of Bachevalier and colleagues (see for review, Bachevalier, 1994). The study was similarly designed, using 12 rhesus monkeys of both sexes: 6 receiving neonatal aspiration lesion of the amygdala at 10-15 days of age and the remaining 6 served as unoperated controls. At 2 months of age, operated animals were not hyperactive but instead displayed less activity and less manipulation of their environment as compared to controls. Social interactions between operated animals and controls were normal but were initiated almost

entirely by control animals. At 6 months, the changes in general activity disappeared, whereas dyadic social interactions became drastically reduced. Also, at the same age control animals became more dominant over the operated animals that responded by an increase in withdrawing from social interaction (Bachevalier, 1994). When animals reached adulthood, there was a complete lack of social interaction between operated animals and controls during dyadic observation test (Bachevalier, 2000). Bachevalier's results confirmed that neonatal lesion of the amygdala produces dramatic behavioral changes which become apparent and more severe over time.

Results from studies examining the social behavioral effects of neonatal aspiration lesions of the amygdala showed that damage produces profound behavioral changes that included increased social fear, lower dominance rank, increased submission, and inappropriate responses to aggression. Yet, these early studies raised a number of issues that need to be taken into consideration. First, the length of observation of the animals after the neonatal damage appears to be critical since Kling and Green (1967), who observed their animals until 2 years of age (prior to sexual maturation), did not find any behavioral changes, whereas Thompson, et al. (1969, 1976, 1977) and Bachevalier (1994, 2000), who observed the animals until they reached sexual maturation, reported severe behavioral changes. Second, the environment in which the infant monkeys were reared may also interact with early-onset damage in the severity of the behavioral changes observed. For instance, in Kling and Green (1967) study, the infants were returned to their mothers, whereas in the studies by Thompson, et al. (1969, 1976, 1977) and Bachevalier (1994, 2000), the infants were nursery-reared and hand-raised by researchers. Last but not least, all of these studies have the limitation that the lesions

were performed by aspiration technique causing damage to surrounding cortical areas as well as connecting fibers passing through and nearby the amygdala that may have led to more severe changes in behavior. This last limitation has recently been empirically investigated.

Neonatal Neurotoxic Lesion of Primates

Emotional Behavior

Prather, et al. (2001) directly examined the effects of neonatal neurotoxic lesion of the amygdala on fear response in primates. The study consisted of 3 rhesus monkeys that had received neurotoxic lesion of the amygdala at 14 days of age and 3 unoperated controls. All infants were reared with their mothers until 5.5 months of age after which they were separated from their mother. At 8.5 months of age, infants were presented with neutral objects (i.e. luggage tag) and fearful objects (i.e. rubber snake replica) in their home cage. Compared to controls, amygdala-operated animals engaged in greater manipulation of neutral objects and took a shorter amount of time to retrieve food adjacent to the fearful objects. These findings indicate that even selective early damage to the amygdala resulted in drastic changes in affective reactivity.

Neuroendocrine Response to Stress

A study by Goursaud, Mendoza and Capitanio (2006) investigated neuroendocrine response to stress in the same animals. At 3 to 5 months of age subjects were separated from their mothers and relocated to individual cages for a 48 hour period of behavioral testing; after testing was completed infants were returned to their mothers. Blood samples were collected by hand-restraining the awake subject and taking the blood from

the femoral vein. On test day 1 the first blood sample was taken between 1.5 and 2.5 hours after subjects were initially separated from their mothers. Then, the second blood sample was taken at the end of the day before an injection of dexamethasone (synthetic glucocorticoid that suppresses natural production of ACTH). On test day 2, the third blood sample was taken and used for comparison with the dexamethasone challenge, which was immediately followed by an injection of adrenocorticotropic hormone (ACTH acts on the adrenal gland to produce cortisol). A fourth blood sample was drawn 30 minutes after the ACTH challenge to assess the function of the adrenal gland. Lesions of the amygdala had no effect on cortisol levels following separation from the mother. The results of the dexamethasone and ACTH challenges show that animals with amygdala lesions responded similarly compared to controls. Thus, unlike rodent studies (Feldman, Conforti, & Weidenfeld, 1995) or adult nonhuman primate lesion studies (Kalin, Shelton, & Davidson, 2004; Machado & Bachevalier, 2008), this study demonstrated no effects of lesion on neuroendocrine response. Yet, results of this study should be viewed with caution since there were no baseline hormonal levels measured for direct comparison with hormonal levels measured under stress conditions. Thus, additional studies are required to further evaluate the effect of neonatal neurotoxic amygdala lesion on neuroendocrine response to stress.

Social Behavior

The study by Prather and colleagues (2001) was also the first to examine the effects of selective neonatal lesion of the amygdala on social behavior in primates. Social behavior was observed under two conditions: mother-infant interactions in home cage until 5.5 months of age and, at 6.5 months of age, in dyadic social interactions.

There were no differences found during mother-infant interactions since the controls and amygdala-operated infants spent similar amounts of time nursing and maintaining contact with their mother's ventrum. Differences in social behavior were observed during the dyadic social interactions. The amygdala-operated infants displayed more fearful behaviors (fear grimace and screams) during the dyadic social interactions compared to controls. This result indicates that even selective neonatal amygdala damage results in an increase in social fear.

Bauman and colleagues (2004a, 2004b) expanded on the work of Prather, et al (2001) by examining the effects of neonatal ibotenic acid lesions of the amygdala on social behavior. The study used 24 rhesus monkeys, 8 of which underwent neonatal lesion of the amygdala at 12-16 days of age, 8 others received neonatal lesion of the hippocampus, and the remaining 8 served as age matched sham-operated controls. For the purpose of this paper, I have only summarize the data from the amygdala-operated and controls animals. All subjects were raised in individual cages with their mothers for the first 6 months of life. During this time, they were given three hours of socialization 5 days a week with 5 other mother-infant pairs. After 6 months of age, infants were weaned from their mothers and permanently placed in small social groups containing 6 experimental animals (2 from each treatment group), 1 adult male and 1 adult female. Behavioral observations were conducted throughout this study under varying conditions: home cage, dyadic, tetradic, and social group. Like Prather, et al. (2001), neonatal amygdala lesion did not disrupt the development of mother-infant social interactions, although amygdala animals did spend significantly more time in contact with their mothers compared to controls. Additionally, Bauman performed a mother preference test

after the infants were weaned at 6 months of age. The results showed that, unlike sham-operated controls, animals with neonatal amygdala lesions did not show a clear preference for their mother over an unfamiliar adult female. That is, they spent a significant amount of time alone or near the unfamiliar adult female instead of remaining close to their mother (Bauman, et al., 2004a). At 6 and 9 months of age, the amygdala-operated animals developed a normal repertoire of social behaviors but consistently produced more fear behaviors than controls. At 12 months of age, all animals were tested with unfamiliar animals in a novel dyad condition, results show that, unlike sham-operated controls, amygdala-operated animals displayed less aggressive behaviors and more affiliative behaviors. The authors proposed that the amygdala plays a critical role in the regulation of social fear responses (Bauman, et al. 2004b).

Together the findings from Prather, et al (2001) and Bauman, et al. (2004a, 2004b) suggest that selective neonatal amygdala damage disrupts the ability to evaluate potentially dangerous social situations resulting in increased social fear. Yet, increases in social fear were not observed during mother-infant interactions. Perhaps, the amygdala may regulate emotional responsiveness when the animals are placed in more complex social environments (such as those seen in the dyadic, tetradic, and social group tests) where social signals may become more ambiguous.

Environmental Considerations

Behavioral development in primates is greatly influenced by the complexity of the environment in which they are raised. Studies have shown that social isolation and/or severe environmental restriction produce deficits in primate behavior (Mason, 1960;

Anderson & Mason, 1974). Thus, both physical environment and complexity of social group play a critical role in the emergence of normal primate social behavior (Rosenblum & Andrews, 1994). Additionally, enriched environments as characterized by increased social interaction, physical exercise, and continuous exposure to learning task (Krech, Rosenzweig, & Bennett, 1960) improve cognitive functioning in normal, neonatal and adult ischemic, and prenatally stressed rodents (Pereira, et al., 2007; Qian, Zhou, Pan, Liu, & Wang, 2008). Therefore, results found after neonatal neurotoxic lesions when animals were observed in a more restricted social environment (Prather, et al, 2001; Bauman, et al.,2004a, 2004b) may differ from those obtained on animals that are placed in a more complex social group. Indeed in these earlier neonatal studies, the social environment was limited to only a few animals and due to its artificial creation contained 3 distinct transitions during a critical developmental period, the first year of life. Subjects were paired housed with their mother for the first 6 months of life and had access to 5 other mother-infant pairs a few hours each day. Then at 6 months of age subjects were weaned from their mothers, lived in single cages until 1 year of age, and again given daily access to 5 other infant subjects along with 1 unrelated adult male and 1 unrelated adult female. Lastly at 1 year of age, subjects again transitioned to a new living environment of a small group with 24 hour access to the 7 other animals described above (Bauman, et al. 2004a, 2004b). While a small group size of 8 is certainly more socially complex than dyadic or tetradic interactions, it is not nearly as socially complex as a natural social group employed by Dicks, Meyers, and Kling (1969). Additionally, the stability of social and physical environment has been shown to be essential for normal primate infant development (Rosenblum & Andrews, 1994). Thus, in the Bauman and

colleagues study infants changing social and physical environments 3 times during the first year of life could have negatively impacted their behavioral development. Thus, overall the results suggest that the effects of neurotoxic amygdala lesion on social behavior and emotional reactivity may be significantly attenuated when the young animals are raised in a rich and complex social environment.

To directly test this proposal, the goal of the present pilot study is to examine the effects of neonatal neurotoxic lesions of the amygdala in male rhesus monkeys living in a large complex social group in a semi-naturalistic setting at the Yerkes National Primate Center Field Station. Results from this pilot study should begin to shed light on what impact the complexity of social environment might have on the affective and social behavior effects of early amygdala damage.

Hypothesis 1: Social Behavior

Neonatal amygdala damage will cause changes in the expression of social behavior. Given the previous studies reported above, we predicted that rearing in a large and richly complex social environment will buffer the negative effects of the neonatal amygdala lesions on social behavior, yielding only subtle differences in social behavior and social fear. To test this hypothesis, 2 males received neurotoxic lesions of the amygdala at approximately 1 month of age and 2 others received sham-operations at the same age. Animals will be kept with their mother during the surgical procedures and recovery, and the mother-infant pair will then be reintroduced to the social group. Weekly focal observations on subjects in their social group will be conducted and analyzed at different time points during development in order to test this hypothesis.

Hypothesis 2: Emotional and Neuroendocrine Responses to Stress

Subjects with neonatal neurotoxic amygdala damage will show changes in their ability to process potentially fearful situations and thereby to mount a neuroendocrine when faced with stressful events. Specifically, neonatal amygdala damage will disrupt the processing of the stressor and the ability for this information to reach the hypothalamus and trigger the normal neuroendocrine response to stress. Thus, we predict that animals with neonatal neurotoxic amygdala lesions will show decreased emotional reactivity to stress associated with blunted neuroendocrine responses to stress. To examine this hypothesis animals, at approximately 1.2 years of age, will undergo tests experimentally designed to elicit a stress response (i.e. Social Isolation, Human Intruder, and Social Intruder), blood samples will be collected before and after the first two tests and later assayed for stress hormone levels.

METHODS

Subjects

Four infant rhesus monkeys (*Macaca mulatta*) were selected from middle ranking mothers living in a large social group of 76 adult females and 96 offspring organized into 12 matrilines. At 30-35 days of age, 2 infants received bilateral neurotoxic lesion of the amygdala and the remaining 2 received sham surgeries. Two to three days prior to surgery the mother-infant pair was removed from their social group at the Yerkes Field Station and transported to the Yerkes Main Center. On the day of surgery, the infant was removed from the mother, and, after 24-hour surgical recovery period for the infant, the pair was reunited. One week later the infant received a follow-up MRI to verify the extent and location of the lesion. The mother-infant pair was then transported back to the Yerkes Field Station where they were placed back in their social group using a staged reintroduction procedure. Intensive behavioral observations of the infants began immediately following the reintroduction and continued until 1.5 years of age.

Magnetic Resonance Imaging (MRI) Procedures and Injection Coordinates

The surgical technique utilized MRI for pre-surgical locations of injection sites and pre-surgical calculations of stereotaxic coordinates of each injection site. On the day of the surgery, the infant was anesthetized (Ketamine hydrochloride, 100 mg/ml), intubated and given isoflurane, 1-2% to effect. An IV drip of dextrose and 0.45% sodium chloride was placed to maintain normal hydration during MRI and surgery. The animal's head was secured in a nonferromagnetic stereotaxic apparatus with ear bars containing vitamin E (opaque contrast) to establish the MRI coordinates of the tip of each earbar, which served as precise reference points. Once the animal was aligned in the magnet

their brain was imaged in each of two stereotaxic planes (sagital and coronal) using a Siemens 3.0 T/90 cm whole body. After a sagittal localizer, a high resolution coronal series was taken at 1 mm through out the brain, and was used to estimate the coordinates of injection sites. Three Fluid Attenuated Inversion Recovery (FLAIR) coronal series were taken at 3.0 mm (each offset of 1 mm) through the entire brain to detect hypersignals from brain edema after surgery and were used to estimate the location and extent of the lesions. After imaging, the infant was kept anesthetized in the stereotaxic apparatus and immediately brought to the surgical suite where it was prepared for aseptic surgical injection of excitoxin, ibotenic acid, in the amygdala.

Injection coordinates were determined by using the high-resolution T1 image that cuts through the largest body of the amygdala, roughly at the level of the chiasm and the middle portion of the anterior commissure. Four injection sites were located 1mm dorsal, 1mm ventral, 1 mm lateral and 1 mm medial to the center point of the amygdala, allowing diffusion of ibotenic acid through the entire amygdala. Coordinates of each injection site were determined by measuring the distance of the target site to each of three referents for each monkey: Anterior/Posterior Coordinates are calculated from the zero point determined from the level of vitamin E filled ear bars; Medial/Lateral Coordinates are calculated from the midline of the brain, usually identified by the midsagittal sinus and third ventricle; Dorsal/Ventral Coordinates are calculated from the dorsal/ventral coordinates of the earbars. These MRI coordinates are then translated into stereotaxic coordinates.

Surgical Procedure

At the end of the MRI session, the infant was kept under deep anesthesia, moved to the surgical suite, and prepared for surgery. A line was drawn at the middle line of the head from the occiput to the occipital ridge and bupivicaine (1.5 ml at 0.25% concentration) was injected subcutaneously along that midline to reduce pain. Under aseptic conditions, the scalp was opened along the midline, and connective tissue was gently displaced laterally to expose the skull. Two craniotomies were made bilaterally, in front of bregma and above the amygdala, and the dura was cut and retracted to expose the brain. Injections were made simultaneously in the two hemispheres using 30-gauge needles attached to 10 μl Hamilton syringes. The needles were lowered slowly at each injections site, and 0.4 μl of ibotenic acid (10mg/ml concentration) was manually injected at a rate of 0.2 ml/min. After injection, the needles were left in place for an additional 3 minute period to allow complete diffusion of the excitoxin at the tip of the needle and minimize its spread in the needle track during retraction of the needles. After the last injection, the dura was closed with silk sutures, the bone opening was covered with Surgicel NU-KNIT (absorbable hemostat), and connective tissues and skin were sutured at the midline. After surgery, the animal was placed in an incubator ventilated with oxygen and was then returned to the nursery upon complete recovery from anesthesia. The following day, when the animal was alert and feeding normally, it was then returned to the mother and the reunion was monitored hourly.

Post-surgical MRI Scan

Infants received a second MRI session one week post-surgery. The animal was anesthetized, repositioned into the stereotaxic frame and both high-resolution T1 and

FLAIR coronal images were again obtained on the coronal plane (see pre-surgical MRI for details above). Comparisons of the FLAIR images pre- and post-surgery were used to assess location and extent of the hypersignals indicating location and extent of brain edema (see Figure 1 & 2).

Sham Surgery

Control animals received a sham-surgery and were treated in the same way as the experimental animals. They were removed from their mothers the day of surgery, anesthetized, positioned in the stereotaxic frame and received both T1 and FLAIR images. They remained under deep anesthesia for roughly the same amount of time as the subjects that received neurotoxin injections. Upon arrival to the surgery suite, their skin, skull, and dura were opened and the needle of the Hamilton syringe was lowered at the estimated amygdala level but no injections were made. Afterward, the tissue was closed in anatomical layers using the same procedure as those described for the experimental animals. They were then returned to their mother the day following surgery, and while they did not receive a one week follow-up MRI, they were separated from their mothers for the same length of time as were the experimental animals.

Lesion Extent Histology

Perfusion

Subjects were deeply anesthetized with Ketamine HCl (10mg/kg, IM), followed by sodium pentobarbital (25-30mg/kg IV or IM) and perfused transcardially with 0.9% phosphate buffered saline, then by a sequence of 4% paraformaldehyde at pH 7.4, 5%

Figure 1: Extent of amygdala damage for subject AMY-1 shown on MR images (A) and histology sections at approximately the same level of MR images and stained for cells and fibers identification (B). From left to right in A, the images illustrate the amygdala as seen on a T1-weighed image acquired just prior to surgery, a FLAIR image performed 5-8 days after surgery and the depicting the extent of hypersignals (white area), and a T1-weighed image acquired 1 year post surgery revealing the shrinkage of the amygdaloid tissue. In B, the Thionin stain demonstrates the cell loss within different amygdaloid nuclei, and the Gallyas stain shows that the fibers crossing through the area of cell loss were spared. Red dashed outline the extent of hypersignals (MR image) and cell loss (Thionin stain) within the amygdala. Black dashed lines outline specific nuclei within the amygdala. Abbreviations: AB, accessory basal; B, basal; CE, central; L, lateral; ME, medial; PAC, periamygdaloid cortex.

