Published in final edited form as:
Behav Neurosci. 2008 October ; 122(5): 1005–1015. doi:10.1037/a0012600.

EMERGENCE OF STEREOTYPIES IN JUVENILE MONKEYS WITH NEONATAL AMYGDALA OR HIPPOCAMPUS LESIONS

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Abstract

The emergence of stereotypies was examined in juvenile rhesus monkeys who, at two weeks of age, received selective bilateral ibotenic acid lesions of the amygdala (N=8) or hippocampus (N=8). The lesion groups were compared to age-matched control subjects that received a sham surgical procedure (N=8). All subjects were maternally reared for the first six months and provided access to social groups throughout development. Pronounced stereotypies were not observed in any of the experimental groups during the first year of life. However, between one to two years of age, both amygdala- and hippocampus-lesioned subjects began to exhibit stereotypies. When observed as juveniles, both amygdala- and hippocampus-lesioned subjects consistently produced more stereotypies than the control subjects in a variety of contexts. Interestingly, neonatal lesions of either the amygdala or hippocampus resulted in unique repertoires of repetitive behaviors. Amygdala-lesioned subjects exhibited more self-directed stereotypies and the hippocampus-lesioned subjects displayed more head-twisting. We discuss these results in relation to the neurobiological basis of repetitive stereotypies in neurodevelopmental disorders, such as autism.

Keywords

amygdaloid complex; repetitive behavior; macaque; psychopathology; neurodevelopment; rhesus

INTRODUCTION

Stereotypies are defined as repetitive and topographically invariant acts without a clearly established purpose or function (Ridley & Baker, 1982). Examples of stereotypies in human populations include, hand flapping, body-rocking, head-rolling etc. (Berkson, Gutermuth, & Baranek, 1995; Rojahn, Matlock, & Tasse, 2000; Rojahn, Tasse, & Sturmey, 1997). These repetitive movements are commonly observed in a variety of developmental, psychiatric and neurological disorders, including autism, Rett syndrome, Fragile X syndrome, mental retardation, schizophrenia, obsessive compulsive disorder, Parkinson disease and Tourette syndrome (Berkson, 1983; Berkson et al., 1995; Bodfish, Symons, Parker, & Lewis, 2000; Lachiewicz, Spiridigliozzi, Gullion, Ransford, & Rao, 1994; South, Ozonoff, & McMahon,

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2005; Wales, Charman, & Mount, 2004). The use of animal models provides one approach to identifying the neural underpinnings of repetitive behaviors observed in clinical populations.

Models of restricted, repetitive behaviors in animals have relied on three main experimental manipulations to induce stereotypies: 1) Social and/or environmental restrictions, 2) Pharmacological manipulations and 3) Targeted insults to the central nervous system (e.g., genetic mutations, viral exposure, lesions) (Lewis, Tanimura, Lee, & Bodfish, 2007). Across species, restrictions or perturbations of the physical and social environment are consistently associated with the production of stereotypies (Capitanio, 1986; Lutz, Well, & Novak, 2003; Powell, Newman, Pendergast, & Lewis, 1999). Likewise, pharmacological manipulations of the dopamine system have also been used to reliably induce repetitive stereotypies in both rodents and nonhuman primates (Bedingfield, Calder, Thai, & Karler, 1997; Lewis, Baumeister, McCorkle, & Mailman, 1985; Presti, Mikes, & Lewis, 2003; Randrup & Munkvad, 1974). Both socio-environmental restrictions and pharmacological manipulations have implicated dysfunction of circuits linking the neocortex and basal ganglia in the pathophysiology of repetitive behaviors. It is not clear, however, how these manipulations act upon the basal ganglia to induce motor stereotypies (Canales & Graybiel, 2000; Lewis, Gluck, Beauchamp, Keresztury, & Mailman, 1990; Lewis et al., 2007; Martin, Spicer, Lewis, Gluck, & Cork, 1991). Moreover, there is some evidence that targeted insults to non-striatal structures within the medial temporal lobe result in locomotor stereotypies in both rodents and nonhuman primates (Bachevalier, 1994; Lipska & Weinberger, 2000).

Here we evaluate the effects of neonatal amygdala or hippocampus damage on the emergence of stereotypies in mother-reared, group-living rhesus monkeys. We have previously reported that early damage to the amygdala or hippocampus did not alter fundamental features of social development, including the development of mother-infant interactions and the ability to interact with peers (Bauman, Lavenex, Mason, Capitanio, & Amaral, 2004a,, 2004b). Though our initial observations in the first year of development revealed few stereotypies, both amygdala- and hippocampus-lesioned subjects began to produce stereotypies in the second year of life. The control subjects did not develop motor stereotypies, suggesting that the emergence of repetitive behaviors is related to the brain lesion rather than the socio-environmental rearing context. We examine these data in relation to the neurobiology of abnormal repetitive behaviors, and discuss possible implications for animal models of developmental disorders.

METHODS

All experimental procedures were developed in consultation with the veterinary staff at the California National Primate Research Center. All protocols were approved by the UC Davis IACUC.

Subjects and Living Conditions

Current data were collected across a six-month span beginning when the average subject was a little over two years of age. A brief summary of rearing/housing conditions is provided below. Twenty-four infant rhesus monkeys (*Macaca mulatta*) naturally born of multiparous mothers were randomly assigned to one of three lesion conditions: bilateral amygdala lesions (five females, three males), bilateral hippocampus lesions (five females, three males) or sham-operated controls (four females, four males). All surgeries were performed at 12–16 days after birth. The infants were returned to their mothers following surgery and provided daily access to a socialization group consisting of six mother-infant pairs and one adult male. The animals in these groups were able to interact for a minimum of three hours per day, five days per week. The four socialization groups were each composed of two amygdala-lesioned infants and their mothers, two hippocampus-lesioned infants and their mothers, and two sham-operated infants

and their mothers. The age range between the youngest and oldest infant within each group was approximately two months. Three of the socialization groups were comprised of one male and one female per lesion condition, and the fourth cohort consisted of two female amygdala-lesioned infants, two female hippocampus-lesioned infants, one male and one female sham-operated infants. When the youngest subject within a socialization group reached six months of age, the infants were permanently separated from their mothers, but otherwise continued to experience the same housing and group socialization in the absence of their mothers. At this time, a new adult female was added to each socialization cohort to provide continued exemplars of adult female social behavior. At approximately one year of age, subjects became permanently socially housed (24 hours per day) with their original socialization cohort in a 2.13 m *W* × 3.35 m *D* × 2.44 m *H* chain link enclosure.

