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AGOUTI VIABLE YELLOW MATERNAL BEHAVIOR, NEONATE BEHAVIOR,
AND LATER OFFSPRING BEHAVIOR

MOLLIE H. WOHLING
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Reviewed and approved* by the following:

Sonia A. Cavigelli
Assistant Professor of Biobehavioral Health
Thesis Supervisor

David J. Vandenberg
Associate Professor of Biobehavioral Health
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Early postnatal interactions between mother and offspring can have lasting effects on offspring behavior, especially in response to novel situations. The present study quantified maternal behavior in *Agouti viable yellow* (A^{vy}); a mice strain in which environmental cues have long-term influences on behavioral development. Offspring nipple attachment behavior was quantified to determine if it predicted rates of maternal behavior, and the long-term effects of maternal behavior and nipple attachment on offspring exploratory and anxiety-related behavior were examined. Careful observations of four litters of A^{vy} dams and offspring interaction were made over the first 8 days postpartum specifically recording maternal nursing, grooming, eat/drinking, nest-building, and licking behaviors as well as offspring nipple attachment. In postweaning and adulthood, pups were behaviorally tested for exploratory tendencies and anxiety-related traits. Results revealed that A^{vy} mothers display similar behavioral trends in regards to nursing, licking, eating/drinking, nest building, and self-grooming in comparison to other inbred strains of mice. The “pup directed” behaviors of nursing and nest building decreased over days 2-8, while maternal eating/drinking increased. A significant positive association between frequency that a pup was licked by the dam and time it was attached to the nipple was found. Frequent licking and nipple attachment were associated with measures of decreased exploratory and increased anxiety-related behaviors in adolescent and adult offspring. While A^{vy} mice are prone to a variety of mutation-induced traits including obesity and anxious behavior, they show similar rates of maternal behavior and connection to later offspring behavior when compared with other inbred and outbred mouse strains.

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Chapter 1

Introduction and Background

Maternal behavior in rodents and humans alike plays a significant role in offspring development, specifically on development of behavioral inhibition and fear associations. It has been demonstrated in rodents that the early postnatal interactions of mother and pups play an important role in inducing long-term behavioral changes. The present study had three main goals. The first was to quantify maternal behavior in a mouse strain for which maternal behavior has not yet been quantified and in which early developmental cues such as diet have been shown to have a strong influence on behavior and brain development (Hagan et al., 2001; Waterland, Tavisano, & Tahilini, 2007). The second was to determine if pup suckling behavior relates to the rate of maternal behavior shown to the pup. Lastly, the long-term effects of maternal behavior and pup suckling on offspring exploratory and anxiety-related behavior was examined. As background, information on the aforementioned behaviors will be provided.

Maternal care

Rodents and humans alike engage in maternal and nurturing behaviors with offspring. In rodents, these behaviors include various nursing postures, nuzzling, licking, and rearranging pups (Stern & Johnson, 1989). Studies comparing certain inbred and outbred mice strains have demonstrated natural variations in postpartum maternal care, but in general show that dams spend the majority of their time during postnatal days 1-6 nursing, followed by eating, then pup grooming. They spend relatively small amounts of time nest building, self-grooming, and drinking (Champagne et al., 2007). Rodent maternal behaviors such as nursing, retrieving, nest

building, and licking decrease in the early postnatal window as pups age (Champagne et al., 2007; Reisbick, Rosenblatt, & Mayer, 1975). Dam eating behavior increases over the first week (Champagne et al., 2007).

Maternal care in the early postnatal days is crucial to offspring behavioral development, such as shaping exploratory behaviors and fearfulness (Caldji et al., 1998). Rodent studies help demonstrate the effects of interactions of mothers and their offspring on behavioral outcomes. In a study of Long-Evans hooded rats, mothers were classified as either high licking/grooming and arched back nursing (LG-ABN) or low LG-ABN. Offspring of high LG-ABN moms show reduced fearfulness in novelty response tests compared to offspring of low LG-ABN mothers (Caldji et al., 1998). Physiologically, those offspring of high LG-ABN mothers showed increased central benzodiazepine receptor density in the central, lateral, and basolateral nuclei of the amygdala as well as in the locus coeruleus, increased alpha-2 adrenoreceptor density in the locus coeruleus and decreased CRH receptor density in locus coeruleus (Caldji et al., 1998).

A cross-fostering study of Norway rats showed that the individual differences in the expression of genes in brain regions that regulate stress reactivity can be transmitted from one generation to the next through behavior rather than genes, and that this mechanism involves differences in maternal care in the first week of life (Francis et al., 1999). Specifically, offspring of low LG-ABN dams that were housed with high LG-ABN dams later showed less fearful behaviors in novel conditions compared to the control offspring of low LG-ABN dams reared by low LG-ABN dams. The shaping of behavioral development in offspring has been attributed to epigenetically mediated processes. Increased licking/grooming of pups alters the methylation status of the promoter regions of glucocorticoid receptors in the hippocampal region of the brain. These differences emerge in the first week of life, could be reversed with cross-fostering, and remained stable into adulthood (Weaver, 2004). Other studies experimentally controlled maternal behavior by reducing the amount of anogenital licking (AGL) of offspring. In Norway rats, zinc

sulfate was applied to the dams' snouts to interfere with the reception of pup chemosignals (Moore & Power, 1992). Saline was added to the dams' water to reduce the appetite for pup urine. Both techniques reduced anogenital licking. Results showed that maternal AGL was a significant predictor of certain juvenile offspring behavior (Moore & Power, 1992). It was found in a study of Long-Evans rats that dams preferentially licked male pups anogenitally more than female pups (Moore & Morelli, 1979). Further research showed that secretions from the preputial gland in male rat urine makes the male scent more attractive to dams than female urine, driving the differential licking of the sexes (Moore & Samonte, 1986).

A recent study that examined within-litter variance in rat maternal behavior by tracking interactions of the mother and individually marked offspring found novel results. Individual offspring that were licked more frequently in the early postnatal days showed decreased exploratory behavior in adulthood (Ragan, Loken, Stifter, & Cavigelli, 2010). The present study was modeled similarly; individually marked offspring were examined to track behavior and maternal interactions. It was hypothesized that pups that were licked more frequently than their littermates may demonstrate decreased exploratory behaviors as adolescents and adults.

The first step in understanding how maternal care plays a role in offspring development is to understand and quantify specific behaviors. The present study aimed to quantify maternal behaviors in a strain of mice with a mutation known to be linked to increased stress responsiveness (Harris, Zhou, Shi, Redmann, Mynatt, & Ryan, 2001). Through observations of dams and their pups on postnatal days 2-8, trends in behavior frequency over the week were examined. Based on information previously collected about 129Sv and C57BL/6J inbred mice strains, it was predicted that A^{yy} mothers would also decrease the amount of time spent on pup-directed behaviors, including nest building and nursing and licking of offspring, over time on postnatal days 2-8 (Champagne, Curley, Keverne, & Bateson, 2007). In turn, she would increase self-focused behaviors including eating/drinking and self-grooming as time progressed.

Influences of maternal care on behavioral inhibition and neophobia

In an effort to develop treatments for anxiety-related disorders in humans, it is important to understand possible causes of the disorders. The relative contributions of genetic and environmental factors in the development of mental disorders have long been disputed. Research suggests that early life stressors such as sexual and physical abuse as well as childhood neglect increase the risk of developing mood and anxiety disorders (Heim & Nemeroff, 1999). This interaction is thought to be partially mediated by corticotropin-releasing factor (CRF), a neuropeptide that plays a role in coordinating endocrinologic, autonomic, immunologic, and behavioral stress responses. Early life stressors lead to CRF hypersecretion which is implicated in mood and anxiety disorders (Heim and Nemeroff, 1999). Appropriate amounts of maternal care are thought to protect against those influences.

Human behavioral inhibition

The scientific label for human personality traits of 'cautious' and 'shy' is 'behavioral inhibition', and 'fearless' and 'outgoing', for 'uninhibited' (Kagan, Reznick, & Snidman, 1987). Behavioral inhibition is characterized according to both social and nonsocial components and is often accompanied by physiologic changes in resting heart rate, hypothalamic activity, and basal cortisol levels (Kagan et al., 1987). Stable behavioral inhibition throughout childhood has been found to predict an increased risk for developing anxiety disorders in adulthood (Hirshfeld et al., 1992). Specifically, studies have shown that behavioral inhibition is correlated with social anxiety and the occurrence of major depressive disorder (Jean-Yves et al., 2010). Van Amerigen et al. (1998) found that social inhibition, compared to nonsocial inhibition, accounted for the relationship between behavioral inhibition as a whole and anxiety disorders.

Exploratory behavior in rodents

Exploratory behavior in rodents is often measured by the time taken to approach a novel object or time spent exploring novel objects and environments. The tendency to avoid the unfamiliar is often assumed to reflect 'neophobia', or the fear of novelty because there is evidence that neophobic rats show greater glucocorticoid response to novelty than neophilic rats, a physiologic change associated with a typical stress response and fear (Cavigelli & McClintock, 2003). Novel environments can be classified into two categories, novel physical and novel social environments, with novel social environments containing an unfamiliar rodent as a novel stimulus, whereas novel physical environments contain unfamiliar objects as the stimuli. Individual responses to physical novelty do not predict individual responses to social novelty (Cavigelli et al., 2007). In addition, male rats identified as neophobic in infancy have shorter lifespans than their non-neophobic brothers. They were 60% more likely to die at any point than the less fearful siblings (Cavigelli & McClintock, 2003). Increased mortality rates demonstrate the severity of the impact of neophobia as a behavioral trait.

Stress response pathways related to exploratory behavior

There is a large endocrine component involved in physiologic reactions to stress that has significant implications in development of fearful behavior. The hypothalamus, pituitary gland, and adrenal gland mediate a complex set of feedback mechanisms characterizing the endocrine system's primary involvement in stress mediation. This system, commonly termed the hypothalamic-pituitary-adrenal (HPA) axis involves a class of steroid hormones called glucocorticoids. In response to a physical or psychological stressor, the hypothalamus releases corticotropin-releasing factor (CRF) that then stimulates the anterior pituitary to release

adrenocorticotrophic hormone (ACTH). In the adrenal cortex, ACTH stimulates the secretion of glucocorticoids. The predominant glucocorticoid in humans is called cortisol, while corticosterone is the functionally equivalent hormone in rodents (de Kloet, Joëls, & Holsboer, 2005). Glucocorticoids play a role in a variety of functions including metabolic regulation and immune response. They are known to affect breakdown of carbohydrate, protein, and lipids while also contributing to immune and inflammatory responses (Buckingham, 2006).

Glucocorticoids play an important negative feedback role in the HPA axis by inhibiting action in the hypothalamus and adrenal cortex. Abnormal function of this feedback loop has implications in overactive stress responses. Sustained high levels of glucocorticoids in the blood are associated with a variety of pathologies including hypertension, immunodeficiency, impaired growth development, and depression (Buckingham, 2006). It has been demonstrated that neophobic individuals have larger HPA axis responses than nonfearful individuals (Cavigelli & McClintock, 2003). Specifically in rodents, it is shown that individuals with low glucocorticoid receptor levels in the hippocampus have a decrease in sensitivity to corticosterone, which diminishes negative feedback inhibition and leads to increased HPA activity (Meaney et al., 1995).

