Brain Structures Associated with Temperament and Social Behavior in Rhesus Monkeys: An MRI Study

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ABSTRACT

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Temperament is thought to be the foundation for normative personality and subsequent behaviors later in life. To assess the relationship of early temperament with variation in structural brain development, this study examined rhesus macaque mother-infant behavior, and then three years later, used MRI to assess neurostructural differences. Individual differences in mother-infant interactions and emotionality were then linked to brain differences. Extensive behavioral data obtained over the first year of life under both resting and stressful conditions was used to assess the quality of mother-infant interactions and emotionality. The MRI focused on brain volume in areas thought to be related to emotional regulation and such as the cingulate gyrus and corpus callosum structures. These structures are often mentioned as areas that modulate emotions, temperament and general social behavior.

The methods involved in this research include behavior coded from group-housed infant rhesus macaques (*Macaca mulatta*). The subjects were 15 mother-reared subjects, each housed in a social group of 12-20 subjects, living in social settings with their mothers, other adult females, two adult males, and other same-aged subjects; conditions that approximate the social composition of the natural setting. Behaviors related to temperament and mother-infant interactions were assessed using an objective behavioral scoring system. Behavior was coded under three conditions, and each behavioral coding session was 5-minute long. Homecage: Two behavioral coding sessions were recorded weekly for each subject as it interacted naturally with its mother and peers over the first six-months of life. Preseparation (month 6): Two weeks before four, sequential, 4-day social separations, behavioral data were collected once each day. Reunion with mother: Following each of the social separations, data were collected twice immediately following return to mother and again on the morning before the separation. Subjects underwent MRIs 1-2 years later when they were 2-3 years of age.

The result of this research was that during pre-separation interactions, anterior cingulate size to brain ratio showed a positive correlation with mutual ventral contact (being cradled and held closely), a measure of the use of mother as a secure base to calm anxiety and fear.

Keywords: cingulate, corpus callosum, temperament, attachment, rhesus

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Temperament refers to infant natural inclinations reflected in emotional and social behaviors. Core temperaments are typically present early in infancy, remain fairly stable over time (Goldsmith et al., 1987), and are predictive of behavioral and mental health problems later in development (Ormel et al., 2005). A recent study suggests that the triadic model of human infant temperament can also be applied to neonatal rhesus macaques (Kay, Marsiske, Suomi, & Higley, 2010). Similarly, temperament can be regarded as the basis of future personality traits such as reactivity, aggression and self-regulation. Temperament is an important indicator of future cognitive, social and perhaps neuroanatomical development (Kay et al., 2010), and could be regarded as the "nature" in the basis of future personality; whereas, the early treatment of the infant by the mother and others could be considered the nurture side. As Thomas and Chess adeptly point out, the two interact to produce subsequent differences in personality (Thomas & Chess, 1989).

Forming and maintaining a strong attachment bond with the mother is crucial to the rhesus infants' survival and emotional well-being. Attachment quality can be defined as the degree to which infants perceive their parents as a reliable, accessible secure base (Kraemer, 1997). Secure attachment is formed as the infant perceives that the mother is responsive, available and sensitive to their needs (Eberly & Montemayor, 1999). There are four distinct characteristics of attachment that have been identified in primates: use of mother as a secure base (having mother as a secure base from which one can explore); use of mother as a safe haven (being able to return to the mother when in need of comfort or in danger); proximity maintenance

(remaining fairly close to the mother), and separation distress (being in a state of stress during the absence of mother) (Bowlby, 1969).

While undoubtedly temperament is the product of inborn neuroanatomical differences (Higley, Chaffin, & Suomi, 2011; Higley, Suomi, & Chaffin, 2011), it is also clear that temperament and attachment interact to affect brain structures (Comer, 2010). Studies, for example, show a relationship between early environment, parental treatment and a resulting effect on cortisol and neurotransmitter systems, which has been hypothesized to result in differences in brain structures (Kalin, Larson, Shelton, & Davidson, 1998; Koch, McCormack, Sanchez, & Maestripieri, 2012; Shannon, Champoux, & Suomi, 1998). Mother-infant treatment also affects temperament, modulating specific genes such as the serotonin transporter gene, and studies show this GxE interaction affects brain development (Bennett et al., 2002; Higley, Chaffin, et al., 2011; Higley, Suomi, et al., 2011; S Spinelli et al., 2007).