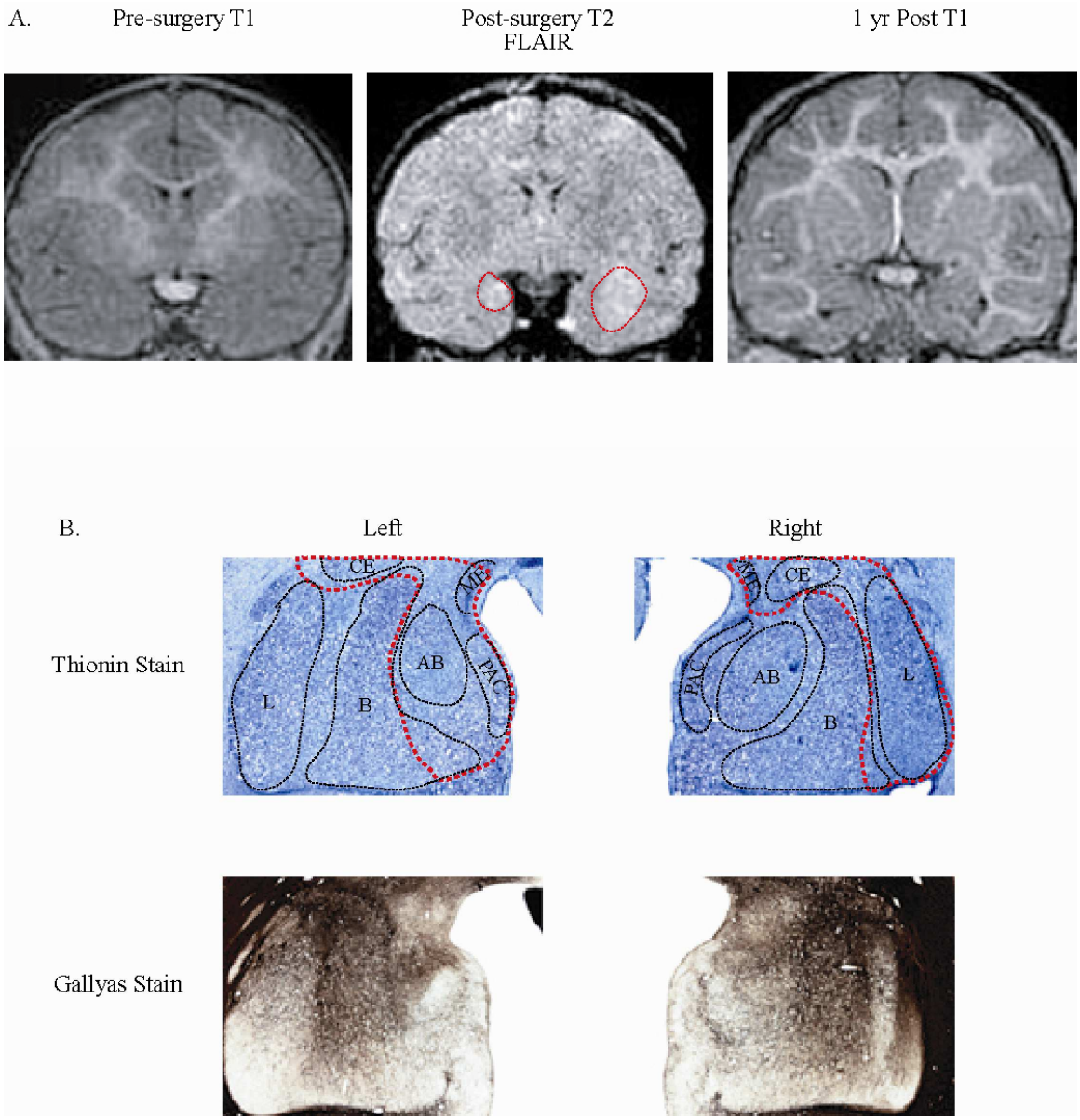
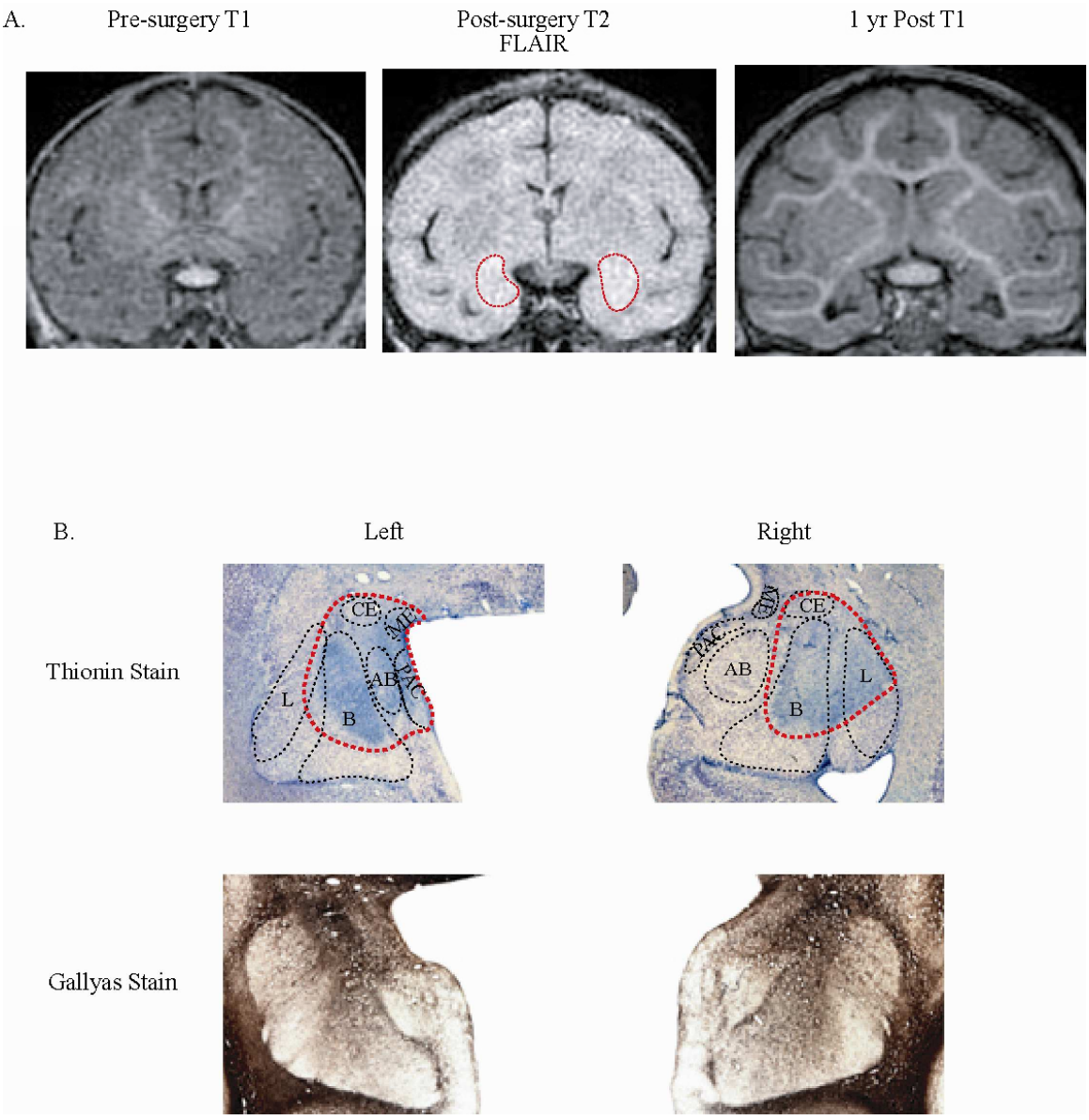

A.
Pre-surgery T1
Post-surgery T2
FLAIR
1 yr Post T1
B.
Left
Right
Thionin Stain
Gallyas Stain
CE
MB
AB
PAC
L
B

Figure 2: Extent of amygdala damage for subject AMY-2 shown on MR images (A) and histology sections at approximately the same level of MR images and stained for cells and fibers identification (B). From left to right in A, the images illustrate the amygdala as seen on a T1-weighed image acquired just prior to surgery, a FLAIR image performed 5-8 days after surgery and the depicting the extent of hypersignals (white area), and a T1-weighed image acquired 1 year post surgery revealing the shrinkage of the amygdaloid tissue. In B, the Thionin stain demonstrates the cell loss within different amygdaloid nuclei, and the Gallyas stain shows that the fibers crossing through the area of cell loss were spared. Red dashed outline the extent of hypersignals (MR image) and cell loss (Thionin stain) within the amygdala. Black dashed lines outline specific nuclei within the amygdala. Abbreviations: AB, accessory basal; B, basal; CE, central; L, lateral; ME, medial; PAC, periamygdaloid cortex.


A.
Pre-surgery T1
Post-surgery T2
FLAIR
1 yr Post T1
B.
Left
Right
Thionin Stain
Gallyas Stain
CE
ME
AB
LA
L
B
PAC
GP

glycerol with 4% paraformaldehyde, and 10% glycerol with 4% paraformaldehyde. The brains were extracted immediately following perfusion and transferred through 10% glycerol with 4% paraformaldehyde, 10% glycerol with 0.1M phosphate buffer, and, finally, 20% glycerol with 2% dimethyl sulfoxide (DMSO) at 4˚C. Lastly, the brains were cut into three blocks in the coronal plane using a histological blade, then wrapped with aluminum foil and stored at –70°C until cutting.

The brains were sliced at 50 μm thickness on a freezing microtome with a custom freezing stage (Model 860; American Optical Corp., Lorton, VA). Every 5th slice (every 250 μm) was collected in standard phosphate-buffer. These sections were mounted on 2 x 3 inch glass slides and air dried in preparation for staining.

Staining

Thionin stain (Nissl) was used to stain cell bodies and identify the main amygdala nuclei and laminar organizations of adjacent cortical areas. The sections (250 μm) were first dehydrated and defatted in xylene for two hours. After re-hydration through a series of descending alcohols, the sections were thoroughly rinsed in diH20, developed in a 1% aqueous solution of thionin for 1-3 minutes, and quickly re-rinsed in diH20 before dehydration through ascending alcohols, clearing in xylenes and being coverslipped.

To ascertain that the concentration and amount of neurotoxin used did not demyelinate the fibers of passage through the amygdala, a silver stain (Gallyas) was used to stain sections at 500 μm. The sections were first rinsed in diH20 then placed in pyridine acid for 50 minutes with constant agitation. Next, the sections were re-hydrated through a series of descending alcohols and run through a series of acetic acid solutions (.05%, .1%, & .5% respectively). The sections were then placed in silver nitrate solution

for 50 minutes with constant agitation, and then developed using a series of .5% acetic acid, ABC solution, potassium ferricyanide, and sodium thiosulfate. Lastly, the sections were dehydrated, placed in xylene, and coverslipped.

Lesion Assessment

Thionin stained sections through the amygdala were used to identify the loss of cells within each amygdala nuclei and adjacent cortical areas, and plot this loss onto corresponding coronal drawings from normal template brain using Adobe Photoshop software (v. 6). These drawings were imported into a Java-based image analysis program (ImageJ®; http://rsb.info.nih.gov/ij/) to measure the surface area (in pixels2) of damage for intended targets, as well as all surrounding structures sustaining unintended damage. For any given region of interest (ROI), the measured surface area of damage on each section through each hemisphere was summed and then multiplied by image thickness (1 mm) to calculate a total volume of damage (Gundersen & Jensen, 1987). The volume of damage was then divided by the normal volume of the ROI (obtained from the normal template brain in a similar manner) to estimate a percent of the total volume damaged.

Extent of Lesion

Damage to the amygdala was bilateral for both subject cases (see Table 1 and 2). The average bilateral damage for each subject was very similar averaging 56.6% for case AMY-1 and 54.5% for case AMY-2 (Table 1). A detailed description of the cell loss for each of the 13 nuclei in the amygdala is given in Table 2.

The lesion of AMY-1 (Figure 1) was bilateral and symmetrical (53.2% on the right and 59.8% on the left). In the right amygdala, cell loss was extensive (>70%) in the lateral, central, medial nuclei, moderate (between 30% and 70%) in the basal and

Table 1. Percentage of cell loss within the entire amygdala and adjacent structures

	Intended Damage				Unintended Damage				
Cases	R%	L%	X%	W%	Hippocampus	TG	TE	ERh	PRh
AMY-1	53.2	59.8	56.5	31.8	0	0	.08	.07	.03
AMY-2	62.5	46.5	54.5	29.1	.58	0	0	0	0

Note: Data are the estimated percentage of normal volume as assessed from histological sections for the intended damage to the amygdala and unintended damage to the hippocampus, ERh (entorhinal cortex), PRh (perirhinal cortex); R% - percentage of damage to the right hemisphere; L% - percentage of damage to the left hemisphere; X% - average of L% and R%; W% = (L% x R%)/100 [weighted index as defined by Hodos and Bobko (1984)]

accessory basal nuclei, and mild (<30%) in the posterior cortical nuclei. In the left amygdala, the cell loss was almost complete in the accessory basal, central, medial, and cortical nuclei, and in the periamygdaloid cortex. The cell loss was moderate in the lateral and basal nuclei. Unintended damage in this case was negligible (<0.1% in area TE, entorhinal and perirhinal cortex bilaterally).

In case AMY-2 (Figure 2), the lesion was slightly more extensive on the right (62.5%) than on the left (46.5%). On the right, the cell loss was extensive in the lateral, basal and cortical nuclei, moderate in the accessory basal and central nuclei, and mild in the medial nucleus and periamygdaloid cortex. On the left, the cell loss was extensive in the accessory basal nucleus, moderate in the basal, central, medial, and cortical nuclei, and mild in the lateral nucleus. Unintended damage in this case amounted to negligible damage to the anterior portion of the left hippocampus (0.58%).

Social Behavioral Observations

Behavioral observations took place in the semi-naturalistic social group compound either shortly after sun rise, continuing until mid-day or late afternoon, continuing until sun down. Observations were continuous, all occurrences focal scans. Data were collected using a palm-based system that records event-sequential data in an actor:behavior:recipient format with an elapsed time since the start of the observations attached to each entry. This method of observation allows for recovery of true frequencies, latencies and durations. A social behavior ethogram targeting 55 social behaviors (Appendix I) was used for observations. Subjects and mothers received four 30-minute observations per subject per week until 3 months of age. At 3 months of age, subjects received three 30-minute focal observations per subject per week until 6 months

Table 2. Percentage of cell loss within amygdala nuclei

	AMY-1				AMY-2			
Nuclei	R%	L%	X%	W%	R%	L%	X%	W%
Lateral	96.9	33.8	65.3	32.7	82.6	27.9	55.3	23.1
Basal	30.2	48.9	39.6	14.8	78.1	55.4	66.8	43.3
AB	51.2	99.1	75.1	50.7	51.6	88.0	69.8	45.4
AAA	0.0	19.9	9.9	0.0	58.5	0.0	29.2	0.0
Central	71.9	87.5	79.7	62.9	65.5	51.9	58.7	34.0
Medial	82.2	89.5	85.4	73.5	9.7	69.0	39.4	6.7
COa	0.0	100	50	0.0	80.7	62.7	71.7	50.6
NLOT	0.0	100	50	0.0	17.5	23.3	20.4	4.1
PAC	0.0	72.9	36.5	0.0	7.1	1.6	4.3	0.1
Den	78.2	95.9	87.1	75.0	10.9	0.0	5.5	0.0
Cop	10.0	43.6	26.8	4.3	0.0	100	50	0.0
PL	5.7	0.0	2.9	0.0	21.7	0.0	10.8	0.0
Post-	67.9	100	84.0	67.9	49.4	59.9	54.6	29.6

Note: Conventions as in Table 1. Abbreviations: AB, accessory basal; AAA, anterior amygdala area; COa, anterior cortical; NLOT, nucleus of the lateral olfactory tract; PAC, periamygdaloid cortex, Den, dorsal endopiriform nucleus; COp, posterior cortical; PL, paralamellar.

of age. From 6 months to 1 year of age, each subject received one 45-minute observation per week. Finally between 1 and 1.5 years of age, each subject received two 45-minute observations a week. Two observers collected all observations between 3 months and 12 months of age, inter-rater reliability to be good at Cohen's kappa [k] 0.78. Only one observer collected all observations between 12 and 20 months of age.

Emotional Reactivity Testing

Social Isolation Test

At approximately 1.2 years of age, the subject was separated from its mother and social group for approximately 28 minutes and placed in a single cage alone in a novel environment to assess their emotional reactivity and neuroendocrine response to novelty. A baseline blood sample was collected within the first 8 minutes of when the separation test began and a second blood sample was taken within 5 minutes after the separation test was completed. During the 28-minute separation, the subjects' behavior was video taped for later analysis using reactivity ethogram (Appendix II). The blood samples were assayed for cortisol and ACTH to examine for differences between treatment groups.

Human Intruder Test

Similar to the social isolation test, at approximately 1.2 years of age, the subject was separated from its mother and social group and place alone in a single cage in a room. However, this test accesses the subject's emotional reactivity and neuroendocrine response to an unfamiliar human intruder. A baseline blood sample was collected within the first 8 minutes of the human intruder test beginning. Following blood sampling, there was a 10 minute acclimation period before the unfamiliar human (researcher wearing a

rubber mask) entered the room, stood two meters from the single cage, and presented his/her side profile to the subject for 10 minutes. At the end of the 10 minutes, the human left the room giving the subject 3 minutes alone in the room after which the unfamiliar human re-entered the room, but this time stared directly at the subject for 10 minutes. The human left the room giving the subject another 10 minutes alone. A second blood sample was taken within 5 minutes after the 43 minute human intruder test was completed. All of the subject's behaviors were video taped during the human intruder test for later analysis using reactivity ethogram (Appendix II). Blood samples were assayed for cortisol and ACTH to examine for difference between treatment groups.