It is important to note that one male amygdala-lesioned subject died at approximately one year of age due to unrelated health reasons and was subsequently replaced with an alternative, neonatally amygdala-lesioned male. The replacement male was born the same year as the other subjects, but was reared alone with his mother for the first year of life. Following weaning at one year of age, he was housed with an age-matched female infant until being introduced to his current cohort at approximately one year and three months of age.

Surgical Procedures

The surgical procedures are summarized below and are described in detail in previous publications (Bauman et al., 2004a,, 2004b). On the day of surgery, the infants were initially anesthetized with ketamine hydrochloride (15 mg/kg i.m.) and medetomidine (30 μg/kg), then placed in an magnetic resonance imaging (MRI)-compatible stereotaxic apparatus (Crist Instruments Co., Inc., Damascus, MD). The infant's brain was imaged using a General Electric 1.5 T Gyroscan magnet; 1.0 mm thick sections were taken using a T1-weighted Inversion Recovery Pulse sequence (TR = 21, TE =7.9, NEX 3, FOV = 8cm, Matrix, 256 × 256). From these images, we determined the location of the amygdala or hippocampus and calculated the coordinates for the ibotenic acid injections. Infants were ventilated and vital signs monitored throughout the surgery. A stable level of anesthesia was maintained using a combination of isoflurane (1.0%, varied as needed to maintain an adequate level of anesthesia) and intravenous infusion of fentanyl (7–10 μg/kg/hour). Following a midline incision, the skin was laterally displaced to expose the skull, two craniotomies were made over the amygdala or the hippocampus, depending on the pre-determined lesion condition, and the dura was reflected to expose the surface of the brain. Ibotenic acid (IBO, Biosearch Technologies Inc., 10 mg/ml in 0.1 M phosphate buffered saline) was injected simultaneously bilaterally into the amygdala or hippocampus using 10 μl Hamilton syringes (26 gauge beveled needles) at a rate of 0.2 μl/min. Complete amygdala lesions required a total of 7–12 μl of ibotenic acid per amygdala. Complete hippocampus lesions required a total of 5.5–7 μl of ibotenic acid per hippocampus. Sham-operated controls underwent the same pre-surgical preparations, received a midline incision and the skull was exposed. The control subjects were maintained under anesthesia for the average duration of the lesion surgeries and the fascia and skin were sutured in two separate layers. Following the surgical procedure, all infants were monitored by a veterinarian and returned to their mothers once they were fully alert.

Lesion Analysis

We obtained T2-weighted magnetic resonance (MR) images ten days after surgery to examine the extent of the edema associated with the lesion. The hyperintense T2-weighted signal for each of the sixteen lesioned subjects was evaluated to confirm the general target and extent of the lesions (i.e., amygdala lesion sparing the hippocampus or hippocampus lesion sparing the amygdala). Their brains were imaged using a General Electric 1.5 T Gyroscan magnet; 1.5 mm thick sections were taken using a T2 weighted Inversion Recovery Pulse sequence (TR = 4000,

TE = 102, NEX 3, FOV = 8cm, Matrix, 256 × 256). Additional lesion confirmation was provided by T1-weighted MR images obtained at approximately four years of age. The animals' brains were scanned using a General Electric 1.5T Signa MRI system; 1mm thick sections were taken using a T1 weighted 3D axial spoiled gradient (SPGR) sequence (TR = 22.0ms, TE = 7.9ms, NEX 3, FOV = 16cm, Matrix, 256 × 256).

Behavioral Observations

Subjects were observed in four distinct behavioral contexts: 1) Solo, 2) Paired with familiar conspecifics, 3) Paired with unfamiliar conspecifics and 4) Group housed in their standard living environment (see Table 1). Both social and non-social data were collected during these tests, but only data relevant to stereotypies will be presented here. The social interaction data from these observations will be described in future publications.

Re-evaluation of Earlier Video Footage—We re-evaluated a subset of our previously reported behavioral observations (Bauman et al., 2004b) using a more sensitive ethogram designed to assess discrete classes of stereotypies (Table 2). The re-scored data are from novel dyads that took place when the subjects were approximately 12 months of age. For each subject, we rescored the first 20 dyadic interactions (400 minutes total observation time per subject).

Solo Context—Subjects were observed alone in their home enclosure to assess stereotypies in the absence of social stimulation. A single test session consisted of observing each animal for two consecutive 5-minute samples. Subjects participated in two test sessions per day over five consecutive days. A total of 20 solo samples were collected on each animal (100 minutes total observation time per subject).

Familiar Dyad Context—Immediately after two subjects from the same social group had concluded solo testing for a particular session, they were paired in their home enclosure and allowed to interact freely for twenty minutes. Each animal participated in two dyads per day for a total of 10 dyadic meetings per animal. Each animal was tested twice with every other animal from its social group according to a predetermined pseudorandom sequence. The identity of the focal animal alternated every 5 minutes during the 20-min period, so that a total of 20 focal observations were collected on each animal across the 10 dyadic meetings (100 minutes total observation time per subject). All dyads were balanced for testing order and time of day.

Novel Dyad Context—The effect of novelty on the occurrence of stereotypies was assessed during pairings of unfamiliar subjects. Novel dyads consisted of two subjects from different social groups that had never met one another. These pairings started two months after the completion of solo and familiar dyad testing. Observations of novel dyads took place in enclosures that were unfamiliar to both subjects but otherwise identical to their home enclosures. Each animal was observed with every animal from a separate social group (two amygdala-lesioned, two hippocampus-lesioned, and two sham-operated control subjects) according to a predetermined pseudorandom sequence. This complete rotation of dyadic meetings was then repeated for five more weeks, resulting in six pairings for each combination of dyad partners or 36 dyads per animal. Each animal participated in two, 20-minute dyads per day, balanced for testing order and time of day. The identity of the focal animal alternated every 5 minutes during a single 20-minute session, resulting in 72 focal observations per animal (360 minutes total observation time per subject).

Social Group Context—Weekly social group observations were conducted within each cohort's home enclosure in order to evaluate the frequency of stereotypies under normative

conditions. Each subject was observed via 5-minute focal observations on 34 separate occasions (170 minutes total observation time per subject).