Recent neuropsychological experimentation has started to tease out the neural underpinnings of attachment and temperament. Numerous studies explore the correlations and connections between temperament, attachment, and brain structure and anatomy (Davis, Bruce, & Gunnar, 2002; Iidaka et al., 2006; Kalin et al., 1998; Kalin, Shelton, Davidson, & Kelley, 2001). Studies have also assessed mother-infant attachment and its relationship to neuroanatomy. Many of these studies make mention of, and show correlations with, the cingulate (Blatchley & Hopkins, 2010; Gillath, Bunge, Shaver, Wendelken, & Mikulincer, 2005; Pujol et al., 2002). This makes sense, as the cingulate is part of the limbic system, linking cortical functioning with areas that modulate emotionality, a central aspect of the attachment bond. Newbury and Rosen (2012), for example, found that mice in an open field test that were more active showed a relatively larger corpus callosum volume. This could suggest a relationship with the

temperamental trait, activity, or more likely in this type of a paradigm, indicate a relationship with emotionality or reactivity. Whittle, et al., (2009), showed that anterior cingulate folding patterns were correlated with temperamental affiliation in juvenile humans. Their findings illustrated that neurodevelopmental processes have an effect on individual differences in temperament. Based on these findings, we hypothesize that:

1- Controlling for head and body size differences, rhesus macaque infants showing frequent positive mother-infant interactions during early development would exhibit a larger corpus callosum relative to their peers with less frequent positive mother-infant interactions.

2- Controlling for head and body size differences, developing rhesus macaque infants with a higher rate of positive mother-infant interactions would exhibit larger cingulate volume relative to their peers with infrequent positive mother-infant interactions.

Literature Review

Temperament

Temperament is thought to be the foundation of personality. Temperamental differences in behavior are thought to be based on our neural makeup. Temperament refers to adolescent natural inclinations reflected in thoughts, emotions, and behaviors. Core temperaments are typically present early in infancy and remain fairly stable over time (Goldsmith et al., 1987). Temperament is defined as individual differences in emotional, motor and attentional reactivity measured by latency, intensity, and recovery of response, and self-regulation processes such as effortful control that modulate reactivity (Rothbart, 2007). The New York Longitudinal Study identified nine dimensions of temperament through interviews with parents about their children (Rowe & Plomin, 1977). These dimensions included activity level, intensity, threshold,

approach/withdrawal, rhythmicity, adaptability, mood, distractibility, and attention span persistence. Since then psychometric studies have refined these categories and it now shows strong correlations with temperament in other animals. These dimensions are related to the Big Five personality factors of Extraversion, Neuroticism, Conscientiousness, Openness and Agreeableness (Evans & Rothbart, 2007). Basic core aspects of temperament appears to be common between humans and non-human primates (Kay et al., 2010).

Brain Structures

Corpus callosum. Studies suggest that emotionality, as measured by freezing vs. activity in an open field test, is correlated with the relative size of the corpus callosum. For example, researchers using mice found subjects with a large midsagittal area of the corpus callosum and showed less freezing and higher levels of activity in an open field apparatus (Newbury & Rosen, 2012). Investigating this in a closely related primate would allow a better modeling of the relationship between the corpus callosum and emotionality in humans. Given that the corpus callosum is larger in humans than in many other animals and the relatively close phylogenetic relationship between rhesus and humans, we hypothesize that the corpus callosum would be larger in those monkeys that were low in reactivity as infants.

Cingulate gyrus. The cingulate cortex is a medial structure that runs between the corpus callosum and the cerebral cortex. This structure is part of the limbic system of the brain and is involved in emotional regulation, executive functioning, as well as learning and memory (Baleydire & Mauguiere, 1980). Studies suggest that the cingulate is linked to mother-infant attachment quality and other social behaviors. Zahn, et al., for example, showed that there is a strong link between attachment and the cingulate gyrus by having subjects read scenarios or

scripts that reflect attachment and/or social behavior and looking at the neural activity that resulted from those scripts (2009). They found that subjects with higher empathic concern, or attachment, showed greater activity in the cingulate when working through the scripts that evoked attachment toward another person. In primates, mothers are used as a secure base is to mitigate or control stress responses and to increase the frequency of social interactions (Barr et al., 2008).