Social Intruder Test

At approximately 1.2 years of age, all four subjects were be separated from their mothers and social group and placed together in a large 10'x20' familiar housing cage. Four novel adult males were placed in single housing cages 3 feet back from the mesh of the large housing cage. This distance was necessary to ensure that the subjects could not gain contact and be injured through the mesh by the adult males. The large housing cage allowed the subjects to explore freely, getting within 3 feet of the novel males or staying back as far as 20 feet from the males. The subjects remained in the presence of the adult males for 30 minutes. The subject's reaction to the males was video taped from multiple angles for later analysis using reactivity ethogram (Appendix II) with added distance codes to calculate approximate distance from the males.

All scoring behavioral data for each emotional testing task (Social Isolation, Human Intruder, and Social Intruder) was conducted by one observer.

<u>Neuroendocrine measures</u>

Blood samples were collected in chilled plastic eppendorf tubes with EDTA (100mg EDTA tetrasodium salt/ml dH_20 per volume of blood). Samples were centrifuged at approximately 3,000 rpm for 15 minutes in a refrigerated centrifuge (at 4ºC). Plasma was pipetted into sterile plastic eppendorfs, immediately placed on dry ice, and maintained at -80ºC until they were assayed. ACTH and cortisol levels in the plasma were assayed with enzyme immunoassay kits and performed by the Yerkes Biomarker Core Laboratory. The ACTH was assayed using the DiaSorin kit (DiaSorin, Inc., Stillwater, MN) with an intra-assay coefficient of variation (CV)% of 2.5%, an interassay CV% of 7.5%, and an assay sensitivity of 10pg/ml. The cortisol was assayed using the DSL kit (Diagnostic Systems Laboratories, Webster, TX) with an intra-assay CV% of 4.9%, interassay CV% of 4.5%, and an assay sensitivity of .5µl/dl.

RESULTS

CHAPTER 1: SOCIAL BEHAVIOR

To investigate the effect of neonatal amygdala lesions on development and expression of social behavior a Multivariate Analysis of Variance was conducted with group (AMY and SHAM) and age (3, 6, 9, 12, and 20 months) as the main variables. A Fisher LSD post-hoc analysis was performed to examine group differences at each age point. Due to the restricted number of animals per group each observation was treated as a sample. To account for the differences in the length of observation at different age points all behavioral results were converted into overall rates of behavior per minute. When homogeneity of variance could not be assumed a log transformation was performed. Means for all behaviors reported in Table 2 are non-transformed means. Finally, covariates were used to ensure that significant group differences on some behaviors would not influence the analysis of other behaviors.

Mother-infant Interactions

As subjects matured they spent progressively less time in full body contact with their mother (Figure 3A). This decline in contact with mother was present in both the sham-operated controls and those with neonatal amygdala lesions, but was more pronounced in the later experimental animals at 9 and 12 months of age [GROUP X AGE: $F(4,250) = 2.45$, $p = .047$, $\eta^2 = .038$ and AMY < SHAM: LSD: $p < .001$, $p = .005$, at 9 and 12 months, respectively).

In addition, infant rhesus monkeys became more independent from their mother (time spent out of proximity to mother) as they matured. This increase in independence from the mother occurred in animals of both groups (Figure 3B), although it was more pronounced in the amygdalectomized males than in the sham-operated controls at 3, 9, 12

Table 3. Mean rates per minute of social behavior

Mean rate per minute ± Standard Errors for each behavior in each behavioral category in sham-operated controls (SHAM) and animals with neonatal amygdala lesions (AMY) at ages 3, 6, 9, 12, and 20 months.

		Age (Months)				
Behavior	Group	3	6	9	12	20
Mother Infant Interactions						
Contact	SHAM	.587±.042	.371±.027	.485±.060**	.317±.037*	.125±.022
	AMY	.488±.056	.385±.029	.267±.044**	.163±.032*	.032±.013
Independence	SHAM	.223±.029*	.439±.026	.371±.054**	.425±.036**	.712±.031**
	AMY	.363±.055*	.479±.031	.632±.045**	.701±.037**	.903±.017**
Groom & Genital Inspect	SHAM	.086±.017**	.058±.010*	.072±.024**	.033±.008	.023±.009
	AMY	.031±.010**	.014±.003*	.014±.004**	.022±.007	.001±.001
Follow & Retrieve	SHAM	.001±$6.8E^{-4}$**	$6.7E^{-5}$±$6.7E^{-5}$	$4.3E^{-5}$±$4.3E^{-5}$	000	000
	AMY	.005±.002**	$8.2E^{-4}$±$7.2E^{-4}$	000	$1.0E^{-4}$±$1.0E^{-4}$	000

Note: LSD post-hoc *p<.05, **p≤.001. T-test †p<.05; Small numbers are expressed in scientific notation $E^{-\#}$

(continued)

		Age (Months)				
Behavior	Group	3	6	9	12	20
Mother Infant Interactions (continued)						
Weaning	SHAM	.003±.002	.021±.005	.038±.010**	.021±.007	.016±.003
	AMY	.001±.001	.016±.004	.015±.004**	.017±.006	.004±.001
Affiliative Behavior with Others						
Contact	SHAM	.003±.001**	.004±.002	.002±.001	.001±.001	.017±.015
	AMY	.071±.020**	.019±.003	.014±.004	.003±.001	.037±.012
Received Groom & Genital	SHAM	.007±.003	.003±.001	.001±.001	.002±.002*	.013±.011**
Inspect	AMY	.012±.003	.007±.002	.001±.001	.054±.033*	.105±.045**
Grooming Express	SHAM	000	$1.6E^{-4}$±$9.8E^{-5}$	$3.9E^{-4}$±$3.4E^{-5}$	.003±.002	.005±.002**
	AMY	000	$2.9E^{-4}$±$2.6E^{-4}$	$2.8E^{-4}$±$2.6E^{-4}$	.003±.001	.025±.009**

Note: LSD post-hoc *p<.05, **p≤.001. T-test †p<.05; Small numbers are expressed in scientific notation $E^{-\#}$

(continued)

		Age (Months)				
Behavior	Group	3	6	9	12	20
Sexual Behavior						
Incorrectly Oriented	SHAM	000	$8.4E^{-4}$±$5.8E^{-4}$*	000*	$8.5E^{-4}$±$8.5E^{-4}$*	.001±$7.0E^{-4}$
Mounts	AMY	.001±.001	.008±.003*	.006±.002*	.007±.003*	.001±$5.9E^{-4}$
Play and Dominance Behaviors						
Total Social Play	SHAM	.054±.011	.215±.030	.253±.051**	.165±.035*	.233±.049*
	AMY	.126±.040	.273±.042	.470±.059**	.333±.066*	.404±.055*
Rough & Tumble Play	SHAM	.016±.004	.072±.011	.084±.024*	.056±.019*	.023±.007
Rate	AMY	.030±.011	.080±.011	.161±.030*	.114±.023*	.050±.011
Rough & Tumble Play	SHAM	.010±.003	.038±.007	.054±.017	.028±.011*	.012±.004
Initiation	AMY	.011±.005	.035±.006	.075±.018	.068±.016*	.034±.008

Note: LSD post-hoc *p<.05, **p≤.001. T-test †p<.05; Small numbers are expressed in scientific notation $E^{-\#}$

(continued)

		Age (Months)				
Behavior	Group	3	6	9	12	20
Play and Dominance Behaviors (continued)						
Dominance Displays	SHAM	000	.003±.001	.007±.003**	.013±.004	.010±.003**
	AMY	.003±.003	.010±.004	.036±.013**	.023±.007	.067±.009**
Aggressive, Fear and Anxious Behaviors						
Aggression Received	SHAM	.009±.004	.009±.002	.008±.003**	.006±.002*	.018±.003**
	AMY	.010±.003	.013±.003	.038±.006**	.020±.005*	.039±.007**
Aggression Expressed in	SHAM	.001±.001	$9.6E^{-4}$±$5.3E^{-4}$	.002±.001	.003±.002	.006±.002†
Proximity to Mother	AMY	.001±.001	.001±$5.9E^{-4}$	.004±.002	.003±.002	.001±.001†
Aggression Expressed	SHAM	.003±.003	.004±.001	.008±.004*	.008±.003*	.029±.006**
while Alone	AMY	.002±.001	.005±.002	.030±.010*	.028±.008*	.062±.009**

Note: LSD post-hoc *p<.05, **p≤.001. T-test †p<.05; Small numbers are expressed in scientific notation $E^{-\#}$

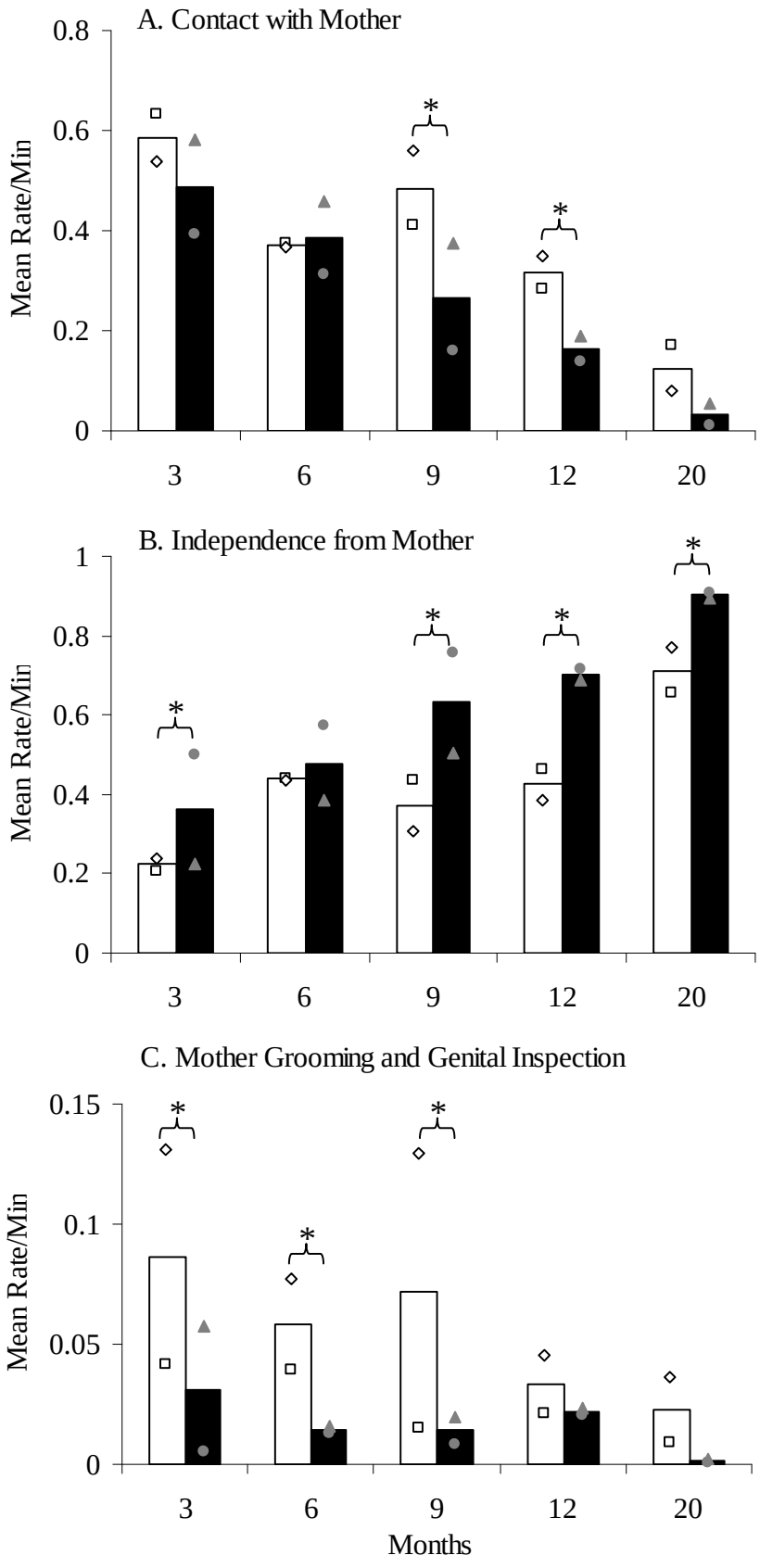
(continued)

		Age (Months)				
Behavior	Group	3	6	9	12	20
Aggressive, Fear and Anxious Behaviors (continued)						
Fear Expressed	SHAM	.002±.001	.018±.004	.040±.013	.035±.008	.168±.015*
	AMY	.003±.001	.019±.005	.052±.009	.052±.012	.137±.011*
Anxiety Expressed	SHAM	.062±.009	.070±.008	.048±.010	.096±.013*	.153±.015
	AMY	.052±.009	.064±.008	.080±.017	.143±.017*	.156±.017

Note: LSD post-hoc *p<.05, **p≤.001. T-test †p<.05; Small numbers are expressed in scientific notation $E^{-\#}$

Figure 3: Mother-infant interactions

Scores are averaged mean rate per minute for body contact with their mother (A), away from their mother (B), and mother grooming and genital inspection of their infant (C), for sham-operated controls (white bar) and animals with neonatal amygdala lesions (black bar) at 3, 6, 9, 12, and 20 months. Scores for each individual in each group are illustrated by an open square and open diamond for those infants receiving sham-operations and by a grey circles and grey triangles for those infants receiving neonatal amygdala lesions. * indicates $p<.05$.


A. Contact with Mother
Mean Rate/Min
0.8
0.6
0.4
0.2
0
3
6
9
12
20
B. Independence from Mother
Mean Rate/Min
1
0.8
0.6
0.4
0.2
0
3
6
9
12
20
C. Mother Grooming and Genital Inspection
Mean Rate/Min
0.15
0.1
0.05
0
3
6
9
12
20
Months

and 20 months of age [GROUP X AGE: $F(4,250) = 3.23$, $p = .013$, $\eta^2 = .049$ and AMY > SHAM: LSD: $p = .010$, $p < .001$, $p < .001$, $p < .001$, at 3, 9, 12 and 20 months, respectively).

As a corollary, rhesus monkey mothers tended to progressively spend less time grooming and genital inspecting their male offspring as they matured (Figure 3C, AGE: $F(4,250)=4.86$, $p=.001$, $\eta^2=.072$). This maturational change was clearly evident for the mother of the sham-operated controls but less so for the mothers of amygdala-operated males, even when controlling for the total time mothers spent in contact and proximity with their infants. However, this group difference reached significance only for the three youngest ages (GROUP: $F(1,250)=28.98$, $p<.001$, $\eta^2=.104$; LSD: $p<.001$, $p=.003$, $p<.001$, for 3, 6, and 9 months, respectively).

When infant rhesus monkeys are very young the mothers maintain contact with them by restraining and following them and protect them from potential harm from other higher ranking animals. As shown in Table 3, this occurred in mothers of normally developing sham-operated controls that spent more time restraining and following their infants at 3 months of age as compared to any other age [AGE: $F(4,250)=4.86$, $p=.001$, $\eta^2=.072$]. Interestingly, mothers of amygdala-operated infants spent even more time (5 times more) restraining and following their infants at 3 months of age than mothers of sham-operated controls, [GROUP X AGE: $F(4,250) = 4.51$, $p = .002$, $\eta^2 = .067$, AMY>SHAM: LSD: $p < .001$ at 3 months of age).

As shown in Table 2, weaning behaviors (rejecting and punishing) in mothers of sham operated controls peaked at 9 months of age, although this peak was much less in magnitude in mothers of amygdala-operated males. The group difference emerged even

when the amount of time spent with mother was controlled for [GROUP: $F(1,250)=11.54$, $p=.001$, $\eta^2=.044$, AGE: $F(4,250)=6.82$, $p<.001$, $\eta^2=.099$, AMY < SHAM: LSD: $p=.001$ at 9 months of age].

Affiliative Behaviors with Non-mother individuals

Because infant monkeys spend most of their time with their mother at least until the juvenile period, the time spent interacting with other members of the group is negligible in infancy but increases in the juvenile period. This progressive increase of interest with other members of the group was evident in the sham-operated control males, but not in the amygdala-operated males.

First, as compared to sham-operated controls that spent very little time in full body contact with non-mother females even until 20 months of age (Figure 4A), animals with amygdala lesions displayed higher frequency of body contacts with non-mother females, especially at the youngest age of 3 months [GROUP X AGE: $F(4,250) = 4.24$, $p = .002$, $\eta^2 = .064$, AMY > SHAM: LSD: $p < .001$ at 3 months of age].

Second, at older ages (i.e. 12 and 20 months), when in contact with non-mother females, animals with amygdala lesions received more grooming and genital inspections by these non-mother females [Figure 4B; GROUP X AGE: $F(4,250) = 4.24$, $p = .002$, $\eta^2 = .064$, AMY > SHAM: LSD: $p = .042$, $p < .001$, at 12 and 20 months respectively]. Finally, at 20 months of age (Figure 4C), animals with amygdala lesions also displayed more grooming toward other non-mother members of the group as compared to sham-operated controls [GROUP X AGE: $F(4,250) = 4.65$, $p = .001$, $\eta^2 = .070$, AMY > SHAM: LSD: $p < .001$, 20 months of age].