Scoring Methods

The presence of stereotypies was assessed using a behavioral ethogram of 11 abnormal behaviors known to exist in laboratory rhesus macaques (Berkson, 1968; Capitanio, 1986) (see Table 2). Stereotypies by definition are repetitive behaviors that often occur in rapid succession which makes them challenging to define and accurately quantify (Gardenier, MacDonald, & Green, 2004). Given that stereotypies generally occur as "bouts" of repetitive behavior, we have adopted a scoring procedure that requires two or more repetitions of a target behavior or repetition of a target behavior for more than 3 seconds to be scored as a stereotypy. Likewise, our scoring procedures require a stereotypy to cease for 3 seconds before another stereotypy can be scored. This approach produces reliable indices of stereotypy bouts for the majority of repetitive behaviors. It is important to note, however, that the frequency of head-twisting may reflect inflated values as an artifact of scoring protocols. Head-twists are often observed while an animal is pacing back and forth, occurring each time the animals reaches the end of a cage and turns. In this scenario, the continuous pacing would be scored as one stereotypy (i.e., pacing must stop for 3 seconds before it can be scored again). However, if the time in between turns is longer than three seconds then each head-twist is scored as a discrete behavior. In order to assess any impact that the inflated frequency of head-twisting may have had on the total number of stereotypies, we analyzed the data with and without this category of stereotypy. The overall results (i.e., lesioned subjects produce more stereotypies than controls) were similar with and without the head-twist data, therefore we only present findings that include head-twisting.

Statistical Analysis

Behavioral data were collected with The Observer software (Noldus, Sterling, VA; (Noldus, 1991) by trained observers demonstrating an inter-observer reliability ≥ 90% (agreements/[agreements + disagreements] × 100). All data were transformed using the ln(x+1) transformation to respect normal distribution requirements. Analyses of variance (ANOVAs) followed by Fisher's protected least significant difference (PLSD) post-hoc tests (with a significance level of $p < 0.05$) were used for data analyses. Repeated Measures ANOVAs were conducted, with testing context as a within subject factor, in order to assess the effect of testing context (i.e., solo, familiar partner, novel partner, social group) on the expression of stereotypies.

RESULTS

Magnetic Resonance Imaging and Histological Evaluation of Lesions

T2-weighted images of coronal sections are illustrated in previous publications, providing substantial reassurance that the ibotenic acid was injected and was focused in the amygdaloid complex or hippocampal formation (Bauman et al., 2004a,, 2004b). The extent of the targeted lesion was confirmed in one amygdala-lesioned subject that died due to an unrelated illness and whose brain was subjected to histological evaluation of the lesion (see Figure 2 in Bauman et al. 2004b). Analysis of a second series of structural MRIs performed when the subjects were approximately 4 years of age provided additional confirmation of the lesions (see Figure 2 in (Bauman, Toscano, Mason, Lavenex, & Amaral, 2006). Qualitative assessment of the lesion extent revealed that all eight amygdala-lesioned subjects demonstrated substantial bilateral damage to the amygdaloid complex, as indicated by clear shrinkage of the amygdala and/or expansion of the ventricles into space formerly occupied by the amygdala. If there was any sparing of amygdala tissue, it was limited to the most caudal aspects of the amygdala, perhaps including the central nucleus. Analysis of the hippocampus lesions revealed nearly complete bilateral damage for all cases, with minimal sparing of the extreme rostral and caudal portions.

These qualitative observations of the lesion extent are further supported by recent PET neuroimaging of these subjects (Machado, Snyder, Cherry, Lavenex, & Amaral, in press).

Presentation of Findings

Data were analyzed and are presented as the average number of stereotypies per five minute observation (Table 3). The frequency of stereotypies was not normally distributed and contained a number of zero values. Therefore, a ln (X+1) transformation was performed in order to normalize the data and respect theoretical assumptions prior to statistical analyses. The graphs represent the non-transformed data in order to better illustrate the animals' actual behavior.

We analyzed the frequency of stereotypies across all contexts and then within each individual testing context (solo, familiar dyads, novel dyads and social groups). We began by analyzing the overall frequency of stereotypies (termed "All Stereotypies" in graphs and tables) for lesion group differences. We then subdivided the stereotypies into three broad categories based on previous descriptions of repetitive behaviors in nonhuman primates (Lutz et al., 2003): 1) Whole-body stereotyped behaviors are active, often repetitive, movements of the animal's entire body (e.g., back flip, bounce, pace, spin and swing referred to as "Whole Body" in graphs and tables) and 2) Self-directed stereotyped behaviors are directed to the animal's own body (e.g., rock, salute, self bite, self clasp and other self-directed behaviors referred to as "Self-directed" in graphs and tables) and 3) Non-categorical (head-twisting). In keeping with the definitions of Novak and colleagues (Lutz et al., 2003), we did not include the head-twist stereotypy in either the whole-body or self-directed categories. Finally, we analyzed each individual stereotypy separately for lesion group differences, across all contexts and within each context.

Re-evaluation of Videos from 12 Months of Age

Using the more sensitive ethogram designed for this study to assess discrete classes of stereotypies, we again found no lesion differences among the experimental groups ($F_{(2,21)}$ = 0.851, p = 0.4410) when they were approximately 12 months of age. Only four of the twenty-four subjects produced a consistently identifiable stereotypy at 12 months of age (i.e., averaging at least one stereotypy per five minute observation). These four subjects included one amygdala-lesioned subject, two hippocampus-lesioned subjects and one control subject. The control subject's repertoire of stereotypies was limited to head-twisting, while the amygdala-lesioned subject demonstrated primarily self-directed stereotypies and the hippocampus-lesioned subjects produced head-twisting combined with swinging and pacing.

All Contexts Combined

Amygdala- and hippocampus-lesioned subjects displayed more total stereotypies ($F_{(2,21)}$ = 4.819, p = 0.0189) than control subjects when all contexts are combined (p = 0.0072, p = 0.0348, respectively) (Figure 1). Both amygdala-lesioned and hippocampus-lesioned subjects displayed more whole-body stereotypies ($F_{(2,21)}$ = 3.931, p = 0.0355) than control subjects (p = 0.0206, p = 0.0289, respectively) (Figure 2a). In contrast, amygdala-lesioned subjects exhibited more self-directed stereotypies ($F_{(2,21)}$ = 6.049, p = 0.0084) than control and hippocampus-lesioned subjects (p = 0.0027 and p = 0.0295, respectively) (Figure 2b). Lesion differences were also found for head-twists. Hippocampus-lesioned animals head-twisted ($F_{(2,21)}$ = 4.196, p = 0.0293) more than control and amygdala-lesioned subjects (p = 0.0225 and p = 0.0185, respectively) (Figure 2c).