Offspring cues

Studies performed with rats have shown that offspring actually provide their moms with stimuli that drive their own nurturance (Stern, 1997). Various tests found that pups' noises provide cues to prevent moms from injuring them as well as playing a role in initial arousal and orientation to litter. Overall, tactile stimuli from pups ventral probing or making snout contact elicit maternal responses involved in retrieval, licking, and kyphosis (upright nursing posture) (Stern, 1997). The present study hopes to provide insight into the maternally-directed behavior of

the pups (probing, snout contact, and nipple attachment) as well as the pup-directed behaviors of the dam (licking) and how these interactions affect exploratory behavioral development. Overall, significant findings of the importance of early mother-offspring interactions will be helpful in understanding the importance of early intervention programs in humans.

Another measure of mother-pup interaction has been offspring attachment to the dam's nipples. It was determined in rats that it is olfactory and/or gustatory cues on the skin of the dam's ventrum that drives infant offspring's orientation and attachment to nipples (Hofer, Shair, & Singh, 1976). No known studies have examined the relationship of infant nipple attachment to later behaviors in rodents. The present study aims to quantify individual pup attachment and examine its relationship to maternal behaviors as well as later exploratory and anxiety-related behavior. It was hypothesized that within litters, pups would be attached for variable amounts of time and those attached to the nipple most frequently would receive the less body and anogenital licks in postnatal days 2-8. Extending the scope of nipple attachment beyond infancy, it was hypothesized that increased nipple attachment would be associated with less exploratory adolescent and adult behaviors.

Agouti mice

The present study made use of four litters of inbred *Agouti viable yellow* (A^{vy}) strain mice. They are called 'Agouti viable yellow' because of a mutation that modifies transcription upstream of the agouti gene, resulting in ectopic expression of the agouti protein. This mutation results in golden yellow coat color and obesity as well as increased risk for diabetes and tumors (Morgan, Sutherland, Martin, & Whitelaw, 1999). Mice with the agouti mutation display a variety of coat colors ranging from completely yellow to pseudoagouti (nearly dark brown) with mottled yellow and brown variants in between. Agouti-mutated mice have also been known to show increased

stress responsiveness compared to a/a black mice (Harris, Shi, Redmann, Mynatt, & Ryan, 2001). Stressors applied for longer periods of time were associated with an increase in corticosterone and more post-stress weight loss than wild-type mice. Mice with the agouti mutation also spent less time in the open arms of an elevated plus maze test. It is thought that the increased stress responsiveness in agouti-mutated mice may be a compensation for chronic inhibition of melanocortin receptors, a group of hormone receptors antagonized by the agouti protein and play a role in both coat color and feeding behavior (Harris, Shi, Redmann, Mynatt, & Ryan, 2001).

Goals and hypotheses

The first goal of the study was to quantify A^{vy} maternal behavior. It was predicted that A^{vy} dams would show similar nurturance behavioral patterns to other inbred mice strains. Pup-directed behaviors including licking, nursing, and nest-building would decrease over time in the immediate postnatal window while maternal eating/drinking and self-grooming would increase. The second goal was to examine the relationship between offspring nipple attachment and maternal behavior, specifically licking. It was predicted that the frequency of maternal licking of offspring would be negatively associated with frequency of time points attached to the dam's nipple. Lastly, it was a goal to examine the long-term effects of maternal behavior and nipple attachment on offspring exploratory and anxiety-related behavior. It was hypothesized that within litters, pups licked more and attached to the nipple less frequently would show decreased exploratory and increased anxiety-related behaviors in adolescence and adulthood.

Chapter 2

Materials and Methods

General methods

Four yellow female A^{vy} mice carrying the mutation on the Agouti locus along with four black males obtained from the Jirtle Lab at Duke University were mated. Resulting offspring had a mix of coat colors ranging from yellow to black. The four litters were born two to three weeks apart. Each of the four litters was housed in a plastic cage in a room maintained at 21°C and with a 12L:12D lighting schedule (lights off at 20:00). Food and water were available *ad libitum* by animal caretakers who checked on the mice daily. The cage remained relatively undisturbed by human contact for the first week postpartum. Studies have shown that handling of pups increases the frequency of maternal licking/grooming and arched back nursing behaviors (Francis et al. 1999). Minimal handling of pups occurred to prevent alterations in maternal behavior. Aside from daily marking of pups, data about mother-pup interactions was recorded by watching through the cage. Naturalistic observations (described below) took place during the first 8 days after birth (Figure 1). The present study focused solely on maternal mouse behavior because male mice are not known to play a significant role in raising offspring. Rather than focusing on variance between litters in the maternal behavior, this study quantified within-litter differences of individual mother-pup interactions. Analyses included data from recordings of days 2 through 8. Previous studies have shown that behavior of mothers within the day following birth is highly variable and after that differences between maternal behaviors stabilize (Champagne, Francis, Mar, & Meaney, 2003). Behavioral testing to examine anxiety related behaviors was conducted

after weaning (adolescence) and in adulthood. Weaning and separation of litters by sex, took place around postnatal day 28 for each litter. Litters were sacrificed on postnatal day 72.

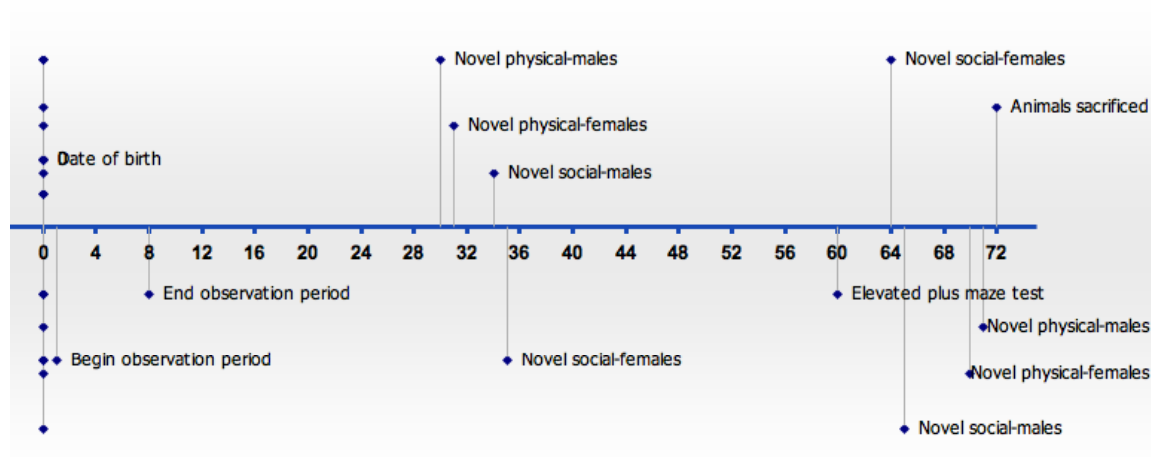


Figure 1: Study timeline (days).

The following methods received approval by the Pennsylvania State University Institute for Animals Care and Use Committee (IACUC #29898, Appendix A).

Maternal and pup behavior observations

Dams and pups were observed for naturalistic behavioral tendencies on postnatal days 1-8, with date of birth defined as day 0. In this time frame, one-hour observations occurred four times per day around 09:00, 13:00, 17:00, and the 21:00 hour. The 21:00 hour observation occurred in red light because the room was in the dark cycle at that time. Non-toxic silver Sharpie® markers were used to mark pups with an individual identifier on their backs and ventral side each day after the 21:00 hour observation. At this time, pups were also weighed. Dams were separated for 15 minutes each day while the marking and weighing occurred. Video recording of the cage and litter occurred around the clock across postnatal days 1-8.

Each surveillance hour required two observers, either graduate or undergraduate students from Dr. Sonia Cavigelli's lab or Dr. David Vandenberg's lab. Observer one was in charge of recording all frequency and duration data, while observer two ran the stopwatch and was the scribe for the every-3-minute time point observations. Observer two looked for maternal behaviors, nursing postures of the dam, maternal states, and pup behaviors (Appendix B). Maternal actions were typically recorded as durations. If the behavior lasted less than 3 seconds, the start time was recorded and underlined, and no finished time was recorded. Observation of pup behaviors included snout contact and ventral probing. Each time one of these behaviors occurred, the ID of the performing pup was recorded. Nursing postures were recorded by their type and durations (marked by start and finish time).

Every 3 minutes of an observation session, beginning at time 0:00 minutes, point samples were collected. For these, the second of the two observers recorded the following: IDs for pups visible and in contact with the litter "huddle", IDs for pups who were away (more than one pup length) from the huddle, and IDs for those who were visibly attached to dam's nipples. If a pup was in easy licking distance from the dam defined by the semi-circular area a centimeter in around the dam's nose, the ID letter was underlined. IDs of pups actively moving at the time of recording were circled; however, this data was not specifically used in analyses. A total of 21 time points were recorded for each hour-long observation.

Behavioral testing

The A^y offsprings' willingness to explore a physically and socially novel environment was determined using exploration arenas called the novel physical and social tests (Appendix C). These are designed to measure general behavioral responses to different kinds of minimally threatening novelty to index behavioral inhibition.

The novel physical test

Testing took place in a room separate from the colony room. The testing arena (50 cm W x 50 cm L x 24 cm H) was made of white Plexiglas walls with a clear Plexiglas cover that was subdivided into a 3x3 grid with tape. The arena floor was padded with corncob bedding. The area was indirectly lit with a red light. Three novel objects of approximately the same size—a plastic tunnel, an upside down ceramic bowl, and right side up plastic bowl—were placed a few centimeters from a different corner of the arena (Figure 2). The five-minute test began when a mouse was lowered into the arena in an empty metal food bowl in the corner unoccupied by a novel object. The cover was slid into place once the mouse was in the arena. The metal food bowl was cleaned with 70% ethanol and allowed to dry between mouse trials to eliminate any distracting scents. Mice were free to explore the arena, and the latency time to approach the first novel object was recorded. Other measures included frequency of: gridlines crossed, digging, rearing, and grooming motions, nose to an object, forepaws on an object, whole body on an object, whole body in an object, and presence of feces at the end of the test. Latency time to first object was the only measure used in the analyses. Novel physical tests were performed around 21:00 hour on postnatal days 30 (for males), 31 (for females), and again on days 70 (for females), and 71 (for males). The tests were video recorded and later coded with the Noldus Observer program (Wageningen, Netherlands).