Play and Dominance Behaviors

Social contact play, such as brief, chase, and rough-and-tumble, is critical for normal social development in rhesus monkeys (Hinde & Spencer-Booth, 1967). As shown in Figure 5A, neonatal amygdala lesions impacted importantly the amount of social plays displayed by the animals. Thus, social play in sham-operated control males sharply increased at 6 months of age but then remained stable until 20 months of age. Males with neonatal amygdala lesions exhibited similar increase in social play at 6 months, but almost doubled the number of social plays as compared to operated controls thereafter [GROUP: $F(1,250) = 22.09$, $p < .001$, $\eta^2 = .081$, AGE: $F(4,250) = 10.01$, $p < .001$, $\eta^2 = .138$, AMY > SHAM: LSD: p = .001, p = .011, p = .010, at 9, 12, and 20 months, respectively].

A similar change was seen for rough-and-tumble play and dominance displays (Figure 5B & 5C). Sham-operated controls exhibited an increase in rough-and-tumble play behavior that peaked around 9 months of age and, then, steadily declined until 20 months of age. Although a similar pattern of changes was observed in Group AMY, their rate of rough-and-tumble play surpassed that of sham-operated controls at the ages of 9 months and 12 months [GROUP: $F(1,250) = 11.56$, $p = .001$, $\eta^2 = .044$, AGE: $F(4,250) = 10.89$, $p < .001$, $\eta^2 = .148$, AMY > SHAM: LSD: p = .002, p = .017, respectively]. These group differences were also observed in the frequency of initiation of rough-and-tumble play [Table 2; GROUP: $F(1,250) = 5.57$, $p = .019$, $\eta^2 = .022$, AGE: $F(4,250) = 7.46$, $p < .001$, $\eta^2 = .107$, AMY > SHAM: LSD p = .010, p = .040, at 12 and 20 months, respectively]. Finally, the normally developing operated control males rarely exhibited

Figure 4: Affiliative behaviors with non-mothers

Scores are averaged mean rate per minute for contact with non-mothers (A), non-mother grooming and genital inspection of the infant (B), and groom of non-mothers by the infants (C) for sham-operated controls (white bar) and animals with neonatal amygdala lesions (black bar) at the ages of 3, 6, 9, 12, and 20 months. Scores for each individual in each group are illustrated by an open square and open diamond for those infants receiving sham-operations and by a grey circles and grey triangles for those infants receiving neonatal amygdala lesions. * indicates $p<.05$.

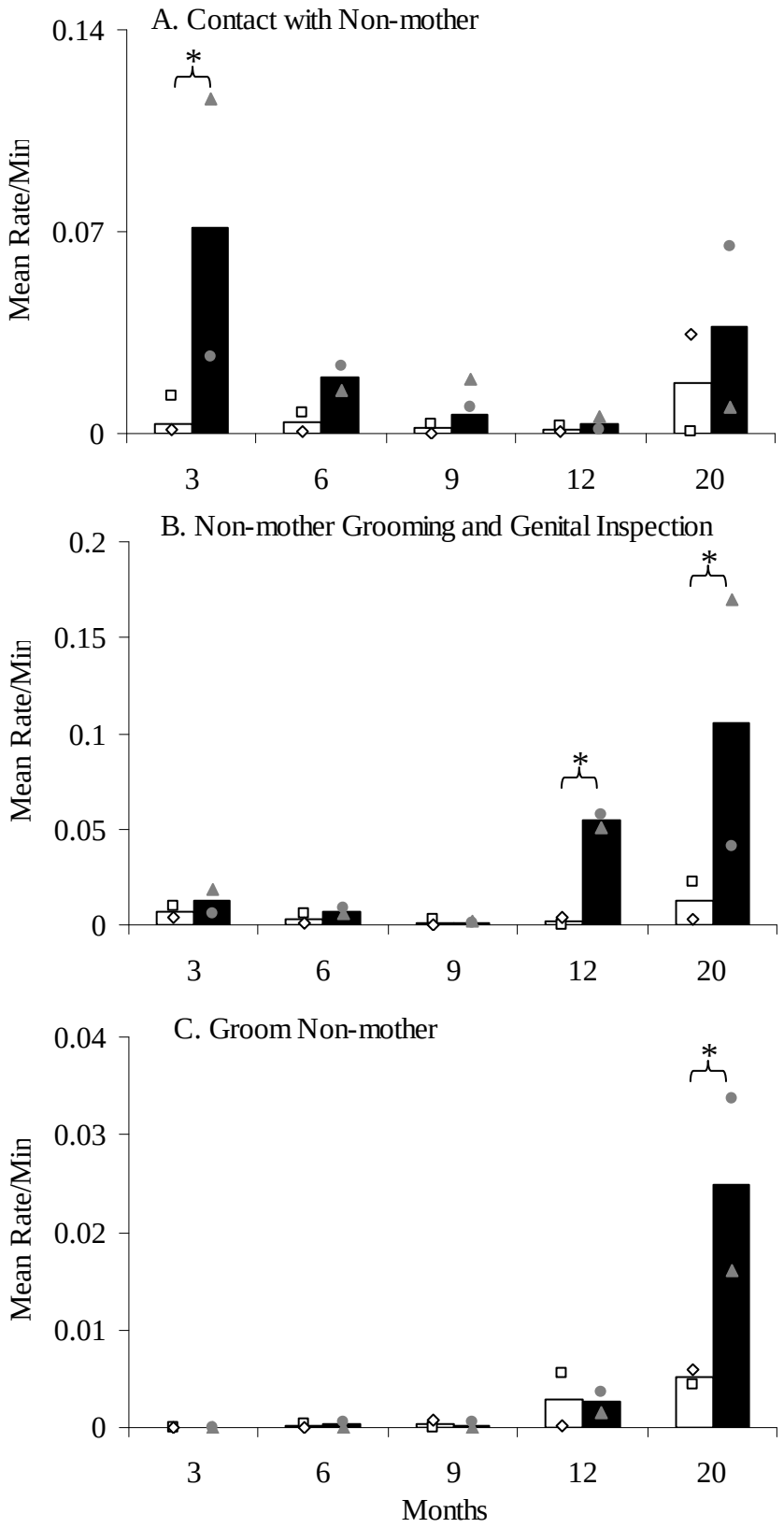
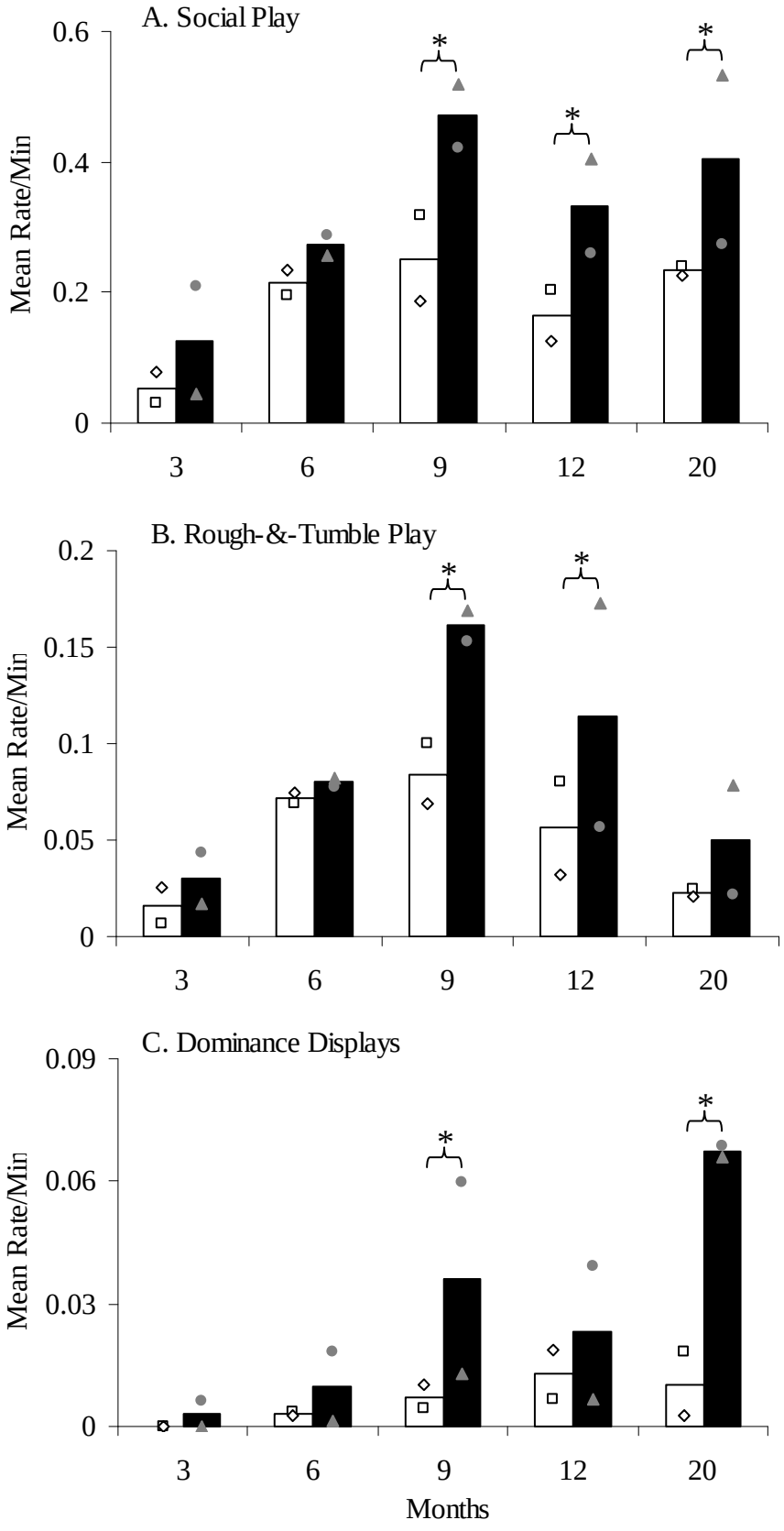

A. Contact with Non-mother
Mean Rate/Min
0.14
0.07
0
*
3
6
9
12
20
B. Non-mother Grooming and Genital Inspection
Mean Rate/Min
0.2
0.15
0.1
0.05
0
*
*
3
6
9
12
20
C. Groom Non-mother
Mean Rate/Min
0.04
0.03
0.02
0.01
0
*
3
6
9
12
20
Months

Figure 5: Play and dominance display behavior

Scores are averaged mean rate per minutes for social plays (A), rough-and-tumble plays (B), and dominance displays (C) for sham-operated controls (white bar) and animals with neonatal amygdala lesions (black bar) at the ages of 3, 6, 9, 12, and 20 months. Scores for each individual in each group are illustrated by an open square and open diamond for those infants receiving sham-operations and by a grey circles and grey triangles for those infants receiving neonatal amygdala lesions. * indicates $p<.05$.


A. Social Play
Mean Rate/Min
0.6
0.4
0.2
0
3
6
9
12
20
*
*
*
B. Rough-&-Tumble Play
Mean Rate/Min
0.2
0.15
0.1
0.05
0
3
6
9
12
20
*
*
C. Dominance Displays
Mean Rate/Min
0.09
0.06
0.03
0
3
6
9
12
20
Months
*
*

dominance displays at the youngest ages of 3 and 6 months and maintained only low but stable rate of this behavior at 9, 12, and 20 months of age. By contrast, animals with neonatal amygdala lesions expressed more dominance displays throughout development. This increase in dominance displays in Group AMY was significant only at 9 and 20 months of age [GROUP X AGE: $F(4,250) = 7.06$, $p = .012$, $\eta^2 = .101$, AMY > SHAM: LSD: $p < .001$, $p < .001$, respectively].

Sexual Behaviors

The typical species-specific mount for rhesus monkeys consists of the mounting partner placing their hands on the hips and foot clasping the calf of the other partner. Thus, any mounts that are not of this orientation are considered to be incorrectly oriented mounts (non-foot clasp and mounting a body part other than hindquarters). Typically developing, sham-operated controls, exhibited very few incorrectly oriented mounts as they mature (Table 2). By contrast, neonatally amygdalectomized males exhibited many more incorrectly oriented mounts as early as 6 months of age and also at 9 and 12 months of age [GROUP: $F(1,250) = 14.88$, $p < .001$, $\eta^2 = .056$, AMY > SHAM: LSD: $p = .003$, $p = .009$, $p = .009$, at 6, 9, and 12 months, respectively].

Aggression, Fear, and Anxiety Behaviors

In macaque monkeys the mothers' rank and social support play an important influence on how non-mother individuals direct aggression on an infant or juvenile (Hinde & Spencer-Booth, 1967). Since all of the subjects in this study were from middle ranked mothers the amount of aggression received should be relatively equal across the

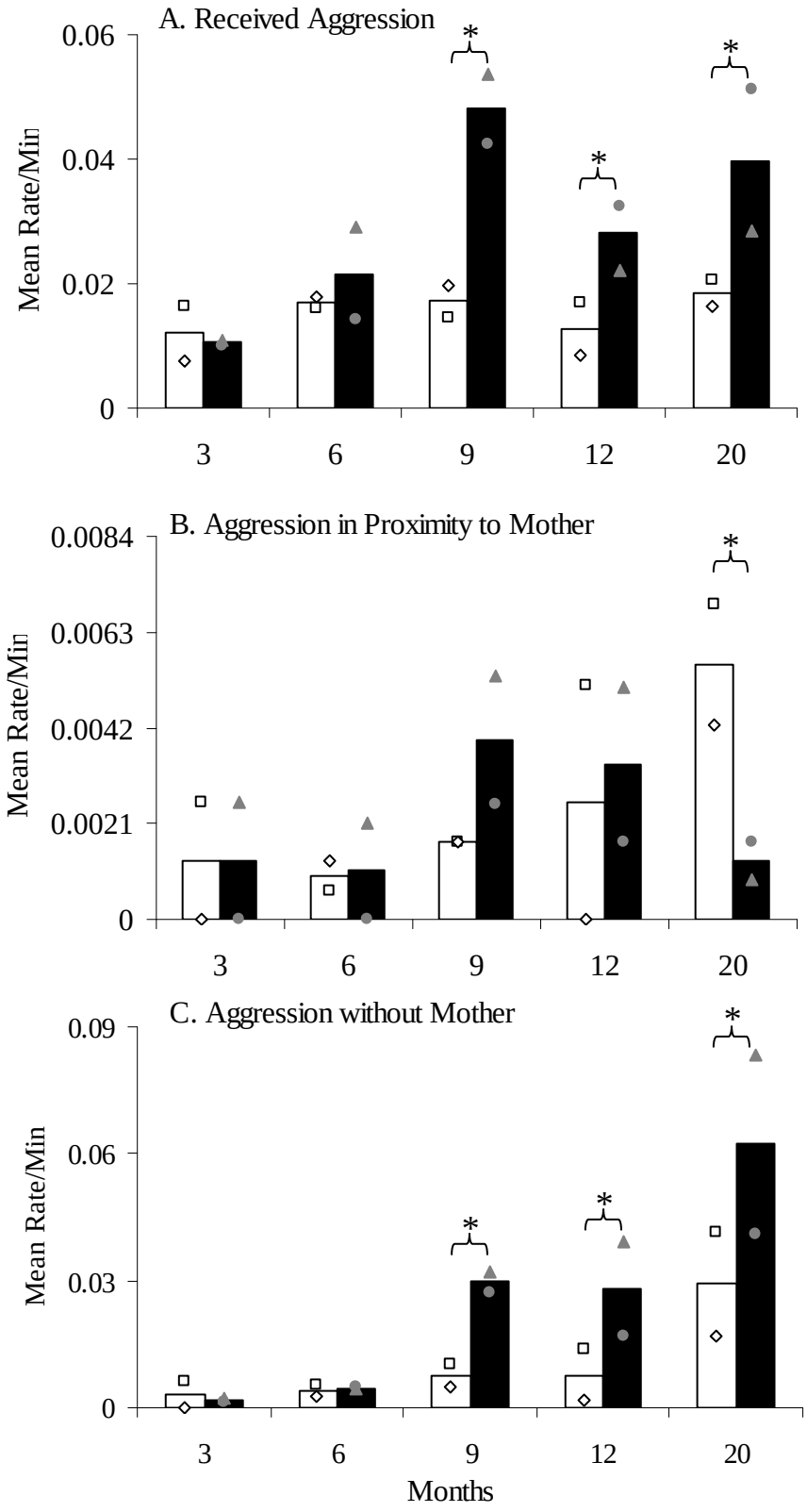
two groups. Figure 6A illustrates that throughout development sham-operated control males received a relatively low, but steady, rate of aggression (threat, hit, slap, bite, grab, and chase). Yet, males with neonatal amygdala lesions received significantly more aggression starting at the older ages of 9, 12, and 20 months [GROUP X AGE: $F(4,250) = 3.95$, $p = .004$, $\eta^2 = .059$, AMY > SHAM: LSD: $p < .001$, $p = .017$, $p = .001$, respectively].

The mothers' rank and social support also play a key role on infant's expression of aggressive behaviors (Imanishi, 1963; Hinde & Spencer-Booth, 1967). Therefore, the mother's proximity to the infant could influence how much aggression the infant expresses. As show in Figure 6B, sham-operated control males demonstrated a progressive increase in aggression toward others when in proximity to their mother, whereas males with neonatal amygdala lesions, although not different from controls at any ages, showed an increase in aggression that occurred at a younger age (9 months) as compared to controls (20 months) followed by a steep decline in this behavior at 20 months of age. Further exploration of this group difference at 20 months of age using an Independent T-test indicated that Group SHAM expressed significantly more aggression while in proximity to their mothers as compared to Group AMY [$t(1,50) = 2.40$, $p = .021$, $d = .664$].