Individual Context Differences

Repeated Measures ANOVAs were conducted in order to assess the impact of test context on the overall production of stereotypies. Results revealed a significant effect of test context ($F_{(3,21)} = 3.224$, $p = 0.0284$) and a significant interaction of lesion condition and test context ($F_{(6,21)} = 2.529$, $p = 0.0295$) on the overall frequency of stereotypies. However, these findings were driven almost entirely by the occurrences of two whole-body stereotypies, pacing and spinning. Both pacing and spinning were affected by the test context ($F_{(3,21)} = 8.261$, $p = 0.0001$, $F_{(3,21)} = 9.462$, $p = <0.0001$, respectively). Subjects, irrespective of lesion condition, spun more in the group setting compared to the solo, novel dyad and familiar dyadic contexts ($p = <0.0001$, $p = 0.0001$, $p = 0.0015$, respectively). Conversely, subjects paced more in the solo context than in the group setting ($p = 0.0037$). There was also a significant interaction between lesion condition and context for pacing ($F_{(6,21)} = 6.390$, $p = <0.0001$). Hippocampus-lesioned, but not control or amygdala-lesioned subjects, altered the amount of pacing based on the context ($F_{(3,28)} = 7.439$, $p = 0.0008$). The hippocampus-lesioned animals paced more in the solo context compared to the group, novel dyad and familiar dyadic contexts ($p = 0.0002$, $p = 0.0008$, $p = 0.0099$, respectively).

DISCUSSION

Delayed Emergence of Stereotypies Following Neonatal Brain Lesions

We have previously reported that monkeys that received lesions of either the amygdala or hippocampus at two weeks of age demonstrated few stereotypies in the first year of life (Bauman et al., 2004a,, 2004b). We have confirmed this finding by re-evaluating videos of the experimental animals at 12 months of age using an ethogram designed to sensitively identify and quantify various stereotyped behaviors. During their second year, it became apparent to staff monitoring these animals that subjects from both lesion groups (but not control subjects) were increasingly producing stereotypies. We therefore formally evaluated the emergence of these behaviors in a variety of experimental conditions, including observations of the juvenile subjects alone, when paired with either familiar or unfamiliar conspecifics and while in their social rearing group. Our observations consistently demonstrated that the amygdala- and hippocampus-lesioned subjects produced more stereotypies than the control subjects (Figure 1) and that the two lesioned groups developed different profiles of stereotypical behaviors (Figure 2). These unique profiles were relatively consistent across different testing paradigms, suggesting that the behavioral context had little influence on the types of stereotypies produced for either lesion group.

Different Profile of Stereotypies for Amygdala and Hippocampus Lesions

Although it may not be surprising that animals with neonatal brain injuries acquire stereotypies after a period of normal development, it is somewhat surprising that damage to the amygdala versus the hippocampus results in a unique profile of repetitive behaviors. Nonhuman primate stereotypies are typically defined as belonging to three broad categories: 1) Whole-body movements (i.e., pacing, bouncing, swinging, etc.), 2) Self-directed movements (i.e., self-clasping, self-biting, etc.) or 3) Non-categorical movements (i.e., head-twisting) (Berkson, 1968; Capitanio, 1986; Lutz et al., 2003). Whole body stereotypies generally involve some form of locomotion, are often associated with small cage size and are potentially reversible; whereas self-directed stereotypies generally do not involve locomotion, are more common in animals that have been socially isolated, and are more resistant to reversal (Mason, 1991). When stereotypies were grouped and analyzed according to these three categories, we found differences between the amygdala- and hippocampus-lesioned subjects. The amygdala-lesioned subjects generally produced a more varied profile of stereotypies compared to hippocampus-lesioned subjects (Table 4). Self-directed stereotypies were more common in the amygdala-lesioned group; two behaviors, salute and self-bite, accounting for more than 65%

of the total. In contrast, the hippocampus-lesioned group produced more head twists across testing conditions and more whole-body stereotypies in the solo condition. Although the head twist data appeared to be driven by three of the eight hippocampus-lesioned subjects (Table 4), we have observed that six of the eight hippocampus-lesioned subjects have continued to develop a similar repertoire of stereotypies (consisting primarily of head twisting and pacing) as the subjects reach adulthood (Toscano & Bauman, unpublished observations).

Our observations of different stereotypy repertoires associated with amygdala or hippocampus lesions expands on previous rodent and nonhuman primate models of repetitive behaviors. Rodents with neonatal damage to the amygdala or ventral hippocampus show different behavioral changes in open field paradigms, though these studies have focused on differences in gross locomotor activity rather than specific stereotypies (Daenen, Van der Heyden, Kruse, Wolterink, & Van Ree, 2001; Wolterink et al., 2001). For example, amygdala-lesioned animals demonstrate increased levels of activity characterized by repetitive circling around the perimeter of the test enclosure, while hippocampus-lesioned animals repeatedly explored objects in the center of the open field (Daenen, Wolterink, Gerrits, & Van Ree, 2002).

Previous nonhuman primate lesion models have evaluated the presence of stereotypies following early lesions of medial temporal lobe structures, but have not assessed discrete classes of stereotypies (see Table 5). For example, peer-reared monkeys that received large medial temporal lobe (MTL) ablations early in life (including the amygdala, hippocampus and surrounding cortex) produced locomotor stereotypies and self-directed behaviors when observed at six months of age (Bachevalier, 1994; Bachevalier, Malkova, & Mishkin, 2001). The abnormal behaviors observed following neonatal lesions of the MTL appear to persist into adulthood (Malkova, Mishkin, Suomi, & Bachevalier, 1997). Early onset stereotypies at six months of age were also reported when the lesion was limited to the amygdala and surrounding cortex, but not when the lesion was limited to the hippocampus (Bachevalier, 1994). Although the animals with neonatal hippocampus lesions did not display early onset stereotypies, these subjects did develop locomotor stereotypies in adulthood (Bachevalier, Alvarado, & Malkova, 1999; Beauregard, Malkova, & Bachevalier, 1995).