Figure 2: Novel physical arena

The novel social test

At postweaning (day 30), four days after mice were tested in the novel physical arenas, a similar measure was performed to examine responses to novel social interaction (on day 34 for males, and day 35 for females). A second round of the novel social tests was run on postnatal day 64 (for females) and day 65 (for males), this time four days prior to the round of novel physical testing. In the same arena, lighting, and room as described above (for the novel physical test), interactions with a novel (unrelated) mouse were observed in the five-minute test. These tests also occurred around 21:00 hour. A mouse from a different litter than the test subject was placed in a small plastic cage with a vented lid. The cage was placed on its side near one corner of the arena (Figure 3). In the same way that the novel physical test began, the test subject was lowered into

the cage in a metal bowl in an empty corner. The primary measure used from this test was the latency to approach the novel mouse cage. Other measures recorded included frequency of grid lines crossed, nose to mouse cage, forepaws mouse cage, body on mouse cage, grooming, rearing, and fecal droppings left. The tests were video recorded and later coded with the Noldus Observer program.



Figure 3: Novel social arena

Elevated plus maze

On postnatal days 60 (for females) and 61 (for males), each litter underwent testing on an elevated plus maze (EPM). This five-minute test is designed to measure anxiety-related behavior. The maze is made of Plexiglas and consists of two opposing open arms (30 cm L x 5 cm W) without walls and two opposing closed arms (30 cm L x 5 cm W x 15cm H) with walls. The arms extend from a common central platform (5 cm x 5 cm) raised 38.5 cm above the floor (Figure 4).

All arms and walls of the EPM were covered in opaque black paper. At the start of each trial, the mouse was placed on the center platform between the four arms and observed for 5 minutes. The primary measure used from this test was the latency to enter one of the open arms. Animals that enter open arms more readily are thought to be less fearful. Two observers recorded a variety of other behaviors as well. An escape behavior, one thought to reveal a form of anxiety and discomfort with being in a new environment, included rearing (standing on hind legs). Other anxiety-like behaviors included freezing (staying still for 2 or more seconds), and self-grooming. Records of the following ethologic behaviors were also taken: head dip, when the mouse peers over edge of arm; stretch attend, when the mouse engages in a forward stretch/lunge where the hind legs remain planted in place; and sway, when the mouse remains in one location and rocks body side to side. The EPM test was performed in the same behavioral testing room as the novel physical and novel social tests at 21:00 hour in indirect red light. Video recordings of each test were coded using the Noldus Observer program.



Figure 4: Elevated plus maze (EPM). From Coulbourn Instruments website. Retrieved March 26, 2011 from <http://www.coulbourn.com/elevatedplusmaze.html>

Methods of data analysis

Data were analyzed with SPSS statistical software. To achieve goal 1, to quantify maternal behavior of A^{vy} mice descriptive measures and graphical representation were primarily used to understand how mothers distribute their time in days 2-8 were used. Paired T-test were used to test for significant differences in mean behavior durations on days 2 and 8. To test hypothesis 2, Pearson correlation tests were used to identify significant associations between nipple attachment and frequency that pups were licked. Repeated measures analyses of variance

(ANOVAs) were used to identify differences in mean litter means of nipple attachment frequency as well as licking frequency over days 2-8. To test hypothesis 3, Pearson correlation tests were used to identify significant associations between nipple attachment and licking and the adolescent and adulthood novelty and EPM tests. Data for licking frequency, snout contact frequency, latency times of behavioral tests, and percentage of time pups attached to dams nipple was centered around the within sex and litter mean to control for litter and sex effect differences. It should be noted that 3 pups did not survive beyond day 8, reducing the sample size (n=36) to 33 for analyses that included data beyond day 8.

Chapter 3

Results

Quantifying maternal behavior

Our observations provided insight into how A^{vy} mothers divide their time between various behaviors. Key behaviors observed included nursing, eating/drinking, nest building, and self-grooming. When comparing the mutually exclusive behaviors, the dams of the four litters spent 70-75% of their time nursing offspring; 13-19% of their time eating and/or drinking; 6-9% of their time self-grooming; and 2% to 3% of time nest-building (Figure 5a-d).

Over the course of postnatal days 2-8, there was variation in the percent of time spent on each behavior (Figure 6). Overall, daily time spent nursing decreased over postnatal days 2-8, but appeared to peak on day 7 and decline by day 8 (Figure 7a). On average, there was a 31% decrease in nursing from day 2 to 8. Time spent eating and/or drinking more than doubled over the week (Figure 7b). Daily self-groom duration remained relatively steady on average over the course of days 2-8 (Figure 7c). On average, time spent nest building decreased by 61% over the week (Figure 7d). Further examination with paired T-tests for each behavior revealed that the changes from day 2 to 8 were not statistically significant, all greater than $p = 0.13$ level, and that the graphical representation of behavior trends provides clues as to how the A^{vy} dams spend their time.

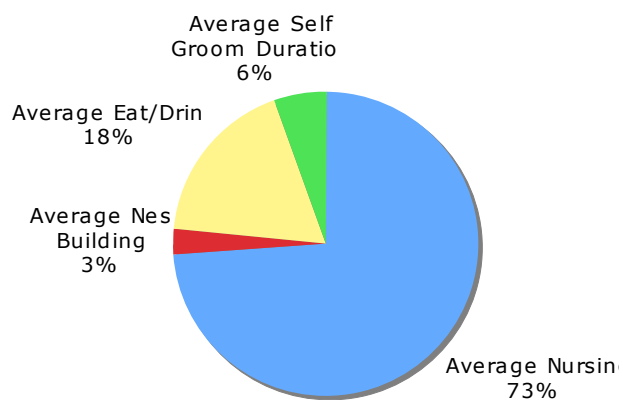


Figure 5a: Maternal behavior durations (litter 55)

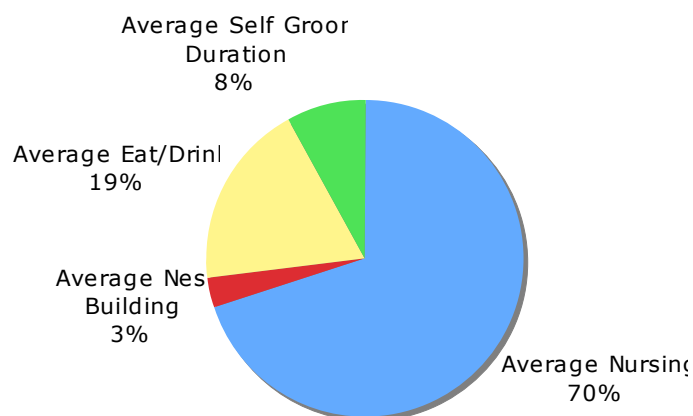


Figure 5b: Maternal behavior durations (litter 63)

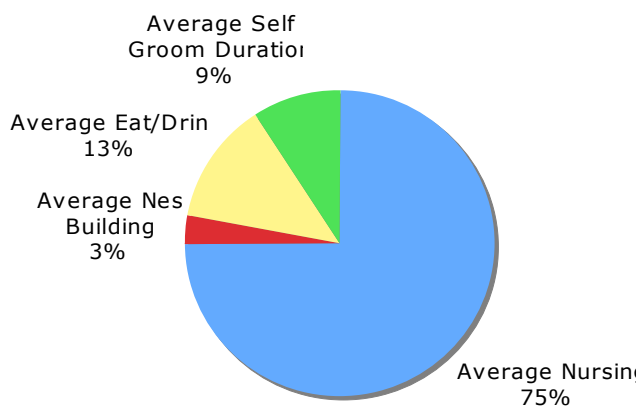


Figure 5c: Maternal behavior durations (litter 87)

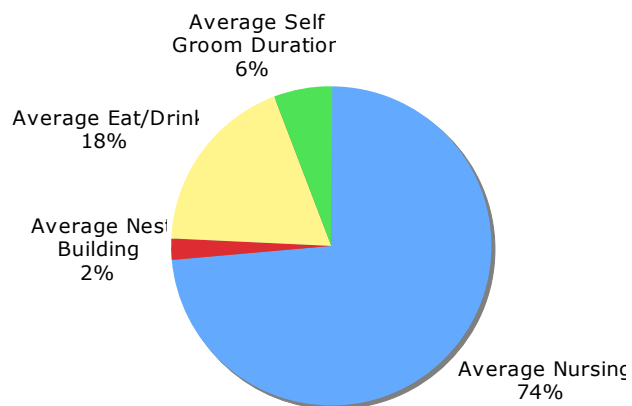


Figure 5d: Maternal behavior durations (litter 91)