Just as mothers rank, social support, and proximity may influence infant's aggressive behavior toward others; one might anticipate that the absence of mother proximity may also influence the infant's willingness to express aggression. As shown in Figure 6C, normally developing sham-operated control males progressively expressed more aggression without mother being in proximity as they mature, whereas those with

Figure 6: Aggressive behaviors

Scores are averaged mean rate per minutes for aggression received from other animals (A), aggression while in proximity to their mothers (B), and aggression toward others when mother is away (C) for sham-operated controls (white bar) and animals with neonatal amygdala lesions (black bar) at the ages of 3, 6, 9, 12, and 20 months. Scores for each individual in each group are illustrated by an open square and open diamond for those infants receiving sham-operations and by a grey circles and grey triangles for those infants receiving neonatal amygdala lesions. * indicates $p<.05$.


A. Received Aggression
Mean Rate/Min
0.06
0.04
0.02
0
3
6
9
12
20
*
*
*
B. Aggression in Proximity to Mother
Mean Rate/Min
0.0084
0.0063
0.0042
0.0021
0
3
6
9
12
20
*
C. Aggression without Mother
Mean Rate/Min
0.09
0.06
0.03
0
3
6
9
12
20
Months
*
*
*

amygdala lesions exhibited the same progressive increase but at a much higher at 9, 12, and 20 months of age [GROUP X AGE: $F(4,250) = 3.55$, $p = .008$, $\eta^2 = .054$, AMY > SHAM: LSD: p = .006, p = .010, p < .001, respectively].

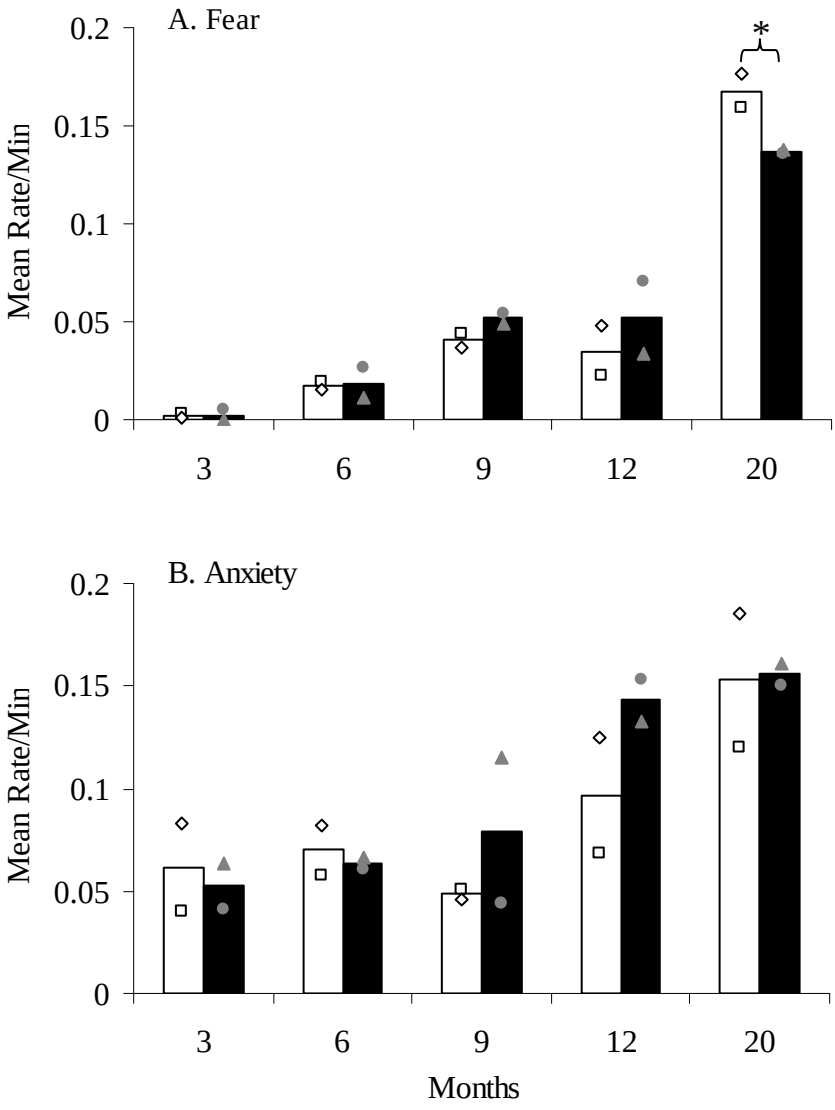
Interestingly, there were almost no changes in the rate of fearful displays (fear grimace and withdrawal) and anxiety behaviors (Figures 7A & 7B) after neonatal lesions. Both groups displayed a slight but significant increase as the animals mature [AGE: $F(1,250)=79.82$, $p<.001$, $\eta^2=.561$, and $F(1,250)=22.42$, $p<.001$, $\eta^2=.264$, for fearful and anxiety behaviors, respectively]. Although the group differences and the interaction between Group and Age did not reach significance in both behaviors, there was a slight, but significant, decrease in fearful behaviors at 20 months of age and a slight increase in anxiety behaviors at 12 months of age in Group AMY as compared to Group SHAM [AMY < SHAM: LSD: p = .019; AMY > SHAM: LSD: p=.010, respectively].

Discussion

Overall, the results indicate that neonatal amygdala lesions impact the expression of behaviors from infancy until early adolescence. The earliest change was the increased independence from the mothers that occurred at a much earlier age in the amygdalectomized males than in the sham-operated control males. Reciprocally, this early infant independence from the mother influenced the mother behaviors towards their infants. Thus, mothers of infants with amygdala lesions spend less time grooming their infants and more time restraining and following their infants. During maturation these changes in mother-infant interactions yielded fewer rejections and punishments expressed by the mothers toward their infants. Furthermore, although animals with neonatal

Figure 7: Fear and Anxiety behaviors

Scores are averaged mean rate per minutes for fearful displays (A) and anxiety behaviors (B) for sham-operated controls (white bar) and animals with neonatal amygdala lesions (black bar) at the ages of 3, 6, 9, 12, and 20 months. Scores for each individual in each group are illustrated by an open square and open diamond for those infants receiving sham-operations and by a grey circles and grey triangles for those infants receiving neonatal amygdala lesions. * indicates $p<.05$.


A. Fear
Mean Rate/Min
0.2
0.15
0.1
0.05
0
*
3
6
9
12
20
B. Anxiety
Mean Rate/Min
0.2
0.15
0.1
0.05
0
3
6
9
12
20
Months

amygdala lesions normally acquired species typical affiliative behaviors, they exhibited greater affiliative behaviors as well as more dominance displays than sham-operated controls.

The neonatal amygdala lesions also altered the levels of aggression, especially when the mothers were not in proximity of their infants, even though the infants were from middle-ranked mothers. This increased aggression, which occurred when the animals reached 9 months of age, was associated with only a slight decrease in fear behaviors. Finally, the neonatal amygdala lesions also negatively impacted the expression and/or learning of sexual behaviors.

Thus, the data suggest that neonatal amygdala lesions did not create socially withdrawn animals, instead they resulted in animals displaying more independence and hypersocial behavior. The lesions also appeared to disrupt the processing of potentially dangerous social situations, leading to an animal with more aggressivity in social context. Overall, our results not only confirm and extent, but also contrast with, those of earlier reports of behavioral changes after neonatal amygdala lesions in monkeys (Bachevalier, 1994; Bauman, et al 2004; Thompson, Schwartzbaum, & Harlow, 1969). The parallels and divergences in the findings will be discussed in turn.

Mother-infant interactions:

Unlike previous studies reporting few behavioral changes in the earliest stages of development after neonatal amygdalectomy in monkeys (Kling & Green, 1967; Prather, et al., 2001), our study demonstrated an early emergence of independent behaviors from the males with neonatal amygdala lesions. This independent behavior was expressed by

less time spent with the mother and more time spent in contact with non-mother females at 3 months of age as compared to sham-operated males. This increased independence observed in animals with neonatal amygdala lesions is not necessarily abnormal but rather the timing of its emergence is. Typically, rhesus infants rely heavily on their mother for nourishment and protection during the earliest developmental stages, and it is not until around 6 months that infants begin to develop independence from their mother (Hansen, 1966; Hinde & Spencer-Booth, 1967). Thus, the early emergence of independent behaviors after neonatal amygdala lesions is a new finding that has never been reported before. In fact, this finding is in stark contrast to the findings from Bauman and colleagues (2004a) who reported that infants with amygdala lesions spent significantly more time in contact with their mothers compared to control infants. This observation held true for dyadic, tetradic, and small social group settings. One possible explanation for this difference between the current study and Bauman and colleagues (2004a) is that physical and social environment stability has been shown to be important for the emergence of infant independence from mother (Rosenblum, 1974; Rosenblum & Andrews, 1994). The current study had a very stable physical and social environment due to the long established troop in which the animals navigated. In contrast, Bauman and colleagues (2004a) utilized more restricted social environments in which infants first lived in cages housed with their mothers the majority of time but were placed in small groups with 6 other mother-infants pairs for 5 hours each day. The transitioning from pair-housing to social group could have contributed to the increased contact with and decreased independence from mother. Alternatively, the difference between studies could reflect differences in physical environment size. The current study utilized a large

outdoor compound of approximately 720 meters squared, whereas animals in the Bauman study were confined to 7 squared meters. The smaller enclosure gave mothers a more confined space to easily keep up with and restrain their infants as compared to the larger outdoor enclosure.

Interestingly, the increased independence from mother noticeably impacted on the normal behavior of the mothers towards their infants. As a result, mother of infants with neonatal amygdala lesions spent more time restraining and following their infants and thus less time grooming them. These changes occurred even when the data were corrected according to the total time the infants spent with their mothers. Finally, this increased independence did not only impact interactions with the mothers but also interactions with other members of the social group. Increased independence from the mothers also gave amygdalectomized males increased opportunity to spend time in contact with non-mother females.

Social interactions with other members of the social group:

Similar to previous studies, the present study also highlighted the more pronounced behavioral and social changes that emerged as subjects aged (Bachevalier, 1994; Bauman, et al., 2004; Thompson & Towfighi, 1976; Thompson, Berland, & Towfighi, 1977). With further maturation, animals with neonatal amygdala lesions began to produce increases in social play, dominance displays, and aggressive behaviors compared to operated controls. Again, animals with neonatal amygdala lesions were able to acquire a normal repertoire of social behaviors, including play, dominance, mounting, and aggression. Yet, it was the rate at which amygdalectomized animals expressed these

behaviors that defined the difference between them and sham-operated controls.

The different rate of behavioral expression from the amygdalectomized males also impacted drastically the way other animals interacted with them. Thus, the amygdalectomized males were both expressing more aggression as well as receiving more aggression from others. However, it is difficult to ascertain at the present time which animals initiated the aggressive behaviors. Future studies utilizing Lag Sequential Analysis will be more appropriate to examine this question in detail. Additionally, this finding contrasts with previous research indicating that neonatal amygdala lesions produced less aggressive behaviors compared to controls, and no differences in the amount of aggression received (Bauman, et al. 2006). Again differences in social and physical environment as discussed above may have played a critical role in the divergent behavior outcomes between the present study and the Bauman study.

Fear and anxious behaviors:

Unlike previous studies indicating that neonatal amygdala lesions produced increased fear behaviors and decreased affiliation with peers (Bachevalier, 1994; Bauman, et al., 2004b, 2006; Prather, et al., 2001; Thompson, Schwartzbaum, & Harlow, 1969). The present results indicated a much different pattern of fear-related changes. Thus, amygdalectomized males exhibited higher levels of affiliation with non-relatives and reduced fear behaviors compared to sham-operated controls. These changes were more prominent as the animals reached 20 months of age and are consistent with previous studies examining the effects of amygdala lesions acquired in adulthood. These later studies reported a reduction in fear after complete and selective removal of the amygdala

(Emery, et al., 2001; Izquierdo & Murray, 2004; Kalin, et al, 2004; Machado & Bachevalier, 2006, 2008; Machado, et al., 2008; Meunier, et al., 1999). Presumably, this reduction in fear behaviors could also account for the premature independence and the increase in social behaviors reported after amygdala lesions acquired infancy.

Sexual behaviors:

The present study revealed changes in sexual behavior that are also similar to those found in large temporal lobe lesions of adult monkeys (Klüver & Bucy, 1939). Males with neonatal amygdala lesions produced more incorrectly oriented mounts (non-foot clasp and mounting a body part other than hindquarters) as compared to sham-operated controls. Typically, sexual mounting behavior first emerges between 3 and 6 months of age, and increases steadily in frequency through the first year of life in rhesus monkeys (Hinde & Spencer-Booth, 1967). In the current study, the emergence of mounting behaviors in animals with neonatal amygdala lesions was normally timed (6 months of age), yet it was the frequency and style of mounting behaviors that were atypical. The increased frequency of incorrectly oriented mounting is a new finding that has never been reported after neonatal amygdala lesions in monkeys.

Factors influencing the different behavioral outcomes found among studies:

Several factors could account for the slight but important differences in the long-term behavioral outcomes of neonatal amygdala lesions in non-human primates. One is the social environment into which the infant monkeys navigate. Previous studies have used limited social environments ranging from single cages (Kling & Green, 1967), pair

housed (Bachevalier, 1994), to artificially created small social groups (Bauman et al, 2004, 2006). Rhesus monkeys naturally live in social troops, consisting of numerous animals of both sexes, ranging from all stages of development (DeVore, 1965; Kaufmann, 1966; Hinde & Spencer-Booth, 1967). The present study utilized an established social group of 174 animals arranged in 12 matrilines, which made the environment richer, more socially complex, and closer to the natural troop environment. Since a complex social environment is essential for normal behavioral development in monkeys (Berman, 1980), one might postulate that it could provide a more buffered environment for social behavior development even in animals with amygdala lesions. Thus, the increased affiliation and decreased fear in infants with neonatal lesions when normally raised in a complex social group attests to the impact of environment on development and expression of social behavior.

Another important factor to consider for the different behavioral outcomes observed across studies is the extent of the amygdala damage. Obviously, all previous studies using aspiration lesion techniques (Bachevalier, 1994; Kling & Green, 1967; Thompson, Schwartzbaum, & Harlow, 1969) damaged not only neurons within the amygdala nuclei but also fibers passing through and around the amygdala as well as cortical areas adjacent to the amygdala. This additional damage is likely to impact the severity, and perhaps even, the direction of the behavioral changes observed. These different outcomes of amygdala lesions according to the amount of tissue damage have already been stressed in studies of amygdala lesions acquired in adulthood (Meunier et al, 1999, 2002). Meunier and colleagues (1999) found that, whereas aspiration and neurotoxic lesions of the amygdala produce similar behavioral changes, the changes were

more pronounced after aspiration lesions, presumably due to damage to surrounding cortical areas (i.e. entorhinal, perirhinal, and area TE) and fibers passing near and through the amygdala. A subsequent study aimed at investigating how damage to rhinal cortices might contribute to behavioral changes found after aspiration lesions of the amygdala, Meunier and colleagues (2002) demonstrated that rhinal lesions yielded emotional changes that were in fact opposite to those described after either aspiration or neurotoxic lesions of the amygdala. Thus, the exaggerated symptoms found after aspiration lesions cannot be a direct additive effect of rhinal cortical damage, but instead reflect a direct and different contribution of the rhinal cortices to emotional regulation. For example, monkeys with rhinal lesions appeared to overestimate potential danger, thus displaying increased emotional reactivity compared to monkeys with amygdala lesions which appeared to underestimate potential danger and thus displaying less emotional reactivity (Meunier, Cirilli, & Bachevalier, 2006). Although the difference in lesion techniques could account for the behavioral outcomes in some of the previous developmental studies, this procedural difference cannot explain the different behavioral outcomes found between the present study and that of Bauman and colleagues (2004a, 2004b, 2006), which, as the present study, used selective neurotoxic lesions of the amygdala. However, it is interesting to note that despite the use of neurotoxic lesion techniques, the amygdala lesions in the Bauman and colleagues' studies yielded much more extended damage than that reported in the present study. For example, examining the FLAIR MR images in the previous studies (Bauman et al 2004a, 2004b, see Figures 2, 1, respectively) revealed the presence of edema well beyond the confine of the amygdala nuclei. Indeed, this edema extended to the entorhinal, area TE, area 35 of perirhinal cortex, superior temporal

sulcus, and anterior hippocampus. Thus, as compared to the selective amygdala lesions reported in the present study (see Figure 1 & 2), it is likely that the additional damage to fibers in passage and adjacent cortical areas could explain the increase in fear behaviors reported by Bauman and colleagues (2004a, 2004b). In fact, the behavioral differences obtained between our study and those of Bauman parallel the behavioral differences found in adult monkeys with selective versus more extended amygdala lesions (see above).

Relationships with studies of early amygdala damage in other species:

Neonatal neurotoxic amygdala lesions in rodents reveal significant changes in social behavior compared to controls (Wolterink, et al, 2001; Daenen, Wolterink, Gerrits, & van Ree, 2002; Diergaarde, Gerrits, Stuy, Spruijt, & van Ree, 2004). In comparison to controls, rats that received neurotoxic amygdala lesion at postnatal day 7 (PD 7) exhibited decreased social play and social behaviors (Wolterink, et al, 2001; Daenen, et al, 2002; Diergaarde, et al, 2004). Decreased social contact, such as anogenital investigation and approach/following, was only exhibited by rats that sustained damage to the medial amygdala nuclei on PD7 (Daenen, et al 2002). Additionally, neonatal amygdala damage in rodents leads to decreased investigation in an open-field test (Wolterink, et al, 2001). These results in rodents showing decreased social play, contact, and investigation, contrast with the present study that demonstrated increased social play and contact behaviors. Perhaps the differing results reflect a species difference, at postnatal day 7 rat pups eyes and ears are still closed whereas rhesus monkeys have their eyes and ears open at birth. Additionally, at postnatal day 7 rats are still undergoing

significant brain development such as cell migration and connections between the amygdala and prefrontal cortex (Verwer, Van Vulpen, & Van Uum, 1996). Therefore, the equivalent to a postnatal day 7 rodent lesion in a rhesus monkey would be performed at an embryonic stage, prenatally.