In contrast to the early onset stereotypies reported by Bachevalier and colleagues, we did not observe differences in the frequency of stereotypies in the first year of life for either the amygdala or hippocampus-lesioned subjects and attribute this difference to the more naturalistic maternal rearing protocol that we have employed (Bauman et al., 2004a, 2004b). We did, however, observe a protracted emergence of stereotypies following neonatal damage to the amygdala or hippocampus that is consistent with the late-onset stereotypies reported for the neonatal hippocampus-lesioned animals in Bachevalier's later studies. It is important to note that differences in lesion technique (aspiration vs. excitotoxic), rearing conditions and behavioral methodology may preclude direct comparison between results from Bachevalier and colleagues and the present study (see Table 5). For example, our studies utilized ibotenic acid to produce discrete lesions of the amygdala or hippocampus (Bauman et al., 2004a,, 2004b; Bauman et al., 2006). Although observations of these animals is ongoing and histological evaluation of the lesions is not yet possible, previous research indicates that the ibotenic acid lesion technique produces more selective lesions than aspiration lesions by sparing fibers of passage coursing to and from adjacent ventral and medial temporal cortical areas (Meunier, Bachevalier, Murray, Malkova, & Mishkin, 1999) . Our studies have also controlled for social-environmental factors known to contribute to the development of abnormal behaviors in nonhuman primates (i.e., rearing conditions, access to social partners, cage size etc.) (Capitanio, 1986; Lutz et al., 2003). Indeed, the control subjects in our studies displayed more stereotypies than lesioned groups in only one of fifteen testing paradigms conducted in the first year of life (i.e., individual home cage observations between 6–12months of age; (Bauman et al., 2004b). This increase in stereotypies of the control subjects was transient

since none of the control subjects have produced consistent stereotypies in any other testing paradigm conducted in the first 7 years of life (Bauman and Toscano, unpublished observations). We thus attribute the development of stereotypies to the lesion condition, rather than socio-environmental restrictions. In contrast, the control subjects in the Bachevalier studies demonstrated stereotypies early in development (Bachevalier, 1994) which became more prominent in adulthood (Bachevalier et al., 1999; Beauregard et al., 1995). Given that the control subjects in the Bachevalier's studies develop motor stereotypies, it is plausible that the rearing environment may be a contributing factor to the emergence of these behaviors in both lesion and control groups.

Potential Causes of Stereotypies

To assess the underlying cause of delayed-onset stereotypies in the amygdala- and hippocampus-lesioned subjects, it is first necessary to consider factors that are known to induce stereotypies in nonhuman primates. Since it is well known that restricted social and nonsocial environments are associated with behavioral pathology in nonhuman primates, we designed our rearing and housing regimens to facilitate species-typical development within the laboratory environment. Briefly, the subjects in the present study were reared by their mothers for the first six months and experienced daily group socialization in large cages for the first twelve months. All subjects, regardless of lesion condition, developed fundamental aspects of social behavior and displayed species-typical mother-infant and peer interactions (Bauman et al., 2004a,, 2004b). After the first year, the subjects were permanently socially housed with their original rearing group. As discussed above, the control subjects never exhibited pronounced stereotypies, indicating that this rearing strategy provided an environment conducive to species-typical behavioral development.

In spite of these efforts, it is plausible that other factors that we were unable to control may have affected the emergence of stereotypies in the amygdala and hippocampus-lesioned subjects. It has been demonstrated that stress-inducing events (such as frequent immobilization or blood draws) can be associated with stereotypies in laboratory animals (Lutz et al., 2003; Rapp, Vollmer, St Peter, Dozier, & Cotnoir, 2004). Though we have designed our housing protocols and experiments to minimize such known stressors, it is plausible that subjects with neonatal lesions perceive and respond to stress differently than control subjects. In this scenario, the subjects with neonatal brain lesions may develop stereotypies as a coping mechanism for events they perceive as stressful. It is unclear what events may have triggered the onset of stereotypies in both amygdala- and hippocampus-lesioned subjects in the second year of life. The only substantial change in environment between the first year (when stereotypies were rare) and the second year (when stereotypies were pronounced in the subjects with neonatal lesions) was a change in housing from daily 3 hour periods of socialization with members of their rearing group to permanent 24 hour housing with the same group beginning at 12 months of age. While we presume that increased cage size and socialization time is beneficial to all the subjects, we cannot rule out the possibility that this change in socio-environmental complexity may have triggered a stress response in the neonatally lesioned subjects. We have observed that the amygdala-lesioned subjects (but not the hippocampus-lesioned subjects) were consistently the lowest ranking members of their social group (Bauman et al., 2006). We did not, however, observe any behavioral indications that the amygdala- and hippocampus-lesioned subjects were responding adversely to increased socialization. Indeed, our preliminary reports on social interactions of these subjects indicates that both amygdala- and hippocampus-lesioned subjects produced species-typical behavior during this observation period (Bauman, Toscano, Mason, & Amaral, 2007). Comprehensive assessments of these social interactions will be described in future publications.

Our observation that stereotypies emerge in the second year of life, following a period of relatively normal development, suggests that the emergence of stereotypies was not directly caused by damage of the targeted structures, but was possibly due to aberrant maturation of connections and or related structures. Indeed, rodent models have demonstrated that lesions of the amygdala or hippocampus on postnatal day 7 result in a delayed emergence of motor abnormalities that are not seen following similar lesions produced at postnatal day 21 (Daenen et al., 2001; Daenen et al., 2002; Daenen, Wolterink, Van Der Heyden, Kruse, & Van Ree, 2003; Wolterink et al., 2001). Although the precise neural mechanism underlying these late-emerging behavioral changes are not known, evidence from rodent models indicates that neonatal damage to medial temporal lobe structures alters the development of the prefrontal cortex and the subsequent regulation of subcortical dopamine function (Baca et al., 1998; Brake, Sullivan, Flores, Srivastava, & Gratton, 1999; Lillrank, Lipska, Bachus, Wood, & Weinberger, 1996; Lillrank, Lipska, Kolachana, & Weinberger, 1999; Lipska, al-Amin, & Weinberger, 1998; Lipska, Jaskiw, Chrapusta, Karoum, & Weinberger, 1992; Lipska, Jaskiw, & Weinberger, 1994; Lipska & Weinberger, 1998).

