2012

The discriminating mother : own v. alien and male v. female

Eric McDaniel

The Discriminating Mother: Own v. Alien and Male v. Female

by

Erin McDaniel

Honors Thesis

In

Psychology
University of Richmond
Richmond, VA

April 29th, 2012

Advisor: Craig Kinsley

The Discriminating Mother: Own v. Alien and Male v. Female

It is often stated that one of the greatest bonds that exists is between mother and child. Previous research has shown that mothers experience modifications in their brain chemistry and structure due to the effects of pregnancy, parturition, and motherhood in animal models (Lambert & Kinsley, 2012). If an individual cannot have children of her own she may chose to adopt a child to fulfill that niche and forge a similar bond. One may ask, however, whether this bond between mother and child is equivalent in strength whether forged in parturition or appropriation/adoption. Are there other discriminations that mothers make in their behavior towards their offspring? How might these discriminations be manifested in animal models?

A Beach and Jaynes (1956) found that lactating rats may show signs of "rejection" of alien young but will ultimately retrieve all pups that are presented to them *and* they will do so more slowly than when they retrieve their own offspring. It was not observed that a mother's own young ever experience such rejection throughout the experiment. The animal paradigm begins to give insight into the strengths of the different bonds: These differences may be subtler in human behavior, but the simplicity of this model allows for the investigation of maternal instinct at the most basal level. What would be observed if a mother is presented with both young simultaneously? Can a mother's instinct allow her to forge as strong of a bond with a child that is not her own despite her greatest intention?

A mother may also discriminate in the manner in which she behaves towards her male and female young. Moore (1982) found that maternal rats spend more time licking and grooming their male pups compared to oil-treated female controls. Even female pups

injected with testosterone experienced an increase in attention from the mother. Clark, Bone, Bennett & Galef (1989) found evidence that simple male/female placement and order in the mother's uterus influenced the degree of maternal attention a pup received: Females who were adjacent to two males during the gestation period of gerbils were exposed to higher levels of testosterone and consequently received an equivalent amount of post-natal interaction from the mother as males. Females who were adjacent to two other females did not receive such additional attention.

The literature has proposed this magnification of attention as a necessity in order for male pups to manifest healthy reproductive habits in the future (Clark et al, 1989; Francis & Meaney, 1999; Meaney, 2001). In two other papers, Hirschfeld, Biederman, Brody, Faraone, and Rosenbaum (1997a, 1997b) discuss that anxious mothers are more likely to have children who are reserved and timid. Meaney (2001) has addressed and endeavored to encode several other maternal behaviors in rats that influence pups as they grow into adults. Again, this research raises questions pertaining to the realm of parenthood within humans: Do mothers innately display a noticeable bias towards their sons? Are there interactions indicative of some future behaviors between genders?

The current experiments sought to address these two realms of maternal discrimination discussed: Own v. Alien and Male v. Female. Rats will be used as an animal model to approach their respective questions and, both, behavioral and neuronal data will ultimately be used to observe these differences. In light of the previous research (Beach & Jaynes, 1956), it was anticipated that females administered entirely their own young will retrieve pups more quickly than if given a group of "alien" pups or a group of mixed origin in Experiment 1. In addition, it was predicted that females would spend the

least amount of time interacting with alien pups, followed by "mixed" and "own" pups. Furthermore, due to the findings of Moore (1982) and Clark et al. (1989), it was hypothesized that females would spend a greater amount of time interacting with male young over female young when administered pups from her own litter in Experiment 2. It was also expected the females would spend more time grooming themselves as an indicator for anxiety when given female young versus male young (Meaney, 2001). It was desired to replicate the strength of previous findings and contribute further to the current body of research exploring these topics.

Experiment 1

Method

Participants

Female, 7-10 week-old, sexually naive, out-bred Sprague-Dawley rats (Harlan, Madison, WI), were singly-housed in polypropylene plastic cages with *ad lib* access to tap water and breeder chow (Harlan). All animals were housed on a 14:10 light/dark cycle with lights on at 0500.

Animals were mated and allowed to deliver their pups. On day 4-6 of lactation they were randomly assigned to one of three groups (see below; n = 8/group), after which they were tested.