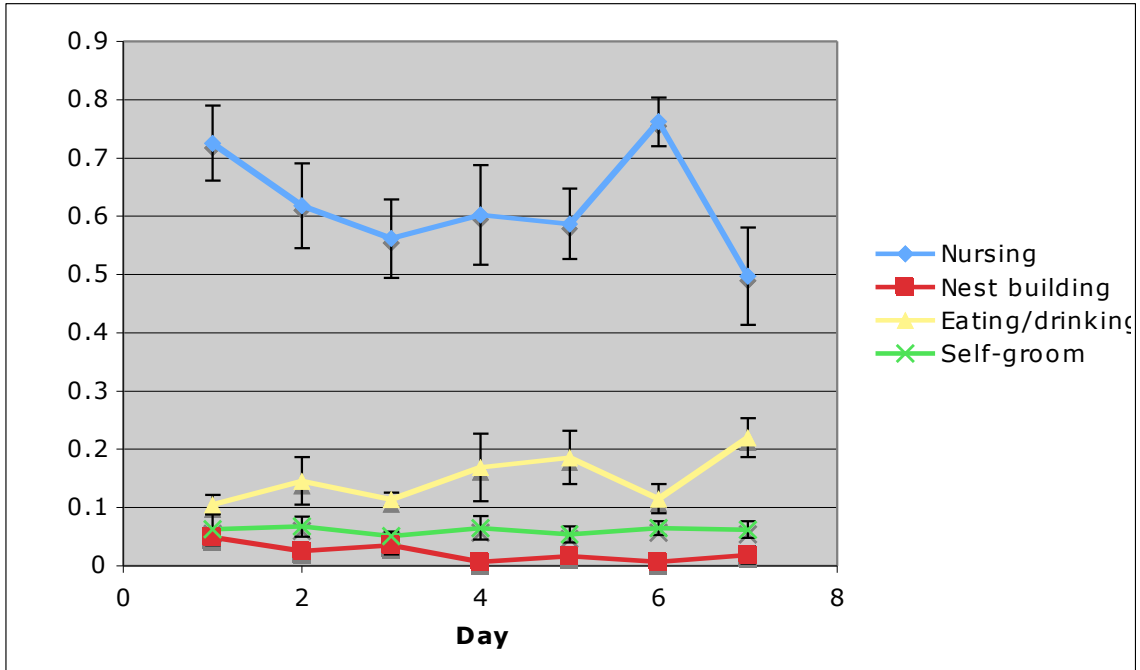


Figure 6: Average maternal behaviors during days 2-8

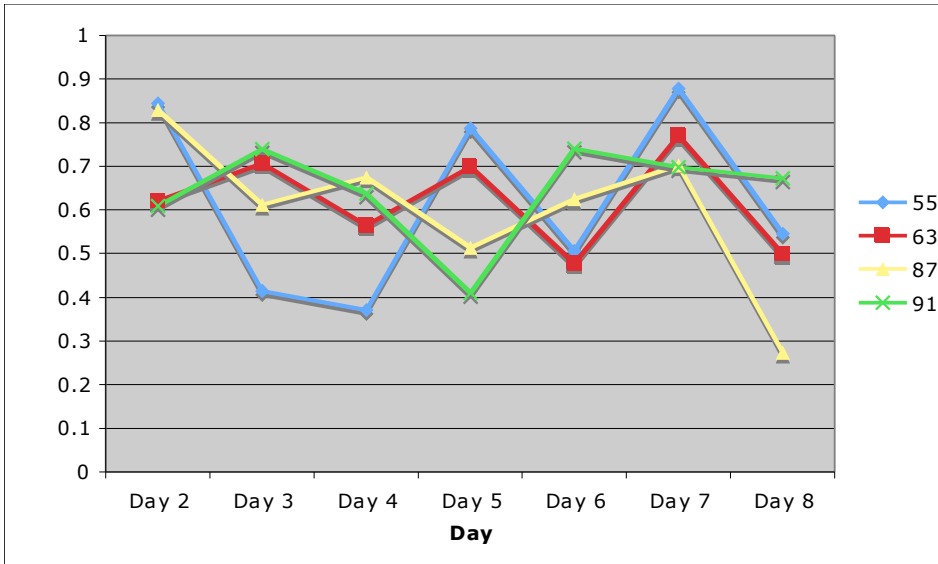


Figure 7a: Nursing by litter, days 2-8

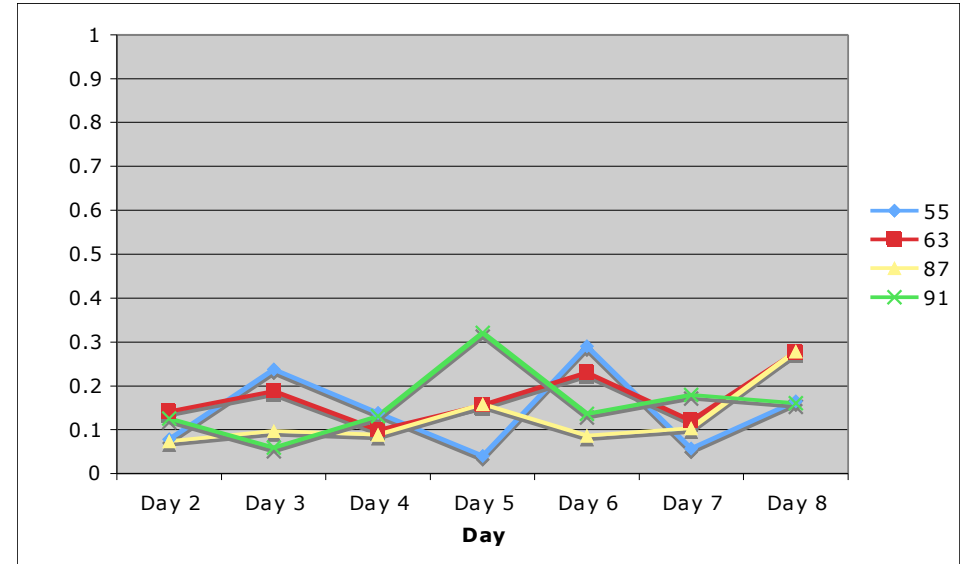


Figure 7b: Eating/drinking, days 2-8

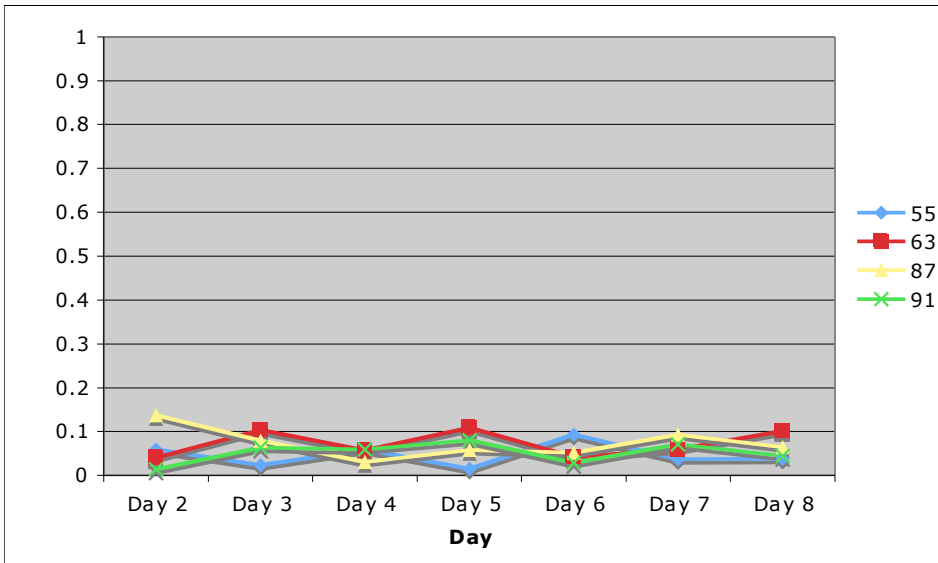


Figure 7c: Self-grooming by litter, days 2-8

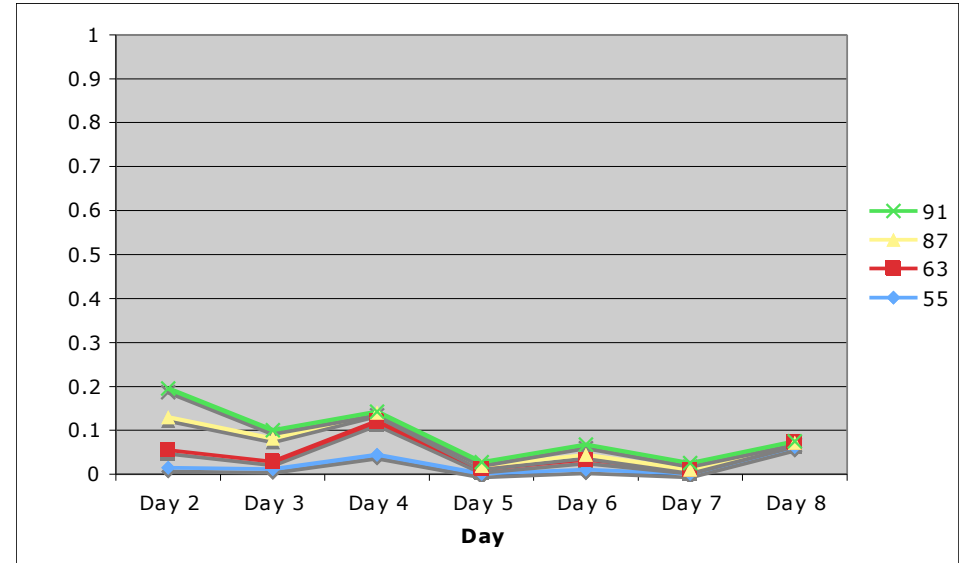


Figure 7d: Nest building by litter, days 2-8

Quantifying licking trends toward individual offspring

No significant relationships between total number of maternal licks received by offspring and maternally directed behaviors like ventral probing and snout contact were observed (ventral probing: $r = 0.158$, $n = 34$, $p = 0.19$; snout contact: $r = 0.22$, $n = 34$, $p = 0.11$). Repeated measures ANOVAs for licking over postnatal days 2-8 revealed that for combined body and anogenital licks, there were differences of licking between litters, but no difference in licking by sex. The litter by sex interaction revealed that within at least one litter there was a dam who differentially licked her male and female offspring (litter effect: $F(3, 33) = 24.9$, $p = 0.00$; sex effect: $F(1, 33) = 0.27$, $p = 0.61$; litter*sex: $F(2, 33) = 3.30$, $p = 0.04$). Looking at strictly anogenital licks over days 2-8, there were only differences in licking by litter, not by sex (litter effect: $F(3, 33) = 9.32$, $p = 0.00$; sex effect: $F(33, 1) = 0.24$, $p = 0.63$; litter*sex: $F(2, 33) = 0.03$, $p = 0.99$). Similarly, with body licks, it was evident that there were no between-subject differences in licking by sex, only litter. However, a litter by sex interaction showed that a sex difference in body licking occurs within at least one litter (litter effect: $F(3, 33) = 34.4$, $p = 0.00$; sex effect: $F(1, 33) = 3.71$, $p = 0.07$; litter*sex: $F(2, 33) = 11.67$, $p = 0.00$). Tukey HSD post-hoc comparisons revealed that male offspring were licked more than females in litter 63. On average, total body and anogenital licking frequency decreased from days 2-8 by 46%. Anogenital licks alone decreased 51%, and body licks alone decreased 39% (anogenital: $t_3 = 4.19$, $p = 0.025$; body: $t_3 = 1.55$, $p = 0.22$; total: $t_3 = 0.10$) (Figure 8).