Converging results from nonhuman primate models also indicates that dysfunction of the prefrontal cortex and subcortical dopaminergic system may be a consequence of early damage to medial temporal lobe structures. For example, nonhuman primates with neonatal medial temporal lobe lesions display decreased levels of N-acetyl-aspartate (NAA; a marker of neuronal viability) in the prefrontal cortex relative to both normal controls and animals that received similar lesions as adults (Bertolino et al., 1997). These neonatal lesions were also shown to disrupt the manner in which the dorsolateral prefrontal cortex regulates dopamine release by the caudate nucleus (Saunders, Kolachana, Bachevalier, & Weinberger, 1998). Subcortical dopamine systems have been strongly implicated in the production of repetitive stereotypies in animal models (Bedingfield et al., 1997; Canales & Graybiel, 2000; Presti & Lewis, 2005; Presti et al., 2003; Saka, Goodrich, Harlan, Madras, & Graybiel, 2004). Collectively, these studies provide a possible mechanism to account for the delayed emergence of stereotypies following neonatal lesions of either the amygdala or hippocampus via disruption of cortical regulation of dopaminergic systems.

Clinical Implications

Two main findings have emerged from the present study that may provide insight into the neurobiology of repetitive behavior disorders in humans. First, the emergence of stereotypies in nonhuman primates that sustained neonatal damage to the amygdala or hippocampus illustrates how early insults to the developing brain can produce specific psychopathology following an initial period of relatively normal development. These results are in accord with previous rodent models (Lipska & Weinberger, 2000), and clarify data from previous primate models (Bachevalier, 1994) by controlling for other social-environmental factors that are associated with the emergence of stereotypies. These findings may provide insight into the temporal course of repetitive behaviors in clinical disorders such as autism. Recent evidence from young children with autism indicate that while some repetitive behaviors may be clearly manifested as young as age 2, these behaviors may take different forms and/or worsen later in development (Charman et al., 2005; Chawarska, Klin, Paul, & Volkmar, 2007; Chawarska & Volkmar, 2005; Lord, 1995; MacDonald et al., 2007; Richler, Bishop, Kleinke, & Lord, 2007). Second, we have found that damage to different brain structures results in a unique behavioral profile of stereotypies. Although the pathophysiology of repetitive behaviors is unknown, it seems unlikely that repetitive behavior disorders stem from a single common pathogenesis (Lewis & Bodfish, 1998). Our observations that self-directed stereotypies are more frequently observed following neonatal amygdala damage, while head-twisting appears more closely related to hippocampal damage indicates that a unique pathophysiology may underlie different patterns of repetitive behaviors. Unfortunately, it is not clear how our

findings relate to repetitive behaviors in humans. Research on stereotypies in human subjects has often relied on indirect measures (i.e., check lists, rating scales, parental reports, etc.) rather than direct observations of stereotypies (Lewis & Bodfish, 1998). Future research in clinical populations that operationally defines and quantifies discrete classes of stereotypies will provide an essential database to determine which features of repetitive behaviors are common across developmental disorders, and which features are unique to specific disorders (Bodfish et al., 2000). This, in turn, will provide information that is needed to develop and evaluate animal models that may reveal the underlying pathology and suggest effective strategies for clinical intervention.

Acknowledgments

This research was supported by a grant from the National Institute of Mental Health (R37MH57502) and by the base grant of the California National Primate Research Center (RR00169). This work was also supported through the Early Experience and Brain Development Network of the MacArthur Foundation. We thank the veterinary and husbandry staff of the CNPRC for excellent care of the animal subjects. We also thank Pierre Lavenex, Jeffrey Bennett and Pamela Tennant for assistance with surgical procedures, and Melissa Marcucci for assistance with behavioral data collection.

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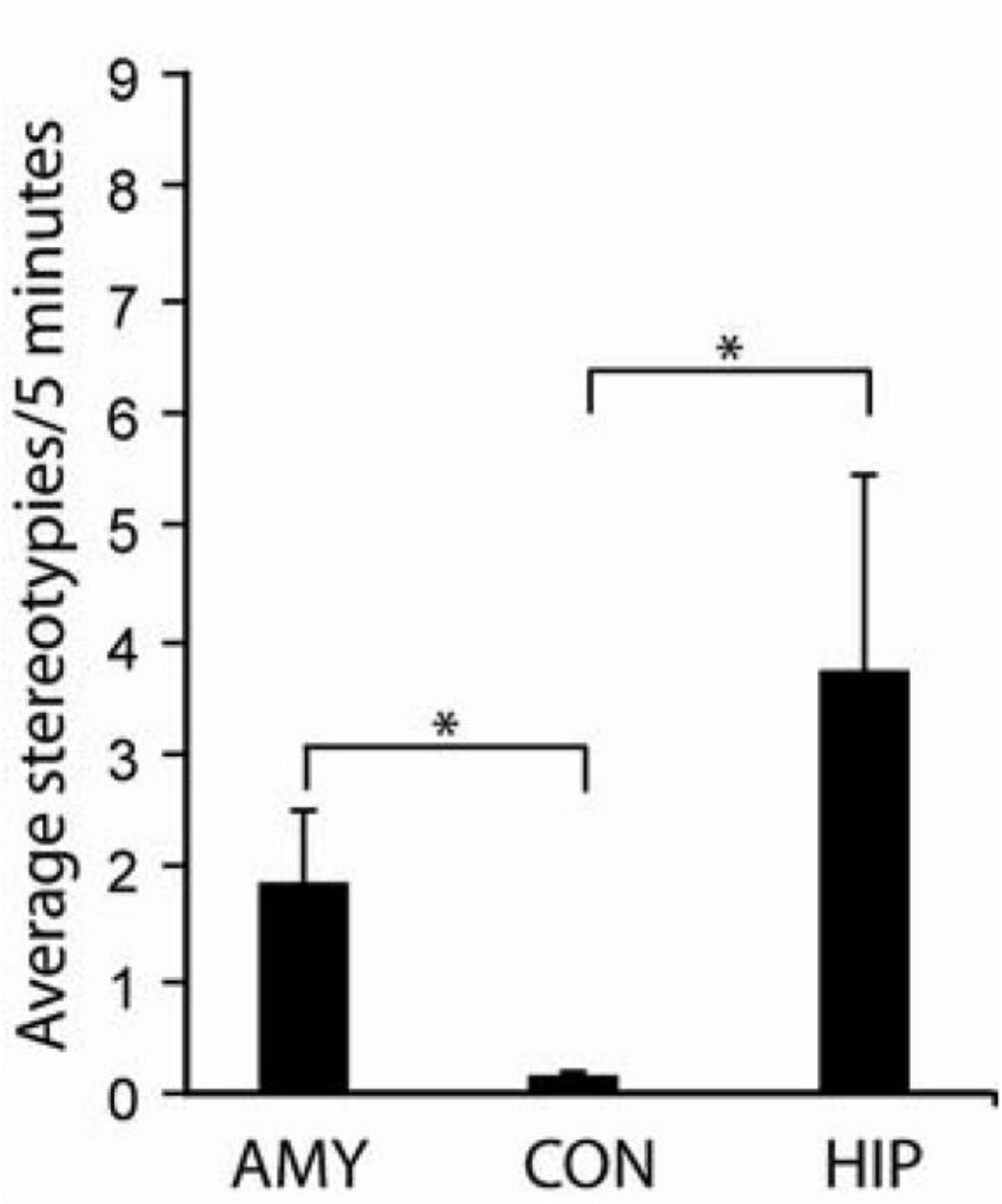