Research examining the effects of amygdala damage in human patients has also revealed significant changes in social and emotional behavior (Adolphs, 2001; Adolphs, Tranel, & Damasio, 1998; Adolphs, et al, 2005; Tranel, Gullickson, Koch, & Adolphs, 2006). One such patient is SM, a woman with a rare disease Urbach-Weithe known to result in mineralization and atrophy of the medial temporal lobe tissue. SM is the purest case of bilateral amygdala destruction without significant involvement of any other neural structures (Tranel, et al, 2006). Examination of SM has revealed impairment in her ability to recognize emotions from facial expressions, which is due to her failure to look normally at the eye region (Adolphs, et al, 2005). The recognition of fear facial expression in humans relies heavily on the eyes being the most important feature for identifying this emotion, which explains why SM has difficulty identifying fear more than other emotional expressions (Adolphs & Tranel, 2004; Adolphs, et al, 2005). Yet, if SM is prompted to explicitly focus on the eyes then her identification of emotional expression becomes entirely normal (Adolphs, et al 2005). In addition with impaired ability to recognize facial expressions, SM is also impaired in her ability to evaluate traits of “approachability” and “trustworthiness” in the faces of strangers in laboratory tasks (Adolphs, Tranel, & Damasio, 1998). Lastly, even though SM has a normal range of social skills, she is almost completely devoid of negative affect, particularly fear and anger (Tranel, et al, 2006).

The current results have many parallels with those of SM and other bilateral amygdala damaged patients (Young, Hellawell, Van de Wal, & Johnson, 1996; Broks, et al, 1998). Neonatal amygdala-operated animals spent more time independent of their mother and in contact with non-mother females as early as 3 months of age, which parallels SM's tendency to classify strangers as trustworthy as compared to human controls. Both amygdala-operated animals and SM developed a normal repertoire of social behaviors across their life. However, both amygdala-operated animals and SM appear to have a deficit in the expression of certain emotions. Amygdala-operated animals expressed fewer fear gestures as compared to sham-operated controls, which is an emotion that SM appears to be lacking. Unlike SM's inability to express anger, the amygdala-operated monkeys easily expressed aggressive behaviors. Although there are many similarities between human patients with bilateral amygdala damage and results of the present study, there are still some differences. Future studies should focus on the areas of fear and aggression (anger) in an attempt to parse out the differences between the human patients and the animal model.

CHAPTER 2: BEHAVIORAL AND NEUROENDOCRINE RESPONSES TO STRESS

As described in Chapter 1, the impact of early amygdala lesions on fear-related behaviors emerged around 20 months of age and resulted in a slight, but significant, reduction in these behaviors. To examine whether these changes could be even more prominent when the animals are placed in more stressful situations and are associated with changes in neuroendocrine responses to stress, animals in Groups AMY and SHAM were subjected to additional tests: The Social Isolation Test, Human Intruder, and Social Intruder. In the Social Isolation Test, animals were simply removed from their group and placed alone in a novel room. In the Human Intruder test, they were separated from their group and were faced with an unfamiliar human presenting his/her profile or starting at them, thus producing two different levels of threat. Lastly, in the Social Intruder Test, animals were separated from their group and given restricted access to four novel male conspecifics in the familiar environment. In addition, during the first two tests, blood samples were collected prior and after the tests and used to measure levels of adrenocorticotrophin hormone (ACTH) and cortisol.

Social Isolation Test

Behavioral Reactivity

Emotional response were analyzed separately for the first half (0-14 minutes) versus the last half (14-28 minutes) of the test. Due to the limited number of subjects a non-parametric Two-Way Friedman test was used to compare groups (AMY vs SHAM) and periods of testing (beginning vs end of the test). Follow up comparisons could not be performed given that Mann-Whitney U test need group sizes of three or larger.

The amount of cage exploration exhibited by animals of both groups were higher at the beginning than at the end of the Separation test [Figure 8A; [$\chi^2(2, N = 4) = 8.00$, *p*

= .018, and the Kendall coefficient of concordance of 1.0 indicates a strong difference among GROUP and PERIOD factors]. Examination of the group means at the beginning of the test indicates a trend for Group AMY (M = 120.39, SD = 50.43) to exhibit less cage exploration as compared to Group SHAM (M = 155.22, SD = 77.22).

Sham-operated controls expressed more hostile behaviors (threat and cage shake aggression) at the beginning of the test compared to the end (Figure 8B). However, animals with amygdala lesions expressed low and steady amounts of hostile behaviors throughout the test [$\chi^2(2, N = 4) = 5.73$, $p = .057$, yet the Kendall coefficient of concordance of .72 indicates a strong effect]. Additionally, examination of the group means reveal a trend for Group AMY (M = 11.00, SD = 14.14) to exhibit less hostility at the beginning of the test compared to Group SHAM (M = 25.50, SD = 19.09).

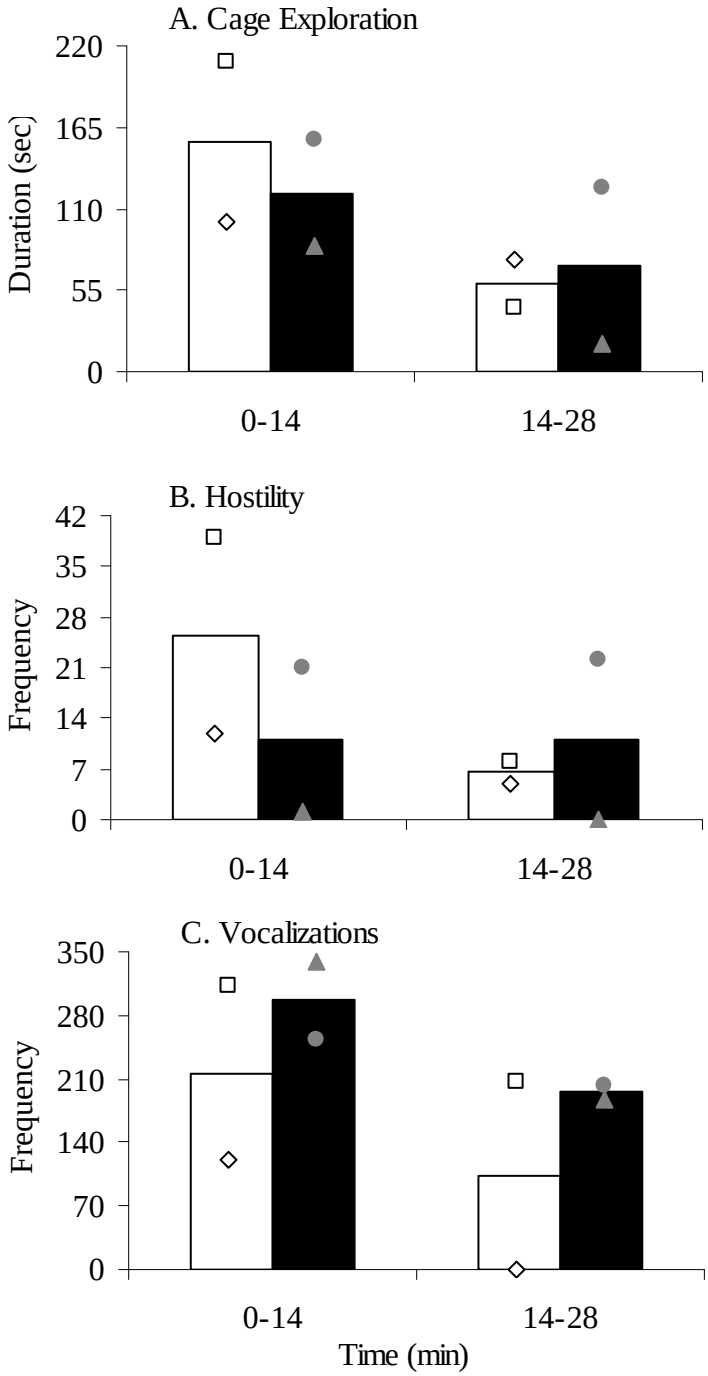
Operated control males produced more vocalizations (coo, threat bark, and other vocalizations) at the beginning of the test than at the end. Amygdalectomized males also exhibited the same pattern of vocalizations [Figure 8C: $\chi^2(2, N = 4) = 6.50$, $p = .039$, and the Kendall coefficient of concordance of .81 indicates a strong difference between the GROUP and PERIOD factors]. Yet, examinations of the means revealed that animals of Group AMY vocalized more than Group SHAM throughout the test (M = 296.50, SD = 61.52, and M = 216.50, SD = 136.47, for start and end of the test, respectively).

Hormonal Reactivity

Repeated Measures ANOVA with Group and Time (baseline vs Post-test) as factors and repeated measures for the last factors were used. Individual variability in basal hormone levels were taken into consideration by using baseline levels as a

Figure 8: Social Isolation Test: Behavioral reactivity

Scores are averaged duration (seconds) for Cage Exploration (A), Hostility (B), and Vocalizations (C) for sham-operated controls (white bar) and animals with neonatal amygdala lesions (black bar) at the beginning (0 to 14 minutes) and at the end (14 to 28 minutes) of the separation test. Scores for each individual in each group are illustrated by an open square and open diamond for those infants receiving sham-operations and by a grey circle and grey triangle for those infants receiving neonatal amygdala lesions.


A. Cage Exploration
Duration (sec)
220
165
110
55
0
0-14
14-28
B. Hostility
Frequency
42
35
28
21
14
7
0
0-14
14-28
C. Vocalizations
Frequency
350
280
210
140
70
0
0-14
14-28
Time (min)

covariate. A Fisher LSD post-hoc analysis was preformed to examine group differences.

Following separation from their social group, both sham-operated control males with neonatal amygdala lesions showed a significant increase in ACTH levels after the 28 minutes of separation as compared to baseline [Figure 7A: TIME: $F(1,1) = 316.83$, $p = .036$, $\eta^2 = .997$]. Yet, this increase was greater in Group SHAM than in Group AMY as revealed by a significant interaction between Group and Time [$F(1,1) = 200.95$, $p = .045$, $\eta^2 = .995$. AMY<SHAM LSD: $p = .048$].

A similar pattern of results was obtained for cortisol levels (Figure 7B). Again Group SHAM appears to mount a greater response to the separation stress compared to Group AMY, although this group difference did not reach significance [TIME: $F(1, 1) = 2.6$, $p = .353$, $\eta^2 = .722$; GROUP X TIME: $F(1,1) = .815$, $p = .532$, $\eta^2 = .449$]. Even though there were no significant effects, the large effect sizes for both the main effect and the interaction suggest that there may be a significant difference, which cannot be detected due to the small sample size of our pilot study.

Human Intruder Test

Behavioral Reactivity

The data were analyzed with a MANOVA using Group, Condition, and Time as the main factors and repeated measures for the last two factors. Post-hoc analysis was performed using a Fisher LSD test.

Interestingly, the two groups behave similarly in response to the Human Intruder (Figure 10 & 11). Thus, when the Human Intruder stared at them, all animals displayed an increase in hostile and affiliative behaviors as well as an increase in freezing responses. Yet, these changes were mainly observed in the first 5 minutes of the test

Figure 9: Social Isolation Test: Neuroendocrine responsivity

Mean blood levels of ACTH (pg/ml) [A] and Cortisol (μg/dl) [B] for sham-operated controls (solid line) and animals with neonatal amygdala lesions (dashed) at baseline and post-separation test. * indicates $p < .05$.

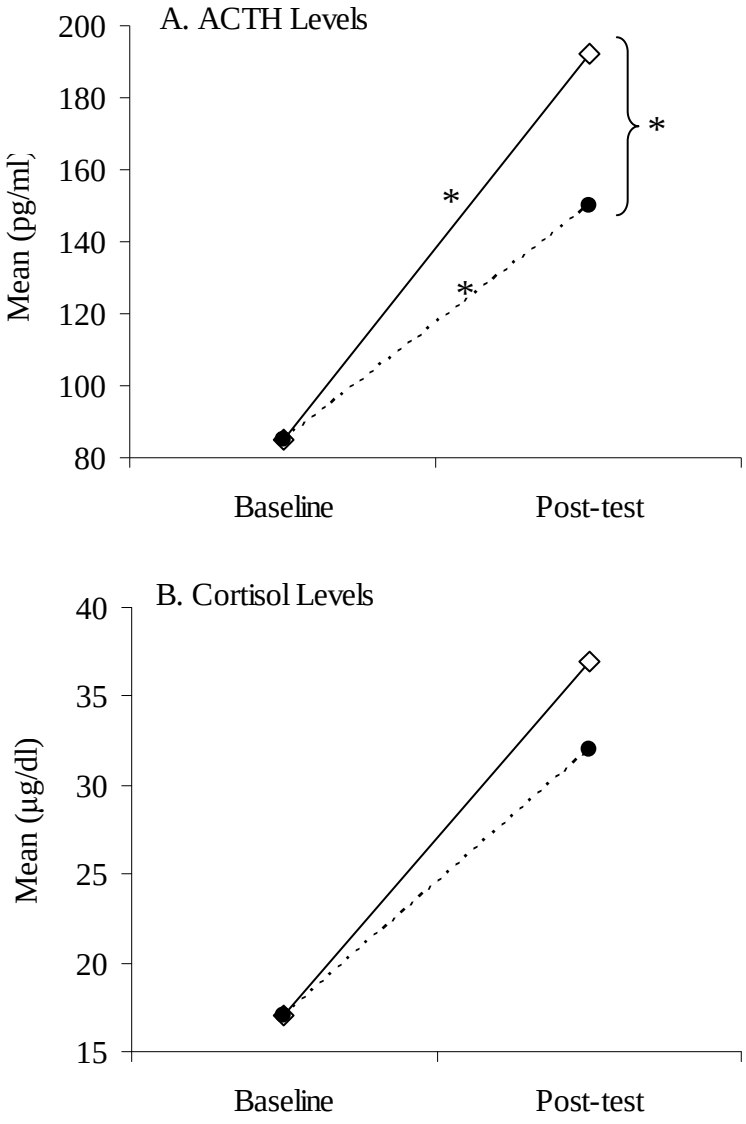

A. ACTH Levels
200
180
160
140
120
100
80
Mean (pg/ml)
*
*
*
Baseline
Post-test
B. Cortisol Levels
40
35
30
25
20
15
Mean (µg/dl)
Baseline
Post-test

Figure 10: Human Intruder Test: Hostile and Affiliative Reactions

Scores are averaged mean frequency of Hostile behaviors (A) and Affiliative behaviors (B) for sham-operated controls (white bars) and animals with neonatal amygdala lesions (black bars) at the beginning (0 to 5 minutes) and the end (5 to 10 minutes) of the Pre-Alone condition (solid bars), the Profile condition (vertical slanted bars), the Stare condition (dotted bars), and the Post-Alone condition (horizontal bars). Scores for each individual in each group are illustrated by an open square and open diamond for those infants receiving sham-operations and by a grey circle and grey triangle for those infants receiving neonatal amygdala lesions.