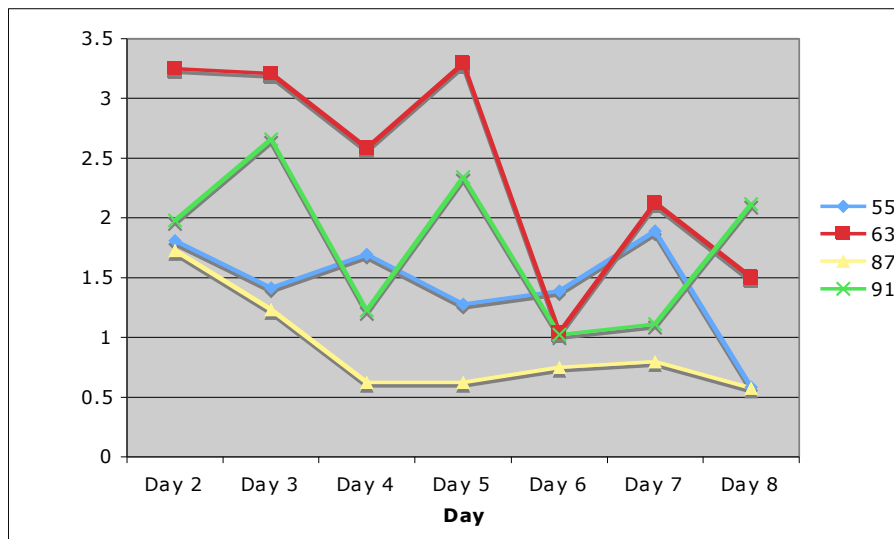


Figure 8a: Body and anogenital licks days 2-8

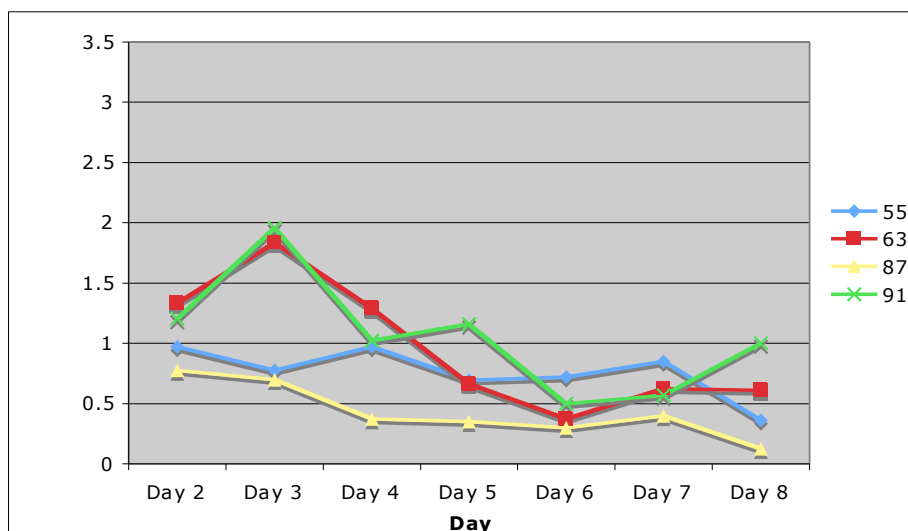


Figure 8b: Anogenital licks days 2-8

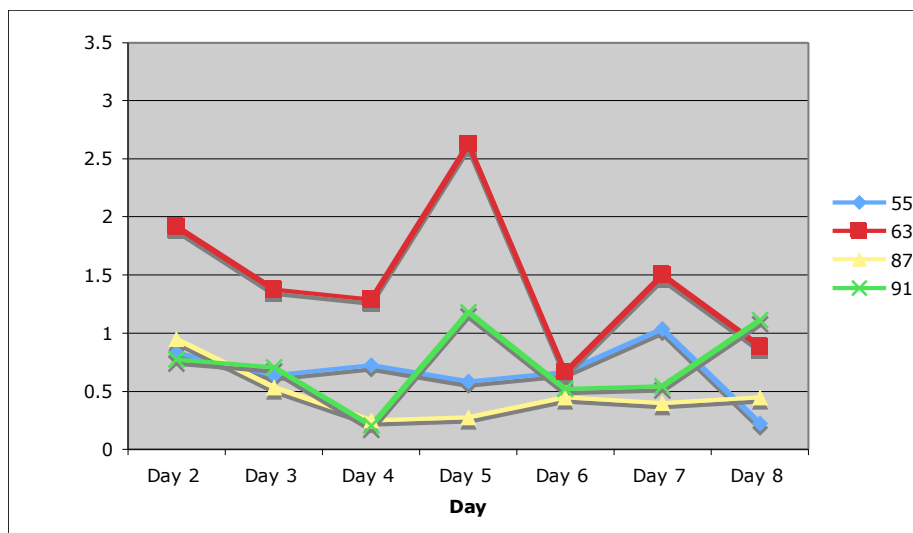


Figure 8c: Body licks days 2-8

Quantifying offspring nipple attachment behavior

Offspring attachment to the dam's nipples was quantified by percent of observation points recorded in which a pup was attached. Pup attachment increased from days 2 to 8 (Figure 9). On average, there was a 37% increase in nipple attachment from day 2 to 8 ($t_3 = 2.50$, $p = 0.09$). There was a spike in attachment on day 7; pups were attached nearly two times more on day 7 than day 2 (99% increase). Analyses of variance tests revealed that pups in different litters showed significant differences in attachment behaviors (see appendix D for bar graph representation of nipple attachment for each litter) [$F = (3, 33) = 9.81$, $p = 0.00$]. Tukey HSD post-hoc comparisons of the four litters indicate that one litter, litter 63, varied significantly from the other three, 55, 87, and 91. Over the course of postnatal days 2-8, a repeated measures ANOVA reveals that there was a significant difference in attachment trends by litter, but not sex (litter effect: $F(3, 36) = 10.48$, $p = 0.00$; sex effect: $F(1, 34) = 1.01$, $p = 0.38$; litter*sex: $F(2, 34) = 11.67$, $p = 0.50$). Male and female offspring were not differentially attached to the dam's nipple [litter and sex effect: $F = (3, 36) = 0.89$, $p = 0.46$] (Figure 10). No significant association between frequency of nipple attachment and body weight in days 2-8 was found ($r = -0.14$, $n = 34$, $p = 0.21$).

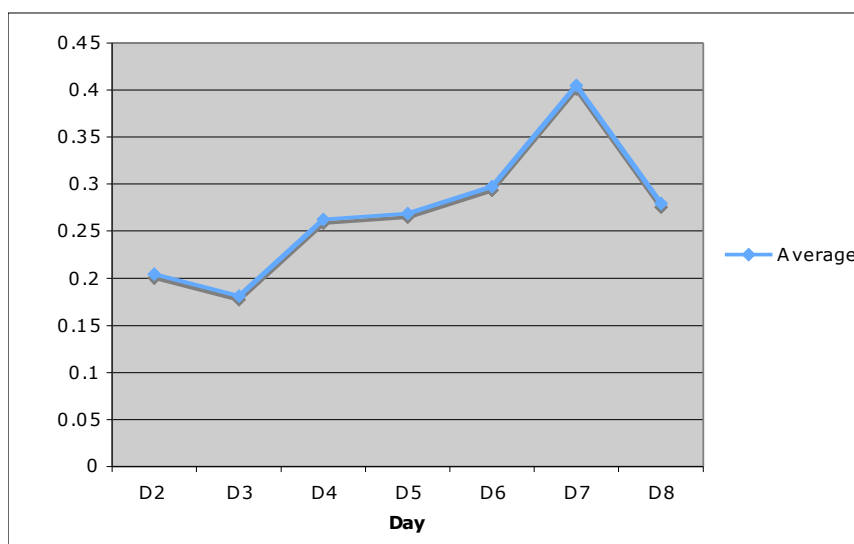


Figure 9: Average fraction of time points attached to nipple days 2-8

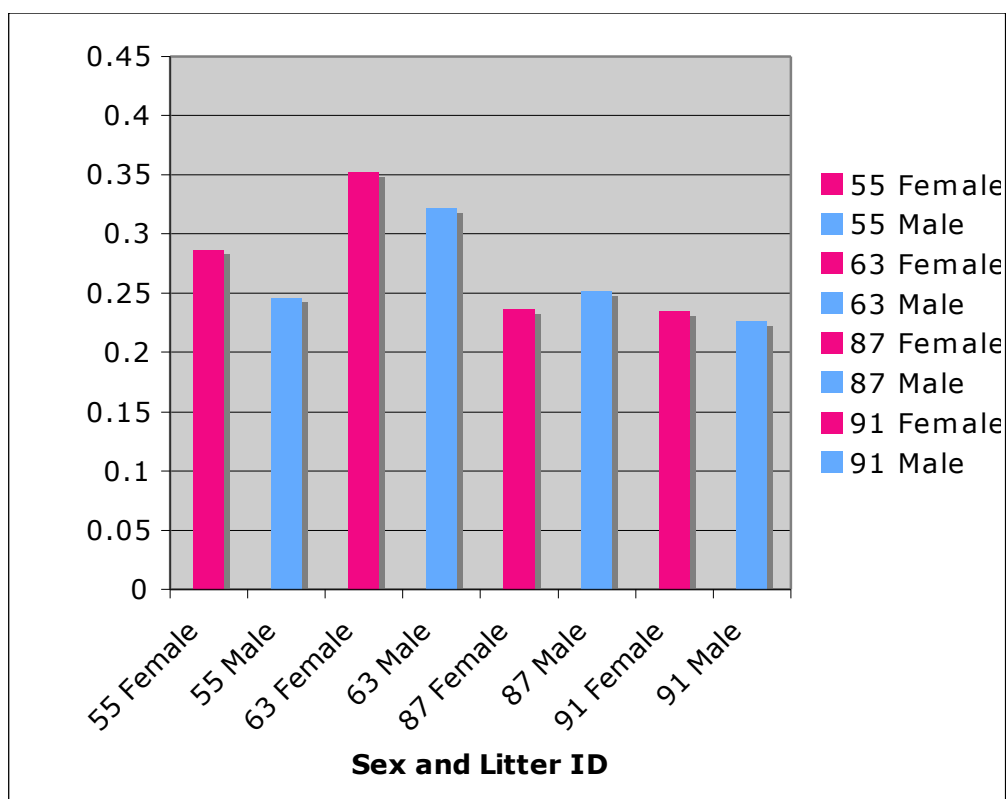


Figure 10: Within litter and sex mean fraction of time points attached to nipple

Nipple attachment relationship to maternal licking

Significant patterns in the relationship between offspring nipple attachment and the frequency a pup was licked in postnatal days 2-8 were observed. The number of time points that a pup was attached to the dam's nipple was positively correlated with the frequency of total licks received in days 2-8. It is apparent that this relationship is driven by nipple attachment's association with anogenital licks (anogenital: $r = 0.34$, $n = 34$, $p = 0.05$; body: $r = 0.10$, $n = 34$, $p = 0.57$, total licks: $r = 0.27$, $n = 34$, $p = 0.06$).

Behavior tests and licking

It was found that offspring that were licked more frequently during days 2-8 displayed decreased exploratory behaviors in response to a novel social stimulus in adulthood compared those licked less frequently (novel social adulthood: $r = 0.39$, $n = 29$, $p = 0.02$). There was not a significant association between licking and response physical and social novelty in adolescence or physical novelty in adulthood (novel physical adolescent: $r = 0.14$, $n = 33$, $p = 0.21$; novel social adolescent: $r = -0.10$, $n = 33$, $p = 0.30$; novel physical adulthood: $r = 0.10$, $n = 33$, $p = 0.28$; elevated plus maze: $r = 0.27$, $n = 33$, $p = 0.06$). When examined separately, frequency of anogenital and body licking was associated with certain behavioral outcomes in adolescence and adulthood. Frequency of anogenital licks was positively correlated with a longer latency to the open arms of an elevated plus maze, an known indicator of anxiety-related behaviors (EPM: $r = 0.47$, $n = 33$, $p = 0.003$). Anogenital licks were not significantly associated with any other the other behavioral tests (novel physical adolescent: $r = 0.08$, $n = 33$, $p = 0.34$; novel social adolescent: $r = -0.14$, $n = 33$, $p = 0.22$; novel physical adult: $r = 0.05$, $n = 33$, $p = 0.39$; novel social adult: $r = 0.18$, $n = 29$, $p = 0.17$). Increased frequency of body licks was associated with decreased physical exploratory behaviors in adolescence and social exploratory behaviors in adulthood (novel physical adolescent: $r = 0.27$, $n = 33$, $p = 0.06$; novel social adult: $r = 0.57$, $n = 29$, $p = 0.001$). Body licking was not significantly associated with the other behavioral tests (novel social adolescent: $r = 0.102$, $n = 33$, $p = 0.29$, novel physical adult: $r = 0.06$, $n = 33$, $p = 0.36$; EPM: $r = -0.4$, $n = 33$, $p = 0.42$).