Figure 1.
Graph illustrating frequency of stereotypies produced across all behavioral contexts (Mean +/− SEM per 5 min observation period) for all stereotypies combined. Asterisks denote significant post hoc Fisher PLSD test ($p < .05$).

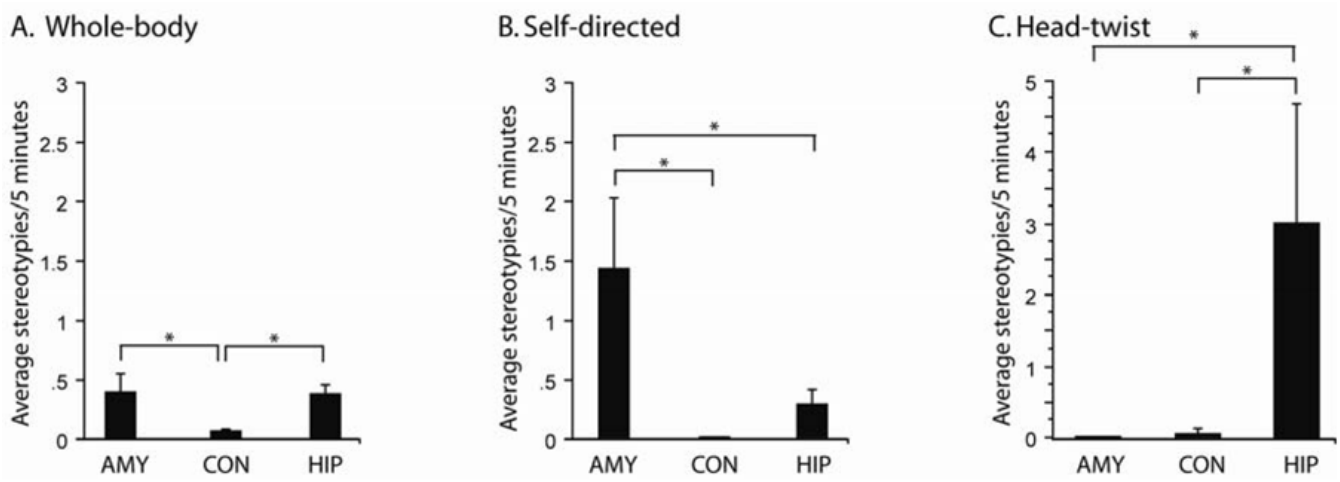

Figure 2.

Graph illustrating frequency of stereotypies produced across all behavioral contexts (Mean +/− SEM per 5 min observation period) divided into categories of repetitive behaviors: a) whole body stereotypies, b) self-directed stereotypies, c) head-twist stereotypies. Asterisks denote significant post hoc Fisher PLSD test ($p < .05$).

Table 1

Summary of Observational Contexts

Test Context	Sampling Method	Description
Solo Observations	5-min focal samples	20 samples per animal alone in home enclosure
Familiar Dyads	5-min focal samples; familiar subjects paired for twenty minutes per session	20 samples per animal while paired in home enclosure
Novel Dyads	5-min focal samples; unfamiliar subjects paired for twenty minutes per session	72 samples per animal paired with a novel conspecific while in a novel enclosure
Social Groups	5-min focal samples; subjects observed undisturbed in group environment	34 samples per animal while in home enclosure

Table 2

Definitions of Stereotypic Behavior

Whole-body Stereotypies	**Definition**
Backflip	At least two consecutive backflips
Bounce	Repetitive hopping or bouncing for at least 3 seconds
Pace	Repetitive, undirected walking or running with the same path repeated for at least 3 seconds
Spin	Repetitive twirling or spinning for at least two rotations
Swing	Repetitive swinging with no progressive movement for at least 3 seconds
Self-Direct Stereotypies	
Rock	Rocking back and forth
Salute (Eye-poke)	Hand held next to or over eye; may include actual poking of eye
Self-bite	Biting oneself; most often of own limbs
Self-clasp	Unusual holding of body part or limb with another body part
Other	Abnormal self-directed behavioral patterns not described above (e.g., nipple clasp)
Non-categorical Stereotypies	
Head-twist	Twisting or rolling of the neck often seen when an animal approaches a corner or barrier