Behavioral Testing

The animals were divided into three groups, OWN versus ALIEN versus MIXED. Approximately 16 hours prior to behavioral testing, all rats and pups were moved from their opaque homecage to a clear testing cage with the same dimensions and

new bedding in order to prevent a display of neophobia during testing. A sheet of paper towel was also introduced at this time to serve as a simple measure of maternal behavior if the female were to shred and use it for nesting at the time of testing. The females had their pups removed for 60-min, whereupon pups were identified for gender and six were marked with an odorless colored marker pen to create three groups: OWN, ALIEN, and MIXED. The OWN group had six pups from their original litter introduced to them and their behavior was observed and scored. The ALIEN group had pups derived from donor females provided to them while the MIXED group was returned three pups derived from their original litter and three from a donor mother, distinguished from each other by two different color markers (randomly changed between animals). All pups were assigned a number 1-6 and were marked with their respective number in four areas across their back; one in the anterior and posterior portions of their back and one on each side of the pup. During behavioral testing, all pups were introduced to the experimental mother simultaneously in what was referred to as a "pup cup." Pups cups were glass storage rounds made by Pyrex® approximately 2-inch in diameter.

The females were exposed to the pups for 30-min and the first 10-min of testing, beginning from the time when the pup cup was dropped into the cage, were used to code the behavioral data. Coding included: The time it took for the female to retrieve the first pup, the time it took for the female to retrieve the all pups, the order in which she retrieved the pups and their respective gender, the total time the female spent interacting with the group of pups*, and the total time the female spent grooming *herself* as a measure of possible anxiety. If not all pups were retrieved at the end of behavioral testing, the female was given a 1800-sec score for the amount of time it took to retrieve

all pups.

*Interaction time was operationally defined as any direct touching or clear attention given by the female to a pup including retrieving, licking, grooming, sniffing, stepping on, or nursing. If the mother was touching a pup, but clearly searching for something else, this time was not included.

Tissue Preparation

Animals were perfused 60-min (± 5 min) following the termination of a test. The rats were deeply anesthetized with sodium pentobarbital prior to intra-cardiac perfusion of .09% saline followed by 4% paraformaldehyde in 0.1M PBS, pH7.4, 100 ml each. Brains were removed, post-fixed overnight in 4% paraformaldehyde, and then cryoprotected in 30% sucrose in PBS. The brains were cut using The Brain Blocker (David Kopf Instruments, Tujunga, CA) and stored in sucrose. The caudal portions were frozen and cut sagittally into 40 μm sections using a sliding microtome (Leica, Microsystems, Heidelberg, Germany), and sorted into two, alternate sets of sections in a 24-well plate containing cryoprotectant solution (PBS containing 30% sucrose, 30% ethylene glycol, and 10% polyvinylpryrrolidone) and stored at -20°C until processing for immunoreactivity. Sections of the hippocampus and medial preoptic area, a region that regulates maternal behavior, were identified and isolated.

Immunohistochemistry

To visualize Fos-IR, one set of alternate sections were washed with PBS in the presence of 0.2% Triton X-100 (PBS-X), blocked in 5% normal goat serum for 1 hour, and incubated for 2 days at 4° C with anti-rabbit cFos antibodies (1:20,000; Oncogene Research Products, Cambridge, MA). Sections were then washed in PBS-X, incubated

for 90 min at room temperature in biotinylated goat anti-rabbit secondary antibodies, washed in PBS-X, exposed to an avidin-biotin complex, washed again in PBS-X, and stained with diaminobenzidine. Stained sections were then mounted, dehydrated, and coverslipped.

Image Analysis

Several measures were taken to ensure Fos-IR was measured consistently between samples [Rhodes et al., 2003]. All sections were exposed to diaminobenzidine for exactly 10 minutes. The background for each cell count was normalized by automatically adjusting light levels. A constant threshold level of staining was used to automatically distinguish Fos-positive cells.

The number of Fos-stained cells in a given region of the brain were quantified by projecting sections in bright-field from an Zeiss Axioimager (Zeiss, Gottingen, Germany), through an Axiocam Zeiss high resolution digital camera attached to the microscope, to a computer running KS300 software (Zeiss). The software performed automated thresholding and cell counting. Each region of the brain was identified and located using standard brain landmarks and counted in a frame of uniform size.

Results

Time to Retrieve First Pup: A one-way ANOVA indicated that there was a significant difference between the groups, $F = (2, 21) = 4.59$, $p = .02$. The Alien group was the slowest to retrieve the first pup on average while the Own group had the quickest retrieval (Own $M = 93.5$ sec, $SD = 130.5$; Mixed $M = 125.6$ sec, $SD = 157.9$; Alien $M = 599.6$ sec, $SD = 614.9$). A Post Hoc Tukey test revealed that there was a significant

difference between the amount of time that the females took to retrieve alien young versus own or mixed young ($p < .05$), however there was not a significant difference between the Own and Mixed groups in retrieving their first pup ($p > .05$).