Postnatal nipple attachment and later adult offspring exploratory and anxiety-related behavior

Pups who were more frequently attached to the dam's nipple in postnatal days 2-8 compared to their siblings were slower to approach novelty in adulthood (novel physical adolescent: $r = 0.21$, $n = 33$, $p = 0.12$, novel social adolescent: $r = 0.16$, $n = 33$, $p = 0.18$, novel physical/novel social adolescent mean: $r = 0.23$, $n = 33$, $p = 0.10$; novel physical adult: $r = 0.18$, $n = 33$, $p = 0.16$, novel social adult: $r = 0.23$, $n = 29$, $p = 0.12$; novel physical/novel social latency mean: $r = 0.35$, $n = 29$, $p = 0.03$). It was also found that pups that were attached more often showed anxiety-related behavior on the elevated plus maze, specifically longer latency to the open arms ($r = 0.39$, $n = 33$, $p = 0.03$).

Chapter 4

Discussion

As part of the first goal of the study to quantify A^{vy} maternal behavior, it was predicted that A^{vy} dams would show similar nurturance behavioral patterns to other inbred and outbred mice strains. Specifically, it was predicted that pup-directed behaviors like licking, nursing, and nest-building would decrease over time in the immediate postnatal window while maternal eating/drinking and self-grooming would increase. Overall, behavioral time budgets and temporal trends revealed that this was, for the most part, true. A^{vy} mothers overall displayed behaviors similar to other inbred mice strains. The second goal was to examine the relationship between offspring nipple attachment and maternal behavior, specifically licking. Contrary to the hypothesis, the frequency that offspring were licked was positively associated with frequency of time points attached to the dam's nipple. Lastly, to extend the scope of maternal-pup interactions beyond the critical developmental window, the long-term effects of nipple attachment and licking were examined in relation to offspring exploratory and anxiety-related behavior. In support of the hypothesis about licking and in contrast to predictions of nipple attachment, pups licked and attached to the nipple more frequently both showed decreased exploratory and increased anxiety-related behaviors as adolescents and adults.

Maternal behavior

Quantification of maternal behavior of A^{vy} mice was possible through careful observations of dams in a relatively undisturbed environment on postnatal days 2-8. It is now widely accepted that maternal care plays a critical role in offspring development. Understanding

how mothers budget their time can provide insight into the mother-pup interactions that are so critical in the early postnatal days. The four *A^{vy}* dams observed in the study displayed very similar duration and temporal trends for licking, eating/drinking, nest building, and self-grooming behaviors. The margin of difference in percent of time spent on each behavior over days 2-8 was small (Figure 7a-d) demonstrating that maternal behavioral patterns are relatively stable among this inbred strain of mice. In accordance with the hypotheses, the “pup-directed” behaviors of nursing, nest building, and licking decreased, and eating/drinking increased from day 2 to day 8. In contrast to the hypothesis, self-grooming behavior remained relatively constant from day 2 to day 8.

It is thought that the maternal behaviors quantified promote offspring survival through thermoregulation and the supplying of nutrients (Champagne, Curely, Keverne, & Bateson, 2007). The physical stimulation that mothers provide to offspring primarily through licking plays a role in reducing offspring body temperature so pups do not have to physically separate from the dam to cool off, allowing urination or defecation, and also promoting more efficient suckling during nursing bouts (Sullivan, Shokrai, & Leon, 1988). The overarching question remains in regards to the basis of these maternal behaviors; is it the pup-induced cues causing alterations in maternal behavior over postnatal days 2-8, or is it an innate maternal instinct to decrease “pup-directed” behaviors? Previous experimental work comparing lactating dams and virgin females in the presence of pups showed similar declines in maternal behavior supporting the hypothesis that maternal behavior is regulated by the pup, rather than a maternal hormone-related instinct (Reisbick, Rosenblatt, & Mayer, 1975). In regards to decreasing rates of maternal behavior during the first postnatal week, it was postulated that offspring may simply need more maternal attention in the early post-natal days, and as the need decreases maternal activity declines over the week. However, nipple attachment behavior increased over the first postnatal week suggesting that nipple attachment is not the driving force stimulating licking which decreased over the week.

On the other hand, the increase in maternal eating/drinking can be attributed to the extra energy expenditure that the dam now spends producing milk for offspring. The stability of self-grooming throughout the immediate postnatal period may simply demonstrate that maternal self-grooming is not influenced by either pup cues or maternal hormones. A future study could compare rates of self-grooming in lactating dams to virgin females to see if maternity has a significant influence.

It may not be valid to make direct quantifiable comparisons to previous studies observing mouse maternal behavior because of variation in data collection methods, but general comparisons can be made. Champagne, Curley, Keverne, & Bateson (2007) examined maternal behavior of two inbred mouse strains, 129Sv and C57BL/6J, and one outbred strain, Swiss. The behaviors recorded in Champagne et al.'s (2007) study were scored based on observations at 3 minute intervals of hour-long observations, four times daily in postnatal days 1-6 resulting in *frequency* data. The present study's *duration* measures of the inbred A^{vy} litters were recorded continuously during hour-long observations, four times daily on days 2-8. In general it was found that the A^{vy} dams in the present study spent the majority of their time nursing followed by eating/drinking, self-grooming, and the least amount of time nest-building. A^{vy} mothers as well as the 129S, C57BL/6J, and Swiss strains examined in Champagne et al. (2007) spend over twice as much time engaging in nursing behaviors than any other behavior. The ranking of these behaviors was the same in both inbred and the outbred mice strains in the Champagne et al. study, with the exception self-grooming being the least in all of strains Champagne studied. However, this difference could simply be caused by methodological differences in the two studies. Scoring behaviors at three-minute intervals, as used in Champagne et al. 2007, may not accurately reflect behaviors that occur infrequently or for short durations in an observation window, for example, self-grooming behavior that occurs in short bouts. In the A^{vy} strain in the present study and in all three strains in Champagne et al. (2007) the frequency of nest-building, licking, and nursing decreased, and eating/drinking increased over the first postnatal week. The general comparison of

the A^{vy} inbred strain to 129Sv and C57BL/6J inbred and Swiss outbred mice strains revealed no outstanding differences in maternal care. In future studies of A^{vy} strains, it would be beneficial to examine not just duration of key maternal behaviors, but also examine frequency of behavioral bouts. The present study was limited by looking solely at duration of many behaviors, and only frequency of licking. Overall, these findings indicate that A^{vy} mothers are similar to other laboratory mouse strain mothers and that further studies on how maternal behavior may influence pup development would be useful in this strain.

Within-litter variance of licking and nipple attachment

Previous studies have examined the influences of maternal care on offspring behavior by classifying the behavioral trends of the mother as high-licking, grooming, and/or nursing mothers (Caldji et al., 1998; Francis et al., 1999). These studies tend to predict that high-licking dams produce offspring that are more exploratory than those reared by low-licking moms. The current study, however, individually tracked pups within each litter and examined frequency that they received licks from the mother, and an opposite affect was found. It was found that individual pups that were licked more frequently than their littermates showed less exploratory and more anxiety-related behaviors. Previous studies showing that high licking moms generally produce high exploratory offspring and the present study finding that pups individually licked more showed decreased exploratory behavior may reflect that mothers that may be giving out more licks are also distributing them unequally among pups within a litter.

The within-litter variance in maternal licking could possibly be a function of diversifying the litter to promote maximum survival. Shaping behavior by licking, for example, may produce offspring that are better at reproducing in one type of an environment, while others may be better reproducing in another. For example, less exploratory offspring may have greater success

surviving in environments that are filled with skillful predators, where as that setting may not be ideal for a bold and exploratory mouse (Dingemanse, Both, Drent, & Tinbergen, 2004). This could contribute to overall success of the litter in unpredictable environmental conditions (Cavigelli et al., 2010). In accordance with the working hypothesis of the temporal trends in maternal behavior, within-litter variance in licking could also be a result of innate differences among neonates that result in different levels of maternal solicitation (Stern, 1997). Those pups that receive more licking may be soliciting the mother more frequently via ventral probing, snout contact, and nipple attachment (Ragan, Loken, Stifter, & Cavigelli, 2010).

Findings revealed that anogenital licking was associated with nipple attachment, but not exploratory behaviors and that both anogenital and body licking were both related to exploratory behaviors. The finding that offspring attachment to the mother's nipple is positively associated with greater licks received on postnatal days 2-8 may be explained by the pup solicitation hypothesis suggested above, for example, "needier" pups are attaching to the nipple more frequently and thereby soliciting more licking from the mother. Two hypotheses about the functions of licking may provide explanation of its association with both nipple attachment and behavioral traits. The first, that anogenital licking in the early postpartum window promotes offspring excretion, may explain why pups that are attached to the nipple more are licked at greater frequencies. Increased attachment could indicate increased eating, and increased eating leads to increased excretion and thus the need to be anogenitally licked. If it is the case that inherently needier pups attach to the mom more frequently, this would explain the relationship between anogenital licking and increased anxiety-related behaviors in adulthood in the current study. This hypothesis would require further testing. The thermoregulatory functions of licking, specifically reduction of brain and body temperature by physical body stimulation, may further explain the association of body licking to decreased exploratory behaviors in adolescence and adulthood (Sullivan, Shokrai, & Leon, 1988). Perhaps the needier pups that spend more time

attached to the mom tend to be buried in the center of the litter, and thus need greater thermoregulation. Rather than pups removing themselves from the cluster to prevent hyperthermia, dams may body lick them to cool them down.

The finding that pups attached to the nipple and licked more frequently than their littermates show less exploratory and more anxiety-related behaviors as adolescents and adults demonstrates that perhaps early neonate “clinginess” to the mother is indicative of later behavioral inhibition. Further exploration of the nipple attachment behavior is warranted to better understand the behavior. Having a temporal view of individual pup attachment behavior would provide insight on the pups’ latencies to attach. The degree to which a pup has to “struggle” or compete with siblings may predict the rate of maternal licking and subsequently its later exploratory and anxiety-related behaviors. To further test the theory that pups innately vary in their neediness, an experimentally-controlled test such as removing the dam from pups and examining how long it takes them to adversely react may present a better view of the origins of their behavior.

Conclusions

The results of the current research show that A^{vy} mothers, though more prone to a host of anomalies such as obesity, diabetes, tumors, and anxiety, demonstrate maternal behavior patterns very similar to other inbred mice strains. They individually licked their offspring at different rates, which is associated with varied behavioral outcomes in terms of response to novelty and anxiety-related behaviors in the pups. Increased nipple attachment and frequency of licks received was associated with diminished exploratory behavior in offspring during adolescence and adulthood. Results have led to the overall theory that the variation in licking and nipple attachment has an innate offspring basis. Perhaps pups with needier dispositions induce greater

maternal attention that may enhance their survival. Those needy pups may end up behaviorally being less exploratory than their peers but possibly less anxious than they otherwise would have been had they not received extra maternal attention through licking. This working hypothesis will require testing in future studies.