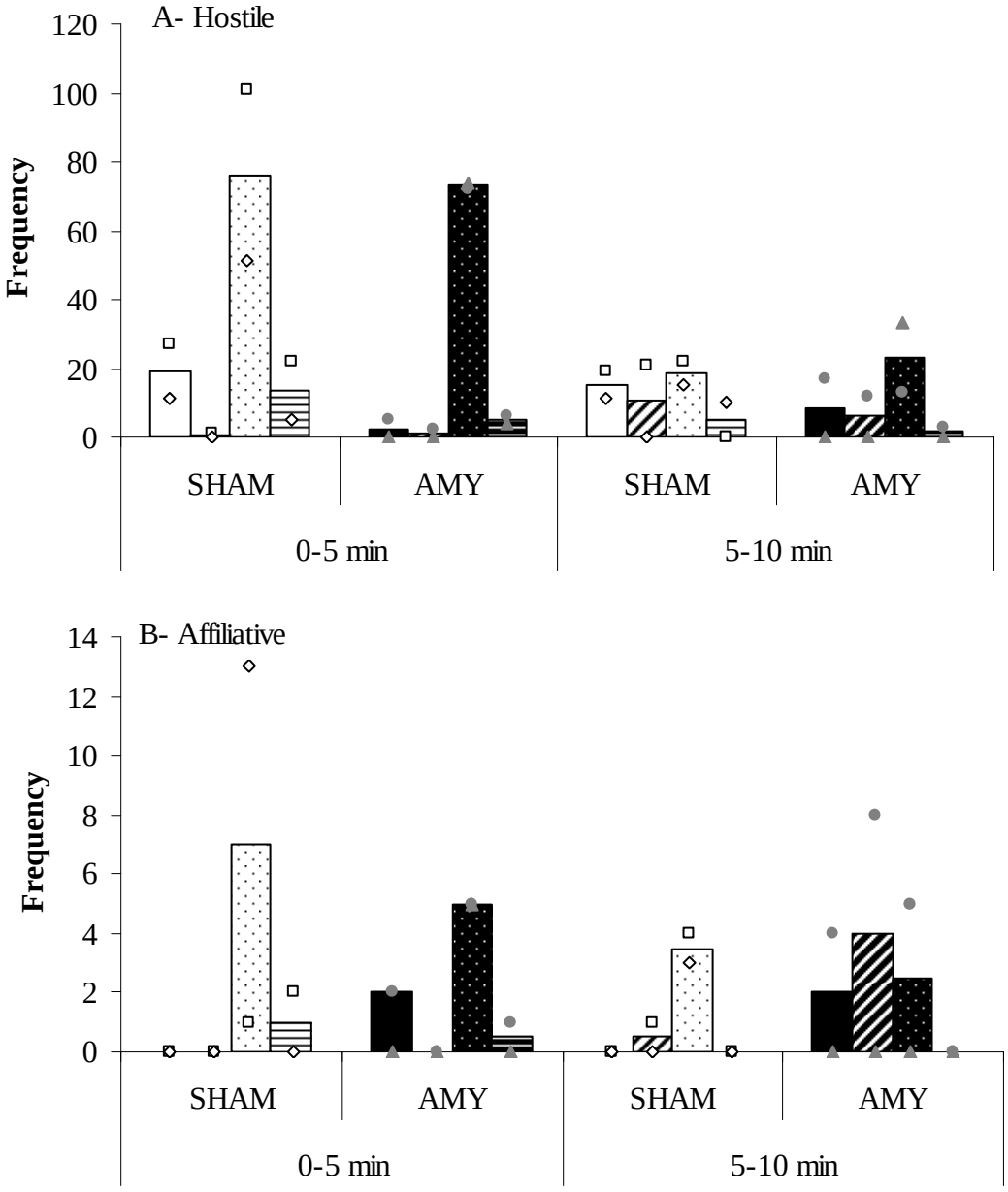
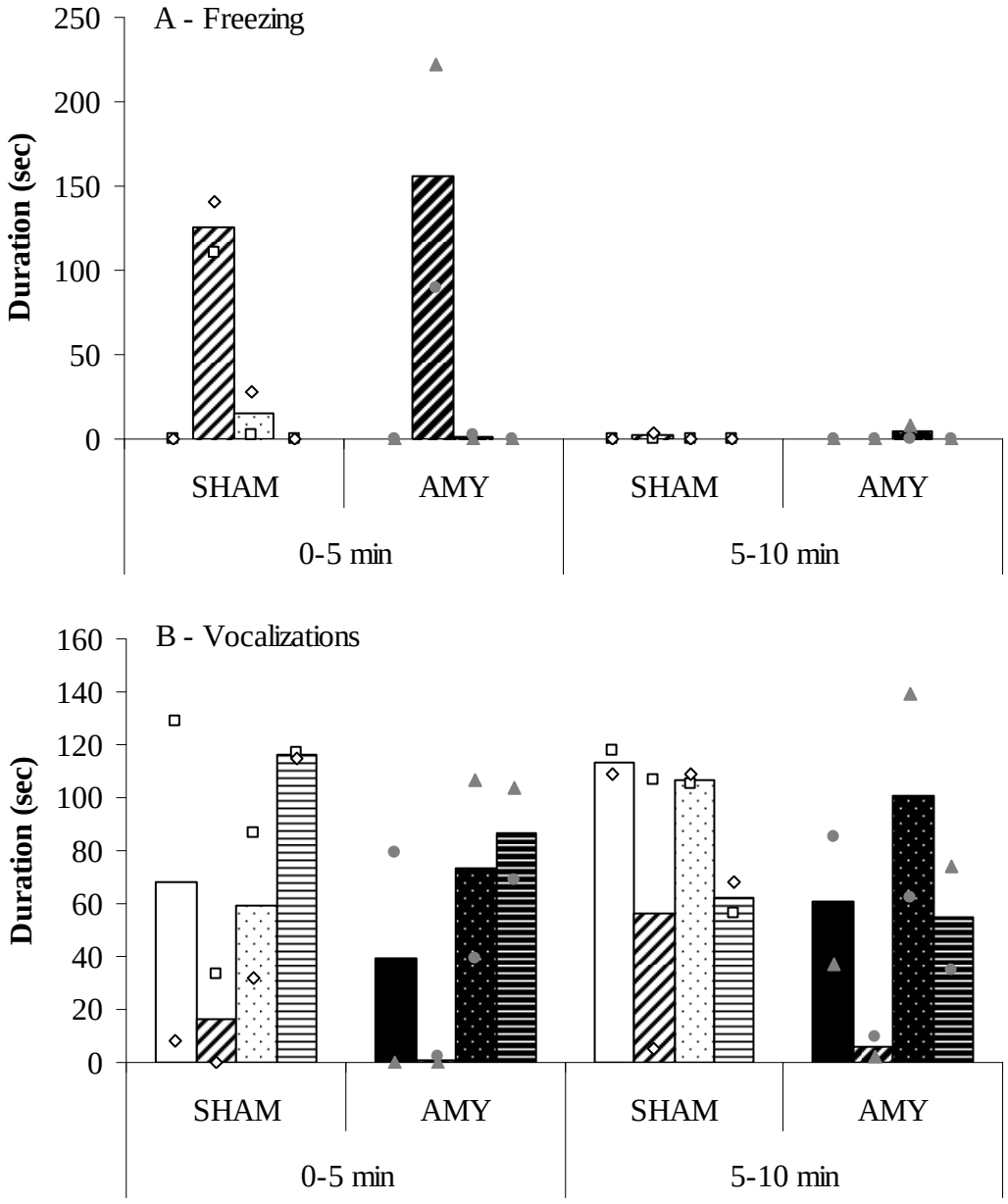

A- Hostile
Frequency
120
100
80
60
40
20
0
SHAM
AMY
SHAM
AMY
0-5 min
5-10 min
B- Affiliative
Frequency
14
12
10
8
6
4
2
0
SHAM
AMY
SHAM
AMY
0-5 min
5-10 min

Figure 11: Human Intruder Test: Freezing and Vocalization Reactions

Scores are averaged mean duration (seconds) for Freezing (A) and frequency of Vocalizations (B) for sham-operated controls (white bars) and animals with neonatal amygdala lesions (black bars) at the beginning (0 to 5 minutes) and the end (5 to 10 minutes) of the Pre-Alone condition (solid bars), the Profile condition (vertical slanted bars), the Stare condition (dotted bars), and the Post-Alone condition (horizontal bars). Scores for each individual in each group are illustrated by an open square and open diamond for those infants receiving sham-operations and by a grey circle and grey triangle for those infants receiving neonatal amygdala lesions.


A - Freezing
Duration (sec)
250
200
150
100
50
0
SHAM
AMY
SHAM
AMY
0-5 min
5-10 min
B - Vocalizations
Duration (sec)
160
140
120
100
80
60
40
20
0
SHAM
AMY
SHAM
AMY
0-5 min
5-10 min

condition. These changes were confirmed by significant Condition by Time interactions for Hostile [$F(3,1) = 10.84$, $p < .001$, $\eta^2 = .670$, Stare > All other conditions: LSD: p = .001, Beginning > End: LSD: p = .002] and for Freezing [$F(3,1) = 15.70$, $p < .001$, $\eta^2 = .746$, Profile > All other conditions: LSD: p < .001, Beginning > End: LSD: p < .001], and a significant main effect of Condition of affiliative behaviors [$F(3,1) = 3.55$, $p = .038$, $\eta^2 = .400$, Stare > All other conditions: LSD: p = .036] and vocalizations [$F(3,1)=4.46$, $p=.019$, $\eta^2=.455$, Profile < All other conditions: LSD: p=.028].

Hormonal Reactivity

Blood levels of ACTH and cortisol stress hormones before and after the Human Intruder are given on Figures 12A and 12B, respectively. Statistical analyses were performed as described for the Social Isolation Test above.

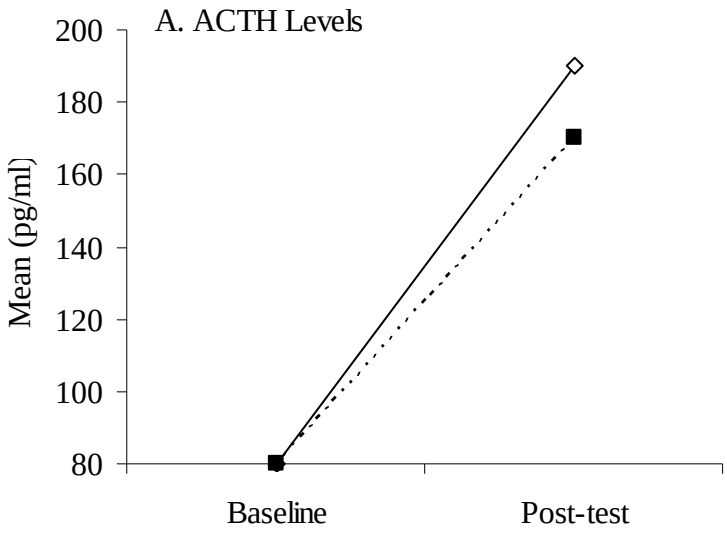
Both sham-operated controls and animals with neonatal amygdala lesions mounted a neuroendocrine stress response to the unfamiliar human intruder, as reflected by the increase in ACTH and cortisol levels from baseline to post-test. Although these changes in hormonal levels did not reach significance [TIME: $F(1, 1) = 10.49$, $p = .191$, $\eta^2 = .913$; GROUP X TIME: $F(1,1) = 2.09$, $p = .385$, $\eta^2 = .676$, for ACTH and TIME: $F(1, 1) = 31.59$, $p = .112$, $\eta^2 = .969$; GROUP X TIME: $F(1,1) = 23.551$, $p = .129$, $\eta^2 = .959$, for cortisol], the effect sizes were large suggesting that potential significant differences could emerge with a larger sample size.

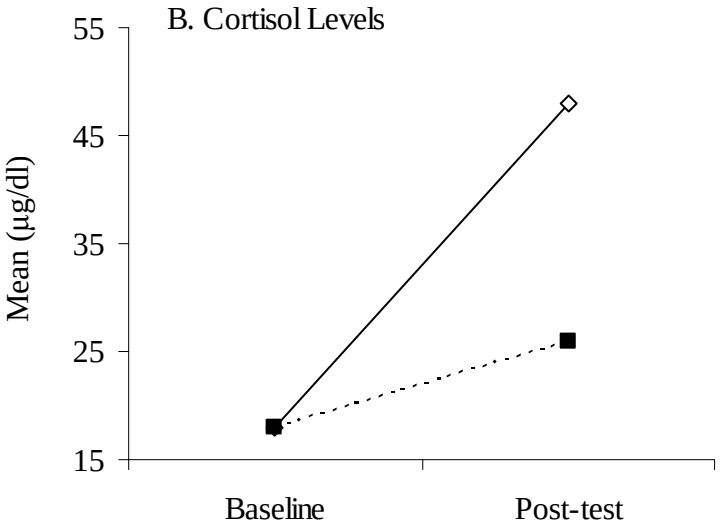
Social Intruder Test

To examine behavioral reactivity toward an unfamiliar conspecific, subjects were

Figure 12: Human Intruder Test: Neuroendocrine responsivity

Mean blood levels of ACTH (pg/ml) [A] and Cortisol (μg/dl) for sham-operated controls (solid line) and animals with neonatal amygdala lesions (dashed) at baseline and post-Human Intruder test.


A. ACTH Levels
200
180
160
140
120
100
80
Mean (pg/ml)
Baseline
Post-test


B. Cortisol Levels
55
45
35
25
15
Mean (µg/dl)
Baseline
Post-test

introduced with limited access to four adult males. The 30 minute test was divided into thirds to examine if subjects had different reactions to the unfamiliar males over time. Statistical analyses used the same tests as those described for the Social Isolation test above.

As shown in Figure 13A, animals with neonatal amygdala lesions spent more time in close proximity to the unfamiliar adult males (within 1 meter) across all time points of the test as compared to sham-operated control [$\chi^2(3, N = 4) = 8.10$, $p = .044$, and Kendall coefficient of concordance of .675 indicates a strong difference among the GROUP and PERIOD factors].

Overall few hostile behaviors were observed during the test (Figure 13B) and they were mostly produced by animals of the two groups during the first 10 minutes of the Social Intruder test [$\chi^2(3, N = 4) = 7.36$, $p = .061$, and the Kendall coefficient of concordance of .6145 indicates a strong difference among the factors].

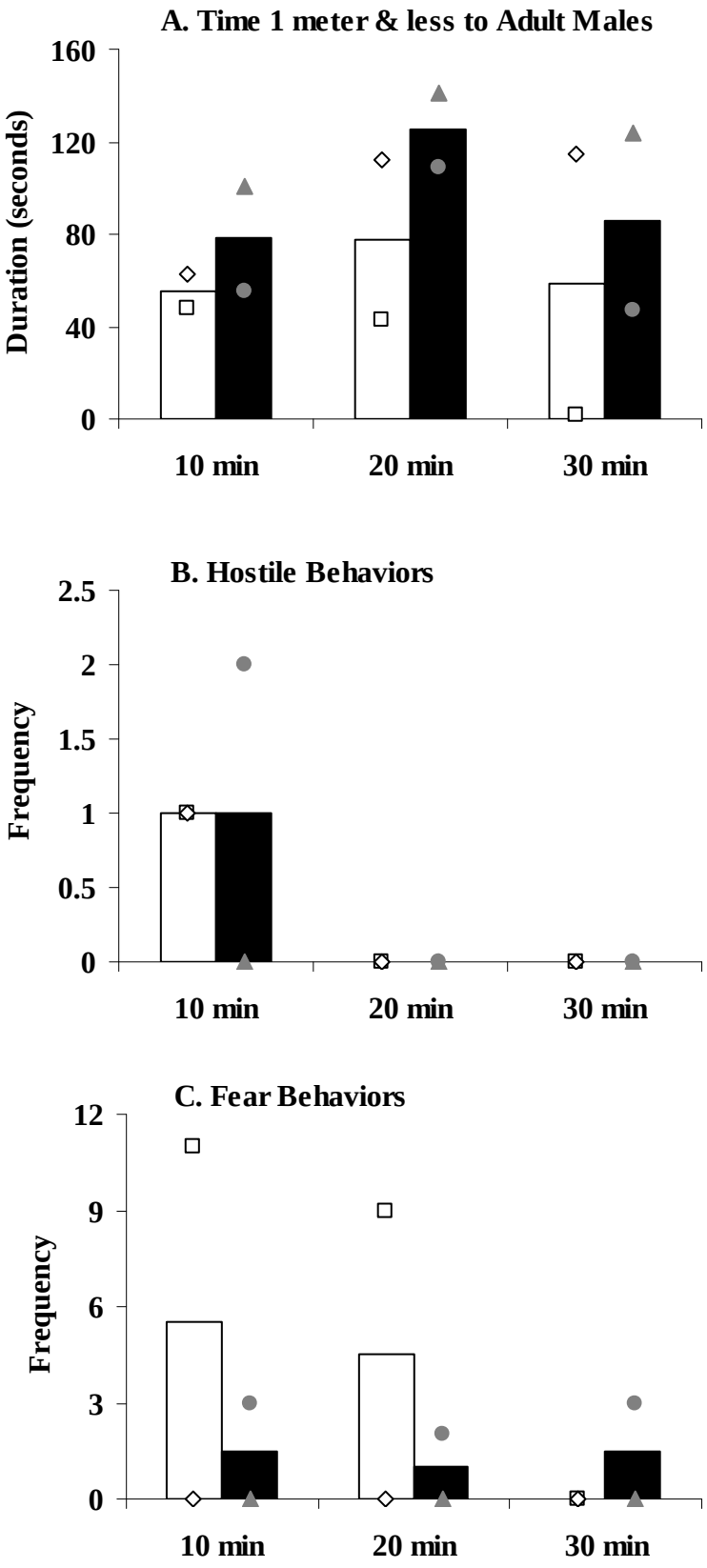
Due to the camera angels during the task, submissive and fearful facial gestures, such as lipsmack and grimace, could not be reliably coded, thus we report only fear behaviors, such as withdrawals, that were observed when an animal was threatened by an adult male. There were also very few withdrawal fear behaviors in both groups that resulted in no significant group differences [$\chi^2(3, N=4) = 1.32$, $p = .724$], even though Group AMY displayed less withdrawals than Group SHAM.

Discussion

Overall, the results indicate that neonatal amygdala lesions impact emotional reactivity and endocrine response to stressful events, although the magnitude of the

Figure 13: Social Intruder Test: Behavioral reactivity

Scores are averaged mean duration (seconds) for being in 1 meter or lesser from the adult males (A), and averaged mean frequency of Hostile (B), and Fear (C) behaviors for sham-operated controls (white bars) and animals with neonatal amygdala lesions (black bars) at three time points (0-10, 10-20 and 20-30 minutes) during the test. Scores for each individual in each group are illustrated by an open square and open diamond for those infants receiving sham-operations and by a grey circle and grey triangle for those infants receiving neonatal amygdala lesions.


A. Time 1 meter & less to Adult Males
Duration (seconds)
160
120
80
40
0
10 min
20 min
30 min
B. Hostile Behaviors
Frequency
2.5
2
1.5
1
0.5
0
10 min
20 min
30 min
C. Fear Behaviors
Frequency
12
9
6
3
0
10 min
20 min
30 min

behavioral and endocrine effects varied among the emotional tasks used.