The Development in the Rhesus Macaque

Rhesus macaques make a good model for human temperament and neuroanatomy as they are a lot like humans in both their neurodevelopment and their social behaviors (Higley, Chaffin, et al., 2011; Higley, Suomi, et al., 2011; Morton, Kyes, Kyes, Dwindler, & Swindler, 2005; Simona Spinelli et al., 2009). Rhesus macaque social and emotional development occurs in a fairly regimented pattern that in many ways parallels that of humans. Newborn rhesus infants start out with functional, but immature, sensory systems (S. Suomi, 1984). Also as in humans, infant rhesus monkeys show an immature regulation of emotion and a high degree of curiosity, particularly when they have a secure attachment (Harry F. Harlow, 2008). As infants mature, they show increased locomotion as well as object play, with a wide variation in rates (S. Suomi, 1984). By three months of age, rhesus macaque infants regularly engage in rough and tumble play, with males showing it more often than females (S. Suomi, 1984). Also at three months monkeys start to demonstrate fear of strangers and an ability to regulate their own arousal and fear (Hinde, Rowell, & Spencer-Booth, 1964 1964). At six to seven months of age, maternal protective defense decreases and aggression by the infant first appears and the infant also begins to have more individualistic differences in behavior via initiating reciprocal grooming with others and displaying sexual behaviors with other age-mates (Eaton, Johnson, Glick, & Worlein,

1986 & Worlein, 1986; S. Suomi, 1984). Because the behavioral growth pattern for healthy rhesus macaques is so well defined, paralleling human developmental patterns, and because they are similar to humans in their attachment style, a monkey displaying aberrant behaviors would likely be easily identifiable, allowing researchers to investigate neuroanatomical correlates of these individual differences to determine the underlying biology. Determining how individual differences in neuroanatomy correlate with individual variation behavior will help us to understand more about the relationship between the temperament and the neurosubstrate.

The mother-infant attachment bond is crucial to infant survival and well being, not only for the delivery of sustenance, but protection of the infant's physical well being. Four unique characteristics of attachment have been identified for primates: 1- use of mother as a secure base (having mother as a secure base from which one can explore); use of mother as a safe haven (being able to return to the mother when in need of comfort or in danger); proximity maintenance (remaining fairly close to the mother), and separation distress (being in a state of stress during the absence of mother) (Bowlby, 1969). A secure attachment in macaques is typically demonstrated via positive mother-infant relationship behaviors such as mutual ventral behavior (Harry Frederick Harlow, 1958). A secure attachment is highly correlated with differences in emotionality (Harry F Harlow & Zimmerman, 1959). Emotionality is the component of emotion that is physiological or observable. It's a measure of an individuals emotional reactivity to a stimulus (Reber, Reber, & Allen, 1985). Because the cingulate gyrus is involved in emotional processing (Damasio et al., 2000) we hypothesized that rhesus macaques with a higher frequency of positive mother-infant relationship behaviors will have a larger cingulate volume relative to their peers.

Neurophysiological Brain Imaging of Temperament

Human studies using neuroimaging show some brain differences in adult subjects that are associated with temperament and/or personality (Cloninger, 2000); however, the literature is limited when it comes to healthy immature subjects. Another shortcoming in the literature is that a lot of the temperament data is based on self-report measures rather than objective long-term observation; this is a common limitation when using human subjects (Davenport, Yap, Simmons, Sheeber, & Allen, 2011). Studies suggest that using self-report measures is less likely to provide accurate and objective behavioral data than objective observation in a controlled environment (Patricia et al., 2004).

Primates as Models of Human Behavior and Neuroanatomy

Primates are used to model many forms of psychopathology and neurobiology and in many different areas of research, from brain imaging (Kawai & Sugiyama, 2010), and psychopathology (S. J. Suomi, 1982), to vaccine studies (Murthy, Salas, Carey, & Patterson, 2006 & Patterson, 2006). Because of their close similarity to humans, nonhuman primates are widely regarded as ideally suited to model many forms of neuropsychology and psychopathology and are often used to test for biological underpinnings (Morton et al., 2005 Dwindler, & Swindler, 2005), because when compared to other animals, their brain, and genetic makeup is more similar to humans (Smith & Minium, 1983). However, monkeys are more expensive to obtain and maintain, therefore making research with them as subjects less common.