Understanding the influence of maternal-pup interactions on behavioral development provides a platform for human studies that may provide useful evidence for further exploration of the phenomenon in humans. Longitudinal studies demonstrate that babies with a strong reaction to novelty as an infant are more likely to grow up and be anxious. The innate temperament is a relatively stable trait (Kagan, Snidman, Kahn, & Towsley, 2007). Other human studies have found that infant cues like crying and “cuteness” provoke maternal care, which is comparable to the rodent offspring cue hypothesis of maternal nurturance. Experimental and longitudinal studies on the mechanisms that underlie this phenomenon in mice may provide more insight in the occurrence in humans.

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Appendix A

IACUC Approval of Protocol #29898

Section of the protocol pertaining to the present maternal behavior study:

Maternal and neonate behaviors: Day of birth will be defined as 'Day 0' of life. Behavioral observations will begin on Day 1. On this day, mothers will be momentarily removed from their cages and each neonate marked with an individually specific mark using a non-toxic 'Sharpie' pen to ensure individual pup identity is maintained. Pups from the center and the periphery of the nest will be selected alternately for sequential marking. For this marking procedure, each neonate will be handled for 90 seconds. During this time, we will also measure neonate weight, and anogenital distance. After all pups are marked the mother will be returned to the cage and the first behavioral observation will begin.

Six, 30-min observations will be conducted daily for the first 7 days of life. We will record maternal grooming (both body and anogenital licking), a behavior known to differ between families and associated with adult offspring behavior and stress physiology. Litters will be observed as a whole to record all grooming bouts and the identity of neonate recipients of each bout. We will also record active 'attention-seeking' behavior in the neonates: maternal snout contact (which elicits maternal behavior), movement, and relative location within the litter at specified time intervals. Quantification of mothers as to the degree of arched-back nursing, licking, and grooming of their pups will be made by videotape recording. This measure only requires positioning of the cages on their racks to maximize visibility when watching the videotape. (Dr Cavagelli and group will perform this data collection and train the Vandenberg laboratory.)

Novel physical behavioral test (at PN 25-35 days and 2.5 months): This test examines a mouse's willingness to explore a novel complex arena. Mice are tested individually during their active period - i.e. during the red-light phase of the light cycle. They are carried from their home cage, placed in the testing arena and their behavior video-recorded for 5 minutes while in the arena. They are returned to their home cage after the 5-minute test. The arena consists of a 122 x 122 cm enclosure with 46 cm walls, a Plexiglas cover, wood chip bedding on the floor, and four rat-sized objects placed 13 cm from each corner. Objects include a plexiglass tunnel, a metal food hopper, a ceramic bowl, a metal tunnel, a brick and a rock, all items that are regularly cleaned in the cage washer. The test arena is made of Plexiglas. When in use, the arenas are wiped down on a daily basis using a solution of Quatricide PV15 (1/2 ounce in 1 gallon water) and allowed to air dry. To ensure novelty of the experience at each trial, objects are replaced with new ones for repeat testing. The test arena will be in a room near the animal colony room, maintained at the same temperature and on the same lighting schedule as the colony room. The arena is indirectly illuminated with a 90-watt red light bulb reflected off the walls of the room. At testing, mice are placed in a body-surrounding ('safe') bowl, thoroughly rinsed with hot water and allowed to air dry. Behavioral videotapes are coded after all mice have been tested. The frequency and total duration of the following behavior is recorded: 'locomotion' (squares entered) and 'inspection' (nose on, touching, climbing on or into an object). Defecation, rearing and latency to leave the

home bowl are also scored for comparison with similar behavioral tests conducted by other researchers. It should be noted that defecation, a classic index of anxiety in animals, is rarely seen in this testing arena. (Dr. Cavagelli and group will perform this data collection and train the Vandenberg laboratory).

Appendix B

Maternal/Pup Behavioral Observations Protocol and Descriptions of Behaviors

Maternal / Pup Behavior Observations Protocol:

S.A. Cavigelli, PSU Behavioral Neuroendocrinology Laboratory

1. Make sure **cameras** around cage is in proper positions (one facing into cage on each side of cage) and correctly focused.
2. One person is in charge of calling **events and time**, and one is the **scribe** to record all the information.
3. Record all maternal behavior as it happens (this is called focal sampling – you are focusing on mother only), identifying time that each new behavior begins. You do not need to record the time licks and carries occur – they happen too quickly.
4. Each row in the data sheet is for a new behavior. Only one behavior per row.
 - a. **Pup lick, carry, ventral probing, and snout contact** are coded by recording the pup number in the column in the next available row. Write an **A** next to the pup ID when pup was licked in anogenital area, body lick is **B**. If the mom licks a pup for more than 3 seconds, mark a **star** next to the pup number.
 - b. **Nursing and contact** is coded as a duration. Record a **starting** and **ending time** and connect them with an **arrow**.
 - c. **Nest building, eat/drink, self groom, and misc.** can be recorded as durations or **time points**. If a behavior lasts more than **3 seconds**. Otherwise record a time and underline it.
5. **Every 3 minutes**, record pups' behavior. **Record all pup numbers** that you can see in the 'peripheral' column, unless they are not in contact with the huddle, in which case record them in the 'away' column. (This will give us a sense of whether a pup was at the center or periphery of the huddle.) Then **circle** the numbers of the pups that are **squirming or moving**. **Underline** pups that are in **easy licking distance** from mom. This is a semi-circle around her snout.
6. End observation at **60 minutes**.

Appendix C

Exploration Arena Testing Protocol

Testing rats on Exploration Arena

S.A. Cavigelli, PSU Behavioral Neuroendocrinology Laboratory

Test during dark phase of the light cycle. Illuminate test room with red light bulb(s) only, deflecting them so they do not cast a glare on the arena cover.

Sprinkle the field with several handfuls of bedding collected from each rat cage in the test animal's colony room.

Center test arena under camera.

For 'Novel Physical' testing, place 3 novel objects in each corner, far enough away from the walls so that the rat may pass easily around all sides of the novel object.

For 'Novel Social' testing, place two wire cages in the corners closest to the 'start bowl'. There is nothing to place in the fourth corner – furthest from the start bowl. In one of the two cages, place another rat from the colony, being careful to control for estrous cycle if testing females (i.e. use a female in the same phase as test subjects), and control for body weight (use a subject that is close to the body weight of the test subjects). Be sure that the cage with the novel social partner is securely closed/latched!

Always double check that you have enough space on DVD before starting new day of recordings.

On data sheet, record amount of light in center and edge of arena (using light meter and recording *lux*), room temperature, date, time, cohort, and initialize.

Rats are tested one at a time for 5 minutes each.

Begin video recording before placing animal in arena. (Press 'REC' on DVD recorder.)

IMPORTANT: Make sure 'DVD' light is illuminated on DVD recorder.

Transport rat from colony room to testing arena using the familiar bowl in their home cage. Make sure to cover rat if traveling through areas with white light.

Place rat & bowl into side-turned clear cage and place the rat, bowl and cage into the free corner in arena. Make sure the opening of the side-turned cage faces the arena corner. Do this as gently as possible. A rough landing for the rat will freak them out and really affect their behavior.

Set timer for 5 minutes.

Code 'lines crossed' during the testing session. Count one line crossed when all four of the rat's paws cross the line. Record the latency (seconds) required to first investigate a novel object or

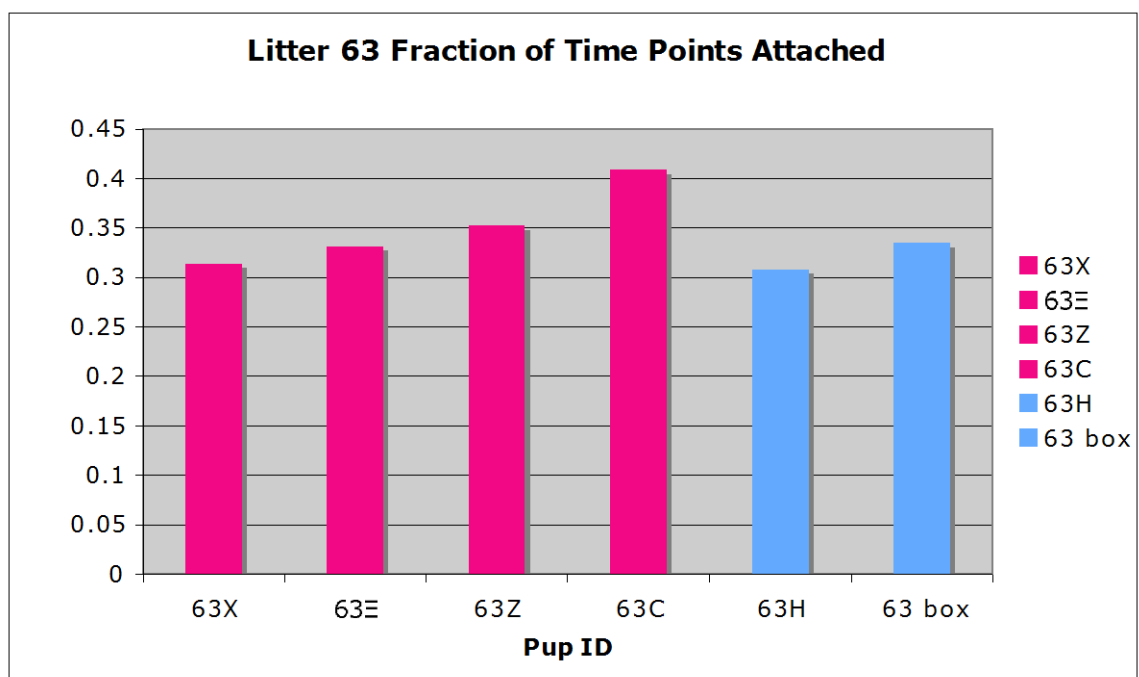
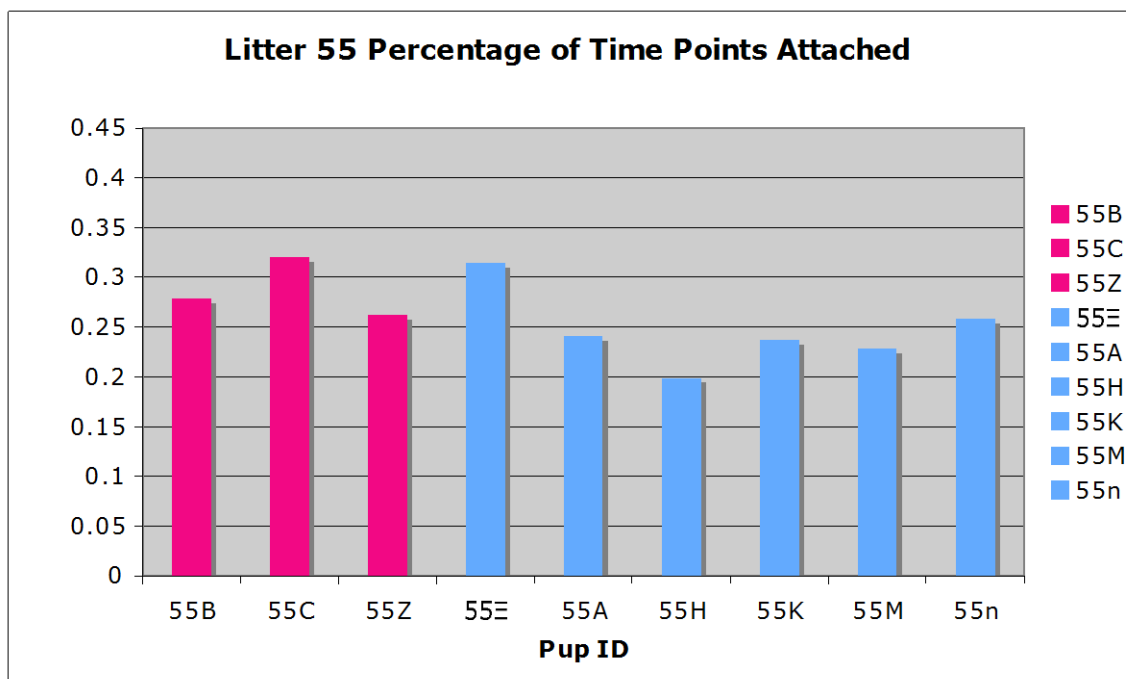
the latency to investigate the novel social partner. Finally, record each time the rat's nose touches novel objects, places both paws on object, and climbs on top of objects. Code the number of times the animal rears as well.