Social Isolation Test:

Separation from the mother and social group yielded increased cage exploration and hostile behaviors in sham-operated control males. These changes could indicate that the separation increased the motivation of control animals to search for an escape to regain contact with the social group. However, these behavioral changes decreased with time as the animals presumably learned that no escape from the cage was possible. Similar changes also occurred in animals with neonatal amygdala lesions although they were less dramatic, resulting in a trend toward significant difference in the amount of cage exploration and hostility between the two groups in the first half of the test session. This reduced amount of behaviors after neonatal amygdala lesions was also accompanied with an increase in vocalizations. Previous studies have shown that animals of many species adopt defensive behavioral responses when placed in a novel environment, such as increased freezing and decreased vocalizations, when presented with sudden stimuli or potential threats, to avoid predatorial detection while assessing the potential danger of the situation (Hansen, 1966; Bolles, 1970; Blanchard, Flannelly, & Blanchard, 1986). Typically developing rhesus monkeys express few vocalizations when placed in a social isolation condition similar to that used in the present study (Kalin & Shelton, 1998). The reduced emotional reactivity following neonatal amygdala lesions has also been described by Thompson and colleagues (1969) who showed that, when exposed to a novel environment, monkeys with neonatal amygdala lesions displayed less emotional reactivity than controls as reflected by their willingness to enter the novel environment

three times faster than the controls. Although both groups displayed an increase in ACTH and cortisol levels from baseline to post-separation test, this increase was greater in the controls than in the animals with neonatal amygdala lesions, but the group difference was significant only for ACTH levels. There are several possible factors that may explain why significant group differences in cortisol levels were not detected in the present study. First, the limited sample size could have impacted our ability to detect small group differences. Second, the timing of blood samples may not have been optimal to detect the hormonal changes. The Social Isolation Test was 28 minutes in length making it ideal for detecting the peak in ACTH, which occurs approximately 30 minutes after the onset of the stressor. By contrast, the peak of cortisol has a much longer time course and cannot be detected until hours after the onset of the stressor. Thus, future studies will need to investigate changes in hormonal levels for several hours after the administration of the stressors.

To date, Goursaud and colleagues (2006) were the first to have examined endocrine responses to stress in rhesus monkeys with neonatal amygdala lesions. They reported no differences in the blood cortisol levels between animals with neonatal amygdala lesions and controls. Although their results conflict with those we report here, the differences may result from differences in experimental procedures. Thus, as compared to our procedure, the lack of cortisol levels changes in Goursaud and colleagues' study could be explained by the absence of a baseline sample, since the first sample was taken at least 2 hours after the stress of social separation from the mother began. The present neuroendocrine findings are in fact in line with those reported in previous studies of monkey acquiring amygdala lesions in adulthood and that have

reported reduced cortisol levels after social isolation and restraint (Machado & Bachevalier, 2008; Kalin, et al, 2004).

Human Intruder Test

The human intruder paradigm has been widely utilized to examine emotional reactivity in non-human primates (Izquierdo & Murray, 2004; Izquierdo, et al, 2005; Kalin & Shelton, 1998; Kalin, et al, 2004; Machado & Bachevalier, 2008). Yet, results have been mixed in studies of the effects of amygdala lesions in adult monkeys. Several studies have found that adult lesions of either the complete amygdala or the central nucleus of the amygdala result in reduced freezing behavior and increased coo vocalizations (Kalin, et al, 2004; Machado & Bachevalier, 2008). Yet, other studies have found no effects of amygdala lesions on emotional responses (Izquierdo & Murray, 2004; Izquierdo, et al, 2005). To date, there have been no studies examining the effects of neonatal amygdala lesions on this paradigm, and the results of the present study indicate a similar emotional reactivity towards the human intruder by both the sham-operated controls and amygdala-operated males. Thus, when a social threat, such as an unfamiliar human, is introduced to the stress of social separation, the amygdalectomized males expressed the same pattern of emotional behaviors as sham-operated males. These results are similar to those of previous studies examining the response of neonatal amygdala-operated animals to a human experimenter. Kling and Green (1967) found that both monkeys with neonatal aspiration lesions of the amygdala and controls responded to humans by withdrawing which "is behaviour typical of the normal infant." In addition, the lack of group difference in emotional reactivity in the Human Intruder paradigm was

also associated with no group difference in neuroendocrine responses. Thus, amygdala-operated animals responded to the presence of the human intruder with a rise in both ACTH and cortisol that was comparable to that found in sham-operated controls. Yet, these results should be viewed with caution. Limitations of our study include a small sample size as well as large individual variability between behaviors of subjects in both the sham-operated and amygdala-operated groups. Therefore, it is possible that a difference between groups might emerge with additional subjects. Another possible limitation was the timing of blood samples, which may not have been optimal to detect the hormonal changes. The Human Intruder Test was 43 minutes in length, which missed the peak in ACTH, occurring approximately 30 minutes after the onset of the stressor, and also missed the peak of cortisol, which does not peak until hours after the onset of the stressor. Thus, one important modification of the test in future studies will be to shorten the task to 30 minutes to capture the peak ACTH, but also continue to collect additional samples several hours after the administration of the stressor to capture the peak in cortisol.

Social Intruder Test

When faced with the threat of a novel conspecific intruder in a familiar environment, animals with neonatal amygdala lesions exhibited a blunted fear response; that is, they spent significantly more time within one meter or less to the unfamiliar adult males than sham-operated controls. This willingness to remain in close proximity to the adult males indicates that animals with neonatal amygdala lesions did not perceive the males as a threat as the sham-operated controls did.

These results parallel those of previous studies examining the reactivity to

unfamiliar conspecifics in animals with amygdala damage. Thompson, Bergland, and Towfighi (1977) found that both neonatal and adult amygdala lesions resulted in a blunted fear response to an unfamiliar 10-year-old conspecific. In fact, the authors commented that operated subjects appeared to be slow to submit to the unfamiliar (more dominant) animal. Additionally, Meunier and colleagues (1999) found that adult animals with aspiration or neurotoxic lesions of the amygdala approached, touched, and occasionally mouthed a conspecific monkey head stimulus, whereas control animals did not. Therefore, the present results of reduced behavioral and neuroendocrine reactivity to stress are consistent with previous research in both adult and neonatal amygdala lesions.

Summary

Although the overall findings pointed to the reduced emotional and neuroendocrine responses to stress in animals with neonatal amygdala lesions, these changes did not occur on all three emotional reactivity tasks. Different results in the three tasks may simply be related to the magnitude of stress provided by the three tasks. Social isolation in a novel environment alone was not enough to produce an emotional response equal to that of controls, nor was exposure to a novel intruder in a familiar environment. Yet, the combination of a novel intruder in a novel environment created the most stressful situation and therefore caused the amygdala-operated animals to mount an equally emotional response compared to sham-operated controls. Alternatively, the different outcomes between the human and social intruder tests may reflect a difference in the threat assessment. Perhaps an unfamiliar human is processed as a more salient threat and potential danger than an unfamiliar conspecific. Future studies should conduct

a more systematic examination of the differences between these two intruder tests.

CONCLUSIONS

The current study demonstrates the critical role played by the amygdala in the development of normal social and emotional responses. The amygdala is not necessary for the acquisition of social behaviors, since animals with neonatal amygdala lesions expressed a normal repertoire of social behaviors. However, the amygdala is important for the appropriate timing and expression of these social behaviors. In addition to confirming findings from previous studies (Bachevalier, 1994; Bauman, et al 2004a, 2004b, 2006; Prather, et al, 2001; Thompson, Schwartzbaum, & Harlow, 1969), the current study also demonstrated that the behavioral changes observed in animals with neonatal amygdala lesions affected how other animals interacted with them (see Table 4).

The rapid emergence of independence from the mother together with approachability towards other individuals and increased aggressivity as the animals matured suggest that infants with neonatal amygdala lesions may have been less fearful than control animals in a rich and complex social environment. These changes are also in line with the blunted emotional and endocrinological reactivity to stress observed in the same animals when placed in isolation from the social group and when facing conspecifics. Therefore, early amygdala damage results in disruption of the hypothalamic-pituitary-adrenal axis (see Table 4).

The amygdala has both direct and indirect connections to the paraventricular nucleus of the hypothalamus which can influence endocrine response (Feldman, Conforti, & Saphier, 1990; Feldman, Conforti, & Weidenfeld, 1995; Davis 2000). Thus, the reduced behavioral and neuroendocrine responses to stressful agents suggest that activation of the amygdala by stressful agents is necessary to modulate the function of the hypothalamus and striatum.

Table 4. Comparison between previous studies and current study

Comparisons between findings from previous studies of acquired amygdala lesions in adulthood (Klüver and Bucy, 1939; Rosvold, Mirsky, and Pribram, 1954; Downer, 1961; Dicks, Meyers, and Kling, 1969; Franzen and Myers, 1973; Meunier et al., 1999; Emory et al., 2001; Kalin et al., 2001, 2004; Machado and Bachevalier, 2006, 2008) or in infancy (Kling and Green, 1967; Thompson, et al., 1969, 1976, 1977; Bachevalier 1994; Prather et al., 2001; Bauman et al., 2004a, 2004b, 2006; Goursaud, Mendoza, and Capitanio, 2006).

	Amygdala Lesion Study Timing		
Behavioral Changes	Previous Adult	Previous Neonatal	Current Neonatal
Social Repertoire	No changes	No changes	No changes
Sexual	Increase	Not reported	Increase incorrectly oriented mounts
Aggressive	Increase or Decrease	No changes or Decrease	Increase
Social withdrawal	Increase	No changes or Increase	No changes
Social activity	No changes	Decrease	Increase
Social Fear	Increase	Increase	Decrease
Neuroendocrine response	Decrease	No changes	Decrease

When compared to previous studies of neonatal amygdala lesion (Thompson, et al., 1981; Bachevalier, 1994; Bauman and colleagues, 2004a, 2004b, 2006), the present findings also point to the important impact that the complexity of the social environment may have on the behavioral effects observed. The complexity of the environment plays a critical role in the emergence of social behavior and/or deficits (Mason, 1960; Anderson & Mason, 1974). Therefore, both physical environment and complexity of social group equally influence behavioral development in primates. Additionally, stability of that social and physical environment is essential for normal primate infant development (Rosenblum & Anderws, 1994). Thus, the restricted social and physical environment of the Thompson and Bachevalier studies as well as the changing social and physical environment of the Bauman and colleagues study could have differently impacted behavioral development. By contrast, the consistent physical and stable social environment provided to the infants in the present study could have fostered the development of more typical primate social behavior. This conclusion seems counterintuitive as one may predict that placing the infant monkeys with neonatal amygdala lesions in a more complex social environment could enhance the behavioral abnormalities observed. Thus, the complexity of the social and physical environment is a significant factor to be considered when assessing the effects of brain damage on social and emotional behaviors. Given the recent discovery that both the physical and social environment significantly impact gene regulation of postnatal brain development (Nikolaev, Kaczmarek, Zhu, Winblad, & Mohammed, 2002; Thiriet, et al., 2008), it is likely that, in the absence of a functional amygdala, a rich environment may modulate

gene functions in other developing brain areas, which in turn may offer some functional compensatory mechanisms.

Although the present results suggest a critical role played by the amygdala in the development of social and emotional behavior in monkeys, there are important issues that deserve comments. Lesion technique has been widely used to examine how specific areas of the brain contribute to behavior. Yet, the results can only tell us what the remaining brain can do in the absence of a given structure. Results are unequivocal when the lesion of a given structure has a profound impact on behavior, suggesting that the structure mediates this function. However, such a strong relation between structure and function may not apply when the behavioral outcomes of a lesion are moderate or milder, especially because permanent lesions as the neurotoxic lesions used in the present study may induce brain reorganization, which in turn may provide partial or complete functional recovery. Thus, one might argue that the milder effects on social and emotional responses and neuroendocrine responses to stress may likewise have resulted from reorganization of the brain following the permanent amygdala lesions. There are several brains areas that might be able to compensate for the amygdala damage in the assessment of fearful situations. Both the prefrontal, more specifically the orbital frontal cortex and perirhinal cortex have been shown to be involved in the regulation of emotional reactivity (Adolphs, 2001; Meunier, & Bachevalier, 2002; Machado & Bachevalier, 2003; Bachevalier & Meunier, 2005). Thus recovery of function after neonatal amygdala damage may involve either or both of these structures. Future studies using other techniques, such as electrophysiological recordings or PET imaging, will be needed to more specifically define the role of the amygdala in the development of social

and emotional behaviors.

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Appendix I: Social Behavior Ethogram

Distance Codes:

px ***proximity*** within arm's reach of another animal's not scored when either animal is in motion

co ***contact*** when two animals have portions of their bodies in contact not only with hands like with touching or grooming

Duration Codes:

fl ***follow*** persistent trailing of another animal with both animals in motion

gm ***groom*** one animal combing through the hair of another with hands or mouth

kn ***kidnap*** an animal other than its mother takes control of an infant and carries it away from its original location

cv **carry ventral**, an animal is carrying another animal against his/her ventrum while standing, walking, or running

cd **carry dorsal**, an animal is carrying another animal that is laying down against his/her back while standing, walking, or running

cj **carry jockey**, same as above but the rider is sitting-up on carriers back

cp ***chase play*** active chasing between two animals, not agonistic

bp ***brief play*** low intensity play, upper body engagement, grappling

rp ***rough play*** high intensity play, whole bode involvement, tumbling and wrestling

sp ***solitary play*** does not involve a partner, vigorous play by oneself i.e. jumping, swinging, flipping, etc.

qp ***quiet play*** does not involve a partner, quiet manipulation of an object

Agonistic/Dominance/Submissive/Other Behavior Codes:

sa ***slap*** aggressive hand contact between two animals

bt ***bite*** aggressive intense mouth contact between two animals

gb ***grab*** aggressive contact intensive grabbing between two animals

tr ***threat*** non-contact aggression, lunge, open mouth woofing, or slap without contact

ch ***chase*** animal chases another animal in a nonplay context

di ***display*** bouncing, shaking vigorously, whole body movement on a substrate

ya ***yawn*** wide-mouth yawn

bs ***bodyshake*** shaking of body as if drying self like a dog (not scored in rain)

ss ***scratch*** self-directed behavior, scored for each bout of scratching

gs ***groom solicit*** posture to solicit grooming from another animal

gr ***grimace*** animal opens its mouth wide to show teeth with a closed jaw

wd ***withdraw*** clear avoidance of another animal

ls ***lipsmack*** facial expression involving rapidly opening and closing lips

Infant directed Codes:

to ***touch*** an animal briefly touches an infant

em ***embrace*** hugging an infant momentarily

gi ***genital inspect*** an animal visibly or physically inspects an infants genitals

ha ***harass*** an animal "messes with" an infant, pulling its tail, grabbing in a non agonistic manor

rs ***restrain*** an animal physically prevents an infant from moving away
rj ***reject*** an animal prevents an infant from making contact or nursing
pu ***punish*** an animal stops an infant from a certain behavior by physically removing it harshly or by mouthing it without causing injury
da ***drag*** an animal drags an infant by a limb across the ground
cu ***crush*** an animal uses its hands to push and hold down an infant on the ground
th ***throw*** an animal throws an infant
hi ***hit*** an animal hits an infant with its hand
ad ***aid*** an animal rescues an infant from any type of abusive behavior by interfering

Vocal Codes:

sm ***scream*** an animal loudly squeals at another animals
oo ***coo*** an infants call for its mother
gk ***gecker*** an infants grunting in protest to another animals actions
ta ***tantrum*** an infants sustained and high intensity protest to another animals actions

Sexual Codes:

ht ***hip touch*** an animal places two hands on the hips of another
pr ***present*** an animal directs its hindquarters toward another often with tail deviated
sn ***sniff genitals*** an animal smells of the genitals of another
mt ***foot clasp mount with thrusting*** one or both feet of the animal are clasped on the outside of the ankles of the recipient so that the actor's pelvis is oriented toward the hindquarters of the recipient
mn ***foot clasp mount without thrusting*** same definition as above only without pelvic thrusting
nf ***no foot clasp and no thrusting*** actor must still be in correct orientation, hips to hips
nt ***no foot clasp with thrusting*** same as above only without pelvic thrusting
am ***abortive mount*** an animal will thrust on anther animal but incorrectly oriented, actor mounts another body part other than hindquarters
if ***interference*** an animal causes the withdrawing or leaving beyond of a male involved in sexual behavior with a female
br ***break*** an animal causes the withdrawing or leaving beyond of a female involved in sexual behavior with a male
in ***intromission*** behavioral pattern when pelvic thrusting become deeper and rhythmic
ma ***mastrubation*** any manual manipulation of the genitals that is rhythmic and repetitious
ej ***ejaculatory reflex*** release of ejaculate or the behavioral response that characterizes such

Appendix II: Reactivity Ethogram

Activity Codes

lo ***locomotion*** animal is initiates movement (walking, running, climbing, etc)
pa ***pacing*** animal is moving around in a stereotypical circular pattern
in ***inactive*** animal doesn't change its body location for more than 3 seconds
fz ***freezing*** animal remain motionless except for some slight head movements and either standing or slightly crouched looking forward at the stimulus

Behavior Codes

tg ***tooth grind*** repetitive, audible rubbing of upper and lower teeth
ca ***cage aggression*** dominance display including vigorous shaking of cage walls, or body slams against the walls
tr ***threat*** lunge or open mouth without woofing
gr ***fear grimace*** exaggerated grin, with teeth showing
ls ***lipsmack*** rapid lip movement with pursed lips
ya ***yawn*** wide-open mouth, displaying teeth
pr ***present*** rigid posture with rump and tail elevated and oriented toward stimulus
te ***tactile exploration*** tactile manipulation of the physical environment
oe ***oral exploration*** use of mouth to explore the physical environment
sd ***self-directed*** manipulatory or grooming a body part with another body part
sr ***scratch*** scratching of a body part by hands or feet

Vocalizations

vc ***coo vocalization*** clear, soft, moderate in pitch and intensity
vo ***other vocalization*** screaming or grunting
vt ***threat bark vocalization*** low-pitched, loud, guttural sound, woofing