Methods

Subjects

The 15 adolescent monkeys (23-32 months) used in the imaging study are drawn from a large ongoing longitudinal study investigating genetic and environmental factors affecting

neurobiology, behavior, and alcohol consumption (Espinel & Higley, 2013). They were born between 2004 and 2005 at the National Institutes of Health Animal Center (NIHAC) at Poolesville, Maryland. The stock progenitors of the subjects originated from the Harlow Primate Laboratory (University of Wisconsin, Madison, WI) in the 1950s, and pedigrees have been traced back to the founders. None of the subjects were closely related. All procedures were approved by the NIH, NIAAA Animal Care and Use Committee.

    Rearing conditions. Following birth, infants were assigned to one of two rearing conditions. Mother-reared subjects (n = 15) were housed with their mother in social groups approximating natural conditions, with 8–10 adult females (including the infant's mother), the infant's father and another adult male, and other similar-aged infants. In the second condition, adopted animals (n = 4), were removed from their mother at birth and fostered to another unrelated, lactating female in a different cage that had recently given birth and the female then raised the infant as her own. The adopted infants were treated identically to the infants reared by their biological mother (Lorenz et al., 2006). Preliminary behavioral and neuroanatomical analyses showed no differences between these two groups; thus for statistical comparisons the adopted infants were treated identically to the infants reared by their biological mother. The behavioral ethogram used for data collection has been used extensively (see description below). Given our hypotheses, in this paper, we only focused on mother-infant interactions and social behavior with other conspecifics.

    These social groups were housed in indoor/outdoor enclosures measuring 2.44×3.05×2.21 m (indoor) and 2.44×3.0×2.44 m (outdoor). The animals had free access to both the indoor and outdoor portions of their enclosures with the exceptions of very cold or inclement weather or cleaning. The floors of the enclosures were covered with wood chips allowing daily

foraging for sunflower seeds and other foods. The lights in the indoor portion of the enclosure were maintained on a 12:12 cycle (7:00 a.m. to 7:00 p.m.). The animals in the social groups were fed Purina High Protein Monkey Chow (#5038) and received water ad libitum. Supplemental fruit was provided three times each week (Shannon et al., 2005).

Data Collection

Behavioral observations.

Focal behavioral scoring was performed in 5-minute sessions during one of three social conditions. Infants were scored twice a week during the homecage phase as they interacted naturally with their mother and peers over the first six-months of life. This phase lasted 24 weeks. This baseline phase typically measures non-stressed homecage mother-infant and peer interactions. Behaviors typical of non-stressful mother-infant interactions are typically seen during this phase. The pre-separation phase lasted 2 weeks and subjects were scored twice a day. While this phase is similar to the homecage phase, the coders are in front of the cage twice as long and twice during this phase, the subjects are captured and anesthetized for physiological sampling. Previous studies have shown elevated cortisol levels during this phase relative to the homecage phase, suggesting a modest degree of stress (Shannon et al., 1998; Shannon et al., 2005). During the reunions, two 5-minute sessions of behavioral data were collected each day. Prior to each reunion, animals were separated from their mothers on Monday morning and caged alone for four days. Mothers were also removed from the social group. Subjects were then returned to their mothers for a weekend reunion and separated again on Monday. This separation-reunion cycle occurred weekly for four weeks. Eleven of our subjects went through the separation paradigm while four were not separated from their mothers but had identical data

collected during the same time period. This paradigm has been published in detail in other publications (S Spinelli et al., 2007). Thus the reunion phase lasted four weeks, with 2 behavioral coding sessions immediately following mother-infant reunion, and one session on the morning before the next separation. In this phase, both mother and infant must readjust to the demands of being returned to a social setting where they have been absent for the past 4 days. Social challenges of the mother by other females are frequent during this period. As has been done in other studies, the mean of the frequency scores of each phase was used as the dependent variable (Barr et al., 2008).