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Table 3

Stereotypy Mean Table

Context	Stereotypy	AMY Mean	HIP Mean	CON Mean	Lesion Effect	Post Hoc
ALL CONTEXTS	**All Stereotypies**	**1.831 ±0.644**	**3.705 ±1.723**	**0.137 ±0.068**	**($F_{(2,21)}$ =4.819, p=0.0189)**	**H>C (p=0.0072) A>C (p=0.0348)**
ALL CONTEXTS	Whole-body	0.386 ±0.153	0.372 ±0.082	0.064 ±0.014	($F_{(2,21)}$ =3.931, p=0.0355)	H>C (p=0.0289) A>C (p=0.0206)
ALL CONTEXTS	Self-directed	1.419 ±0.589	0.287 ±0.121	0.011 ±0.007	($F_{(2,21)}$ =6.049, p=0.0084)	A>C (p=0.0027) A>H (p= 0.0295)
ALL CONTEXTS	Head-twist	0.022 ±0.012	3.023 ±1.670	0.062 ±0.062	($F_{(2,21)}$ =4.196, p=0.0293)	H>C (p=0.0225) H>A (p=0.0185)
SOLO OBSERVATIONS	**All Stereotypies**	**2.013 ±0.755**	**6.113 ±2.252**	**0.106 ±0.059**	**($F_{(2,21)}$ =8.519, p=0.0020)**	**H>C (p=0.0005) A>C (p=0.0321)**
SOLO OBSERVATIONS	Whole-body	0.244 ±0.134	1.100 ±0.272	0.069 ±0.048	($F_{(2,21)}$ =12.938, p=0.0002)	H>C (p <0.0001) H>A (p=0.0010)
SOLO OBSERVATIONS	Self-directed	1.750 ±0.770	0.575 ±0.451	0.000 ±0.000	($F_{(2,21)}$ =4.349, p=0.0263)	A>C (p=0.0082)
SOLO OBSERVATIONS	Head-twist	0.019 ±0.019	4.438 ±1.995	0.037 ±0.037	($F_{(2,21)}$ =6.378, p=0.0068)	H>C (p=0.0058) H>A (p=0.0052)
FAMILIAR DYADS	**All Stereotypies**	**2.463 ±0.988**	**3.031 ±1.459**	**0.063 ±0.031**	**($F_{(2,21)}$ =5.285, p=0.0138)**	**H>C (p=0.0099) A>C (p=0.0108)**
FAMILIAR DYADS	Whole-body	0.481 ±0.235	0.469 ±0.210	0.013 ±0.008	($F_{(2,21)}$ =3.002, p=0.0713)	----------
FAMILIAR DYADS	Self-directed	1.875 ±0.967	0.487 ±0.155	0.038 ±0.025	($F_{(2,21)}$ =4.331, p=0.0266)	A>C (p=0.0078)
FAMILIAR DYADS	Head-twist	0.075 ±0.044	2.075 ±1.289	0.013 ±0.012	($F_{(2,21)}$ =2.947, p=0.0745)	----------
NOVEL DYADS	**All Stereotypies**	**1.819 ±0.701**	**4.486 ±2.859**	**0.153 ±0.081**	($F_{(2,21)}$ =2.659, p=0.0934)	----------
NOVEL DYADS	Whole-body	0.417 ±0.205	0.222 ±0.061	0.068 ±0.031	($F_{(2,21)}$ =1.860, p=0.1805)	----------
NOVEL DYADS	Self-directed	1.391 ±0.613	0.186 ±0.094	0.000 ±0.000	($F_{(2,21)}$ =5.471, p=0.0122)	A>C (p=0.0047) A>H (p=0.0240)
NOVEL DYADS	Head-twist	0.012 ±0.006	4.030 ±2.787	0.085 ±0.085	($F_{(2,21)}$ =2.841, p=0.0809)	----------
SOCIAL GROUPS	**All Stereotypies**	**1.379 ±0.491**	**1.033 ±0.328**	**0.165 ±0.098**	**($F_{(2,21)}$ =4.609, p=0.0219)**	**A>C (p=0.0091) H>C (p=0.0329)**
SOCIAL GROUPS	Whole-body	0.349 ±0.091	0.202 ±0.041	0.085 ±0.045	($F_{(2,21)}$ =4.286, p=0.0275)	A>C (p=0.0080)
SOCIAL GROUPS	Self-directed	1.015 ±0.445	0.213 ±0.144	0.026 ±0.017	($F_{(2,21)}$ =5.408, p=0.0128)	A>C (p=0.0048) A>H (p=0.0270)
SOCIAL GROUPS	Head-twist	0.015 ±0.011	0.618 ±0.308	0.055 ±0.055	($F_{(2,21)}$ =3.335, p=0.0552)	----------

Average number (frequency) of self-directed, whole-body and all stereotypies ± *SEM* is shown for All Contexts Combined, Solo Observations, Familiar and Novel Dyads and Social Groups per amygdala-lesioned subjects (AMY), hippocampus-lesioned subjects (HIP), and sham-operated controls (CON).

Table 4

Lesion Group	ALL	Whole-body	Self-directed	Head-twist
AMY SUBJECT #1	TOTAL = 199	90%	3%	7%
AMY SUBJECT #2	TOTAL = 57	18%	81%	2%
AMY SUBJECT #3	TOTAL = 116	37%	53%	6%
AMY SUBJECT #4	TOTAL = 138	1%	99%	0%
AMY SUBJECT #5*	TOTAL = 762	15%	85%	0%
AMY SUBJECT #6	TOTAL = 3	100%	0%	0%
AMY SUBJECT #7	TOTAL = 294	9%	91%	0%
AMY SUBJECT #8	TOTAL = 570	13%	87%	<1%
AMY TOTAL	**TOTAL =2139**	**21%**	**78%**	**1%**
HIP SUBJECT #1	TOTAL = 125	81%	18%	<1%
HIP SUBJECT #2	TOTAL = 33	67%	0%	33%
HIP SUBJECT #3	TOTAL = 634	16%	<1%	83%
HIP SUBJECT #4	TOTAL = 1298	5%	10%	85%
HIP SUBJECT #5	TOTAL = 163	31%	39%	30%
HIP SUBJECT #6	TOTAL = 133	30%	70%	0%
HIP SUBJECT #7	TOTAL = 9	89%	11%	0%
HIP SUBJECT #8	TOTAL = 1933	2%	<1%	97%
HIP TOTAL	**TOTAL = 4328**	**10%**	**8%**	**82%**
CON SUBJECT #1	TOTAL = 15	100%	0%	0%
CON SUBJECT #2	TOTAL = 5	100%	0%	0%
CON SUBJECT #3	TOTAL = 88	17%	1%	82%
CON SUBJECT #4	TOTAL = 11	100%	0%	0%
CON SUBJECT #5	TOTAL = 14	100%	0%	0%
CON SUBJECT #6	TOTAL = 3	100%	0%	0%
CON SUBJECT #7	TOTAL = 4	0%	100%	0%
CON SUBJECT #8	TOTAL = 20	60%	40%	0%
CON SUBJECT	**TOTAL = 160**	**47%**	**8%**	**45%**

Percentage of head-twist, pace, whole-body, self-directed out of total stereotypies exhibited (x/total #) is shown for each amygdala-lesioned subject (AMY), hippocampus-lesioned subject (HIP), and sham-operated control subject (CON) for all Contexts Combined.

* Replacement amygdala-lesioned subject reared by mother without access to peers for the first 10 months.