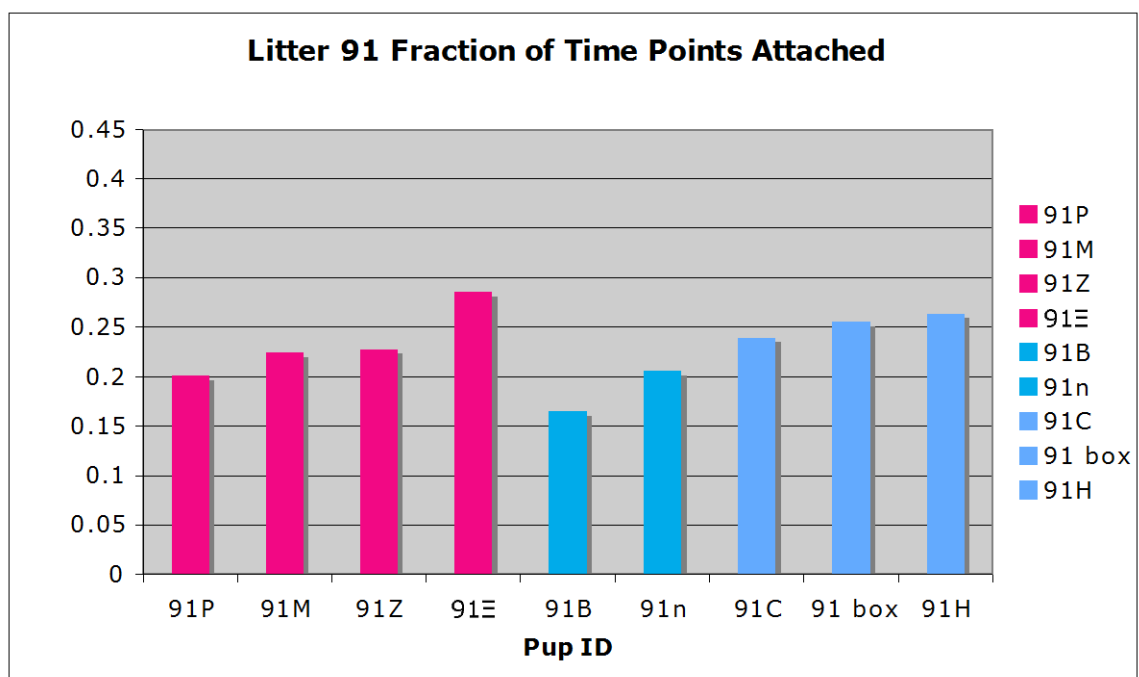
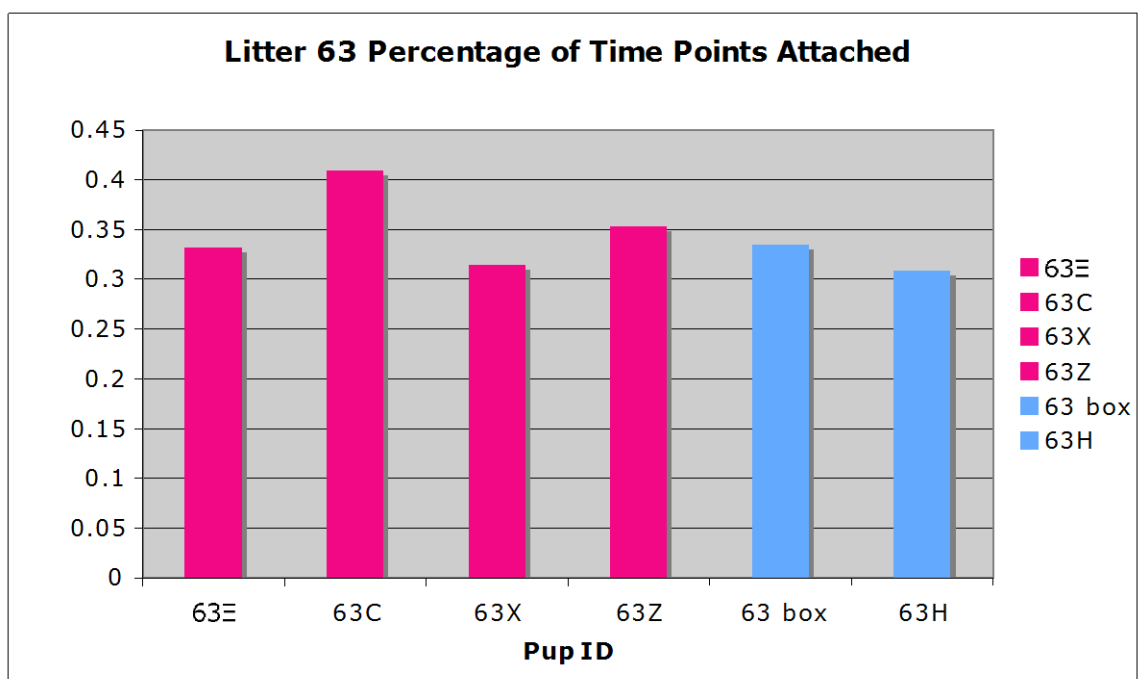
After an animal has been in arena for 5 minutes, turn off video recording, remove animal from arena and return to home cage. Turn lights on in testing room and count and remove fecal samples and record number on data sheet. Check for urine in test bowl and record whether there was urine or not (Y or N) on data sheet. Rinse larger bowl with tap water and dry for next rat.

At the end of the day, burn data onto DVD and label DVD with date, test name, and animal IDs.

Appendix D

Offspring Nipple Attachment by Litter





ACADEMIC VITA OF MOLLIE H. WOEHLING

115 Browning Lane, Rosemont, PA 19010 ♦ mwoehling@gmail.com ♦ 610-745-5292

EDUCATION

The Pennsylvania State University—Schreyer Honors College University Park, PA
Bachelor of Science, Biobehavioral Health Class of 2011
Minor in Biology

Instituto Lorenzo de' Medici, Florence, Italy Spring 2010
Completed courses in Italian language, arts, and culture

HONORS

Student Marshal, College of Health and Human Development Spring Commencement 2011
Alumni Recognition for Student Excellence Award 2011
The Evan Pugh Scholar Award (top 0.5% of class) 2010, 2011
The President Sparks Award for Academic Excellence 2009
Helen Skade Hintz Biobehavioral Health Scholarship 2009
The President's Freshman Award for Academic Excellence 2008

RELATED EXPERIENCE

Penn State University Behavioral Neuroendocrinology Lab, University Park, PA
Undergraduate Research Assistant Spring 2009-Present

- Conducting research with Dr. Sonia Cavigelli in behavioral neuroendocrinology examining how individuals differ in behavioral and physiological responses to common challenges and to understand how this influences individual susceptibility and/or resilience to disease and aging

Public Health Management Corporation (PHMC), Intern Philadelphia, PA
PHMC Care Clinic June-July 2010

- Developed curriculum and taught a medication treatment adherence course/HIV basics education to newly diagnosed patients
- Created lesson plans and led nutrition education sessions
- Shadowed doctors and physicians assistants

CHANCES intensive outpatient substance abuse recovery center

- Taught a stress management class, observed group therapy sessions, and assisted therapists with administrative tasks
- Developed health policy to better integrate physical and behavioral health between CHANCES and the Care Clinic

Penn State's WISE (Women in the Sciences and Engineering) University Park, PA
Summer Camp, Camp Program Assistant and Residence Assistant June 2008

- Responsible for 36 high school-age young women for a camp exploring a variety of science fields

ACTIVITIES/LEADERSHIP

Penn State Dance Marathon—Four Diamonds Fund 2007-2011
Largest student-run philanthropy in the world: over \$9.5 million raised for families of children with pediatric cancer in 2011

THON Family Relations Captain (THON 2011)

- Served as a liaison between THON and The Four Diamonds Fund Families while providing emotional support

THON Donor Relations Captain (THON 2009 and 2010)

- Conceptualized and implemented corporate donor solicitation strategy and a post-THON stewardship mailing system
- Coordinated and empowered the Penn State community to fundraise effectively
- Led and trained a committee of 25 direct reports who worked interdependently to benefit all THON donors (2009)
- Conducted research on donor trends in order to improve donor retention (2010)

Atlas THON Independent Fundraising Team (2007-2011)

- Family Relations Committee member
- Number one fundraising organization in 2011

Rules and Regulations Committee (THON 2008)

- Security Leader: Tour leader and point of contact for other committee members

Alpha Epsilon Delta Honors Society 2009-2011

- Member of Penn State's pre-health profession honors society and club

Biology 110 Peer Mentor Fall 2009

- Provided weekly biology tutoring and review sessions for Penn State students

Second Mile College Friend Program 2008-2009

- Mentor to an elementary-aged child in need of additional support and contact

Penn State Fresh START Team Leader August 2008

- Team leader for Penn State's new student day of community service

Schreyer Honors College Student Council 2007-2008

- Recruitment Committee: led tours and information sessions for prospective students
- Service Committee: participated in service projects to benefit the local community

INTERNATIONAL EXPERIENCE

Amerispan International Studies: Quito, Ecuador May 2008

- 3-week intensive language study program and homestay with local family

Globe Aware Adventures in Service: El Sur, Costa Rica July 2007

- Volunteer teaching English, building footpaths, woodworking, and painting to create a sustainable future through ecotourism