An exhaustive, mutually exclusive, objective scoring system described in other studies (Shannon et al., 2005) was used to record behavior. The behaviors represent the typical mother-infant ethogram of a rhesus macaque, and record behavior categories that characterize anxiety, emotion, activity, mother-infant relationships, and psychopathology. Definitions for behaviors that were recorded are listed in Appendix A. All behaviors were recorded as average frequency per five minutes per week. Multiple observers, who were trained by a senior researcher and achieved interobserver reliabilities of at least 85%, made all behavioral observations. In rare instances, behaviors were not scored because of a necessity to remove an animal from the group for medical observation or because observers could not reliably visualize the infant to score it among the other members of the social group (Barr et al., 2008). In these cases a regression was performed using the overall average for that individual to replace the missing value.

Brain volume acquisition.

The monkeys ranged in age from 23-32 months when brain images were taken. Each monkey was initially anesthetized using intramuscularly administered ketamine, 10 mg/kg, removed from its cage, and prepared for its scan. Anesthesia was maintained throughout the MRI

scan with continuous infusion of intravenously administered propofol, 30 to 50 mg/kg/h. An

individually molded thermoplastic facemask was secured to a custom-made monkey head holder

to reduce head movement during scanning. Vital signs were monitored continuously during the

study.

Images were acquired using a 3.0-T scanner (Allegra; Siemens Medical Solutions, Inc,

Malvern, Pennsylvania). The parameters for the 3-dimensional, T1-weighted magnetization

prepared rapid gradient-echo acquisition were as follows: repetition time/echo time/inversion

time, 2500/3.49/1000 ms; 1 slab of 224 sections, 0.60-mm section thickness, 0.30-mm spacing,

8°-flip angle, 256256-pixel acquisition matrix, and 4 signals acquired. The acquisition was run at

4 excitations using dual-surface coils (Nova Medical, Inc, Wilmington, Massachusetts). The 3-

dimensional slab was placed over the entire brain, centered, and angled on the anteroposterior

commissures line.

Image processing was performed with Analyze 7.5 (Biomedical Imaging Resource, Mayo

Foundation, Rochester, Minnesota). For manual tracings of the regions of interest (ROIs), T1-

weighted images were converted to cubic voxel dimensions of 0.39 mm. All images were

oriented in a standardized oblique plane to eliminate any bias in section angle. In the

standardized orientation, the transaxial plane was parallel to the anteroposterior commissures line

and perpendicular to the inter-hemispheric fissure.

Anatomical Subdivisions

The ROIs were defined using the rhesus macaque atlas of Saleem and Logothetis (Saleem

& Logothetis, 2007) and were measured by 1 rater blinded to subject sex and rearing condition; a

second independent rater performed identical measurements to establish reliability and accuracy

of the measurement, which was calculated for every ROI on each brain side. The minimum value

for interrater and intrarater reliability, calculated as intra-class correlation coefficients, was 0.89 to 0.97, respectively. Manual tracing for the cingulate cortex was performed in the coronal plane, edited in the sagittal or axial planes, and reedited in the coronal view; the CC was drawn using the mid-sagittal view, and the intracranial volumes (ICVs) were traced on the axial view.

The ICVs were calculated by tracing each axial section, excluding the skull and the dura. A semi-automated, threshold-based, region-growing algorithm was used to outline the brain in each axial section. The ICV was defined and subsequently measured as all gray and white matter tissues and CSF volumes in both hemispheres, including part of the midbrain. The inferior border of the pons was chosen for demarcation because it is readily and reliably identifiable on monkey brain images (Lyons, Yang, Sawyer-Glover, Moseley, & Schatzberg, 2001 Moseley, & Schatzberg, 2001).

Data Analysis

Commercially available software (SPSS 21) was used for statistical analysis. Behavioral frequency scores for the behavior collected (full list is included as Appendix A) were used as dependent variables. Analyses were performed using the average frequency of the behavior over the course of the specific phase (homecage, pre-separation and reunion). The data were segregated according to the condition that data were collected (homecage, pre-separation, and reunion respectively) and separate sets of analyses were performed for each of the testing conditions. The average frequency for each of the behaviors referenced in the appendix was used as the dependent variable and run using multiple regression. The independent variable was the relative volume of the brain structure (cingulate or corpus callosum). Regression controlled for sex and separation condition, and the coefficients are reported as partial correlations. We controlled for separation condition, as four of our subjects were not subjected to the separation

condition. The behavior of these macaques was measured over the same period but they were not separated from their mothers. We did not want that to be a confounding variable in the analyses. Multiple regressions were used to investigate the relationships between the core temperament behaviors and the three ROI volumes (Anterior Cingulate, Medial Cingulate, and Corpus Callosum). Data were summarized for the ROI as both a gross volume and also as a ratio in relationship to the overall brain volume. We ran the multiple regressions using both overall ROI volume and ROI as a ratio of overall brain volume but only rate the ratio figure as brain volume could be a confounding variable. In running the partial correlation we controlled for both sex and separation condition. To control for the number of pairwise comparisons we used a Bonferroni correction.

Results

Table 1- Pearson correlation coefficients were computed using the anterior cingulate as a ratio of brain size to various behaviors outlined previously and available in Appendix A.

Anterior Cingulate/Brain	Pre-separation- Mutual Ventral
	$r = -.734$, $p = .04$ (after the Bonferroni correction)

Cingulate Gyrus

Prior to the Bonferroni correction, five of the mother-infant behaviors showed statistical significance with the anterior cingulate to brain ratio. After applying the Bonferroni correction our central measure of mother-infant attachment, the number of times that an infant used it mother as a secure base (mutual ventral cradling) remained significant, showing a significant negative correlation with anterior cingulate to brain ratio ($p = .04$). Infants leaving their mother who showed a smaller anterior cingulate to brain ratio, were also more likely to show high rates of play. While it failed to reach traditional levels of significance, ($r = -.676$ and $p = .1$), there was

a negative relationship between the relative size of the anterior cingulate and the pre-separation play behavior.

Discussion

Cingulate

We hypothesized that rhesus macaque infants with a high frequency of positive mother-infant interactions would exhibit a larger cingulate volume relative to subjects with infrequent positive mother-infant interactions and our data showed just the opposite. During the pre-separation phase, there was a negative correlation with rates of mutual ventral mother-infant cradling, and a trend for more play in the subjects showing high rates of mutual ventral contact. High rates of mutual ventral contact with mother are seen as evidence of a subject that is capable of reducing its arousal through maternal contact, allowing it to return to play with its cage mates. a possible indication of a secure attachment (Kraemer, 1997). Our data suggest a connection between having a secure base, or attachment, and the size of the cingulate, although in a direction opposite to that predicted.

Corpus Callosum

We hypothesized that greater amounts of activity would be positively correlated with relative corpus callosum volume. While the existing literature on the subject is somewhat sparse, we were hoping our data would replicate the previous work performed by Newbury and Rosen (2012) where they showed less freezing and more active exploration in mice during an open field test in subjects with a relatively larger corpus callosum. While there were correlations between several behaviors indicative of emotionality and the relative corpus callosum volume, the correlations disappeared after applying our Bonferroni correction. With our sample size and the large number of behaviors, we may be underpowered to detect such relationships.

Conclusion

In general, the results of our experiment suggest that there is a connection between the relative volume of a developing rhesus macaque's cingulate gyrus and positive mother-infant interactions. More specifically there seems to be a connection between the cingulate size and the use of mother as a secure base.

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Appendix A

Behavior Definitions

Social Other– Includes sitting, standing, locomoting, or lying within arms reach of another animal (in reference to the focal animal). Has precedence over locomotion. Is not scored with stereotypy or stypic.

Social with Mother or Infant – Same as social with other except that focal animal is within arms reach of mother or infant depending on which is the focal animal.

Social with Mother or Infant and Other– Same as social with Mother or Infant depending on which is the focal animal and includes social with an other animal.

Social with Mother or Infant and Social Groom – Same as social with Mother or Infant depending on which is the focal animal and includes giving or receiving groom from another animal. Cleaning/grooming of focal animal by another or by focal animal to another animal, including scratching, biting, liking, or rubbing. Social groom assumes social contact. Does not include grooming between the mother and the infant.

Play – Performance of any play behaviors including: initiating play by "play face," wrestling (rough and tumble), chasing, tagging, swatting, bobbing, biting, pulling, lunging, mouthing, and responding positively to play from another animal. If focal animal is initiating play with an unreciprocating partner it is still scored as play. "Unwanted play," meaning if the focal animal is the receiver and is not reciprocating is scored as social and vocals (if vocalizing). Social play assumes social contact and includes sexual play or mounting of one other animal, whether appropriately or inappropriately oriented, with or without thrusting, mount attempts, rump presenting, and receiving a mount from another animal.

Self-Directed Behaviors– Includes firm manual or pedal gripping of self, which is not a component of an ongoing behavior. Also includes self-grooming, self-scratching, etc.

Self-Mouthing Behaviors – Includes sucking of self, not biting, at any bodily appendage.

Environmental Exploration– Any active manual, oral, or pedal examination, exploration, or manipulation of the physical environment, or the attempt to do the same. Includes manipulating or playing with chow while eating or drinking. Does not include active play on the substrate, chewing chow, or passively holding an object (food or other).

Non-Contact Aggression– Performance of aggressive behaviors including: aggressive chases and threats.

Contact Aggression– Performance of aggressive behaviors, which include bites, slaps, and aggression that results in physical contact to the recipient. Must actually contact the subject.

Receive Non-Contact Aggression– The focal animal is the recipient of aggressive chases of threats.

Receive Contact Aggression– The focal animal is the recipient of aggressive behaviors including bites, slaps, and aggression that results in physical contact.

Inactive– Absence of directed movement, social behaviors, and environmental manipulation (i.e., no simultaneous scoreable social or non-social behaviors, except self-directed and self-mouthing behaviors and vocalizations). Includes bouncing in place.

Locomotion– Any self-induced change in location of self. Includes changes in location by mans of walking, running, dropping from ceiling to floor, swinging across the cage (but not chain swinging), and bouncing, rolling, hopping on all fours, bouncing around the cage, and "displays." Also includes any movements across the substrate. Social has precedence over locomotion. Note: if a motor pattern is repeated more than 3 times it is scored as a Stereotypy.

Stereotypy/Stypic– Stereotypy: Any repetitive, patterned, and rhythmic locomotive movement. Stereotypy is only scored following the third repetition of the motor act. Thereafter, during the test session, it is scored whenever it occurs. Repetitive, stereotyped behaviors that do not involve locomotion are scored as Stypics: Idiosyncratic non-locomotive stereotyped actions such as repetitively saluting, picking the teeth, or strumming the mesh. As with Stereotypies, Stypic is only scored after three repetitive acts.

Distress Vocalizations– Any vocal sound emitted by the subject. Includes coo, bark, screech, squeal, etc. Can be scored with any other behavior. Sounds made by coughing or sneezing are not vocalizations.

Social Groom– Cleaning/grooming of focal animal by another or by focal animal to another animal, including scratching, biting, liking, or rubbing. Social groom assumes social contact (except social with mother or infant). Does not include grooming between the mother and the infant.

Mutual Ventral– Infant is belly to belly with mother, on the mother's nipple, or has one arm around mother.

Mutual Ventral and Social Other– Infant and mother are belly to belly and focal animal is within arms length of another animal.

Mutual Ventral and Social Groom– Infant and mother are belly to belly and the focal animal is receiving or giving groom to another animal. Does not include grooming between the mother and the infant.

Mutual Ventral and Locomotion– Scored only when mother is the focal animal and mother and infant are belly to belly while the mother is changing the location of herself and her infant.

Mutual Break– Occurs when Infant and Mother stop being ventral or belly to belly.

Reject/Withdraw– Mother rejects approaches made by the infant for mutual ventral contact. It is also scored if there is mutual ventral contact and one of the animals attempts to break and the other does not want to.

Give Mother Infant Groom– Cleaning/grooming by the focal animal to the mother or infant depending on which is the focal animal. It includes scratching, biting, liking, or rubbing. Includes grooming between the mother and the infant only.

Receive Mother Infant Groom– Cleaning/grooming to the focal animal by the mother or infant depending on which is the focal animal. It includes scratching, biting, liking, or rubbing. Includes grooming between the mother and the infant only.

Approach by Infant– Occurs when infant creates social contact when already out of social contact.

Leave by Infant– Occurs when infant breaks social contact after being social with Mother.

Approach by Mother– Occurs when mother creates social contact when already out of social contact.

Leave by Mother– Occurs when mother breaks social contact after being social with Infant