Time to Retrieve All Pups: A one-way ANOVA also indicated that there was a significant difference between the groups in this variable, $F = (2, 21) = 9.02$, $p = .001$. The Alien group was, again, the slowest to retrieve all pups on average while the Own group had the quickest retrieval of those pups that were introduced (Own $M = 230.3$ sec, $SD = 222.8$; Mixed $M = 234.6$ sec, $SD = 201.6$; Alien $M = 1075.9$ sec, $SD = 735.2$). Similarly to the "time to retrieval first pup" variable, a Post Hoc Tukey test revealed that there was a significant difference between the amount of time that the females took to retrieve all alien young versus all the young that were of the own or mixed group ($p < .05$), however there was not a significant difference between the Own and Mixed groups in the time to retrieve all of the pups ($p > .05$).

Interaction Time with Pups: The Alien group spent the least amount of time interacting with the pups in the first ten minutes of behavioral testing when compared to the Own and Mixed groups (Own $M = 226.6$ sec, $SD = 144.9$; Mixed $M = 237.0$ sec, $SD = 123.0$; Alien $M = 138.0$ sec, $SD = 131.2$), however a one-way ANOVA did not indicate a significant difference between the groups despite this trend, $F = (2, 12) = 0.88$, $p > .05$. It is important to note that there were less subjects analyzed for total interaction time than the previous two variables (n = 8/group) with n = 4, 5, or 6 subjects within the mixed, own, and alien conditions, respectively.

Time Grooming Herself: Females within the Alien group spent the most amount of time grooming themselves within the first ten minutes of behavioral testing followed by the Own and Mixed groups (Own $M = 27.0$ sec, $SD = 24.0$; Mixed $M = 18.8$ sec, $SD = 14.8$; Alien $M = 53.0$ sec, $SD = 71.5$). A one-way ANOVA did not indicate a significant difference between the groups despite the trend, $F = (2, 12) = .70$, $p > .05$. Again, it is important to note that there were less subjects analyzed for this variable than the first two variables which reached significance (*Time to Retrieve First* and *All Pups,* n = 8/group) with n = 4, 5, or 6 subjects within the mixed, own, and alien conditions, respectively.

Tissue Analysis: All collected tissue has been stained and mounted but currently awaits complete image analysis. The medial pre-optic and hippocampus areas will be analyzed for differences in neuronal activation across conditions and these results will be reported in future work.

Discussion

In regards to the time it took for females to retrieve the first pup and total time to retrieve *all* pups, it is interesting that the Alien group was significantly slower in their retrieval of both variables but there was *not* a difference observed between the *mixed* and own groups. These results suggest that there is some mechanism occurring within the females where a group that is only partially foreign is essentially integrated as if they are entirely her own. However, when a group is totally foreign to a mother, a discrepancy may be observed. Further research may desire to investigate the point at which this

discrepancy appears; does it only take one pup from the mother's original litter in order to induce normative maternal behavior? In addition, this study did not address which mechanisms the females are using in order to identify the group of pups (i.e.- olfactory or auditory). Future research could investigate whether the presence of these senses are critical to a mother's discrimination and whether their absence, separately or simultaneously, renders a female unable to make judgments between her own and foreign pups.

Despite that the interaction time between the female and pups has not yet achieved significance, the present trend seems promising with an increase in subjects. The current data for this variable has 2-4 less subjects within each condition than the two variables discussed previously (time to retrieve first and all pups)- it's possible that there were not enough subjects within each condition to achieve significance. Given that, the trend indicates that females spend less time interacting with Alien young than Mixed or Own young. Again, Mixed and Own females demonstrate similar behavior given the average amount of time they spend interacting with the pups.

While an Alien female spends less time interacting with the pups in the first ten minutes of behavioral testing, she appears to be allocating her time to other activities, such as grooming herself. In light that self-grooming has served previously as a possible measure for anxiety, in this study Alien females seem to experience the most anxiety in comparison to Own and Mixed females. This would seem to be a reasonable trend if the females are aware that the given pups are not their own and may be instinctually wondering where they can find their own pups. To explore this further, one variable that might be looked at in the future is the amount of time the females spending "searching."

Given this idea, it might be predicted that Alien females dedicate a greater amount of time in search of her surroundings than caring for the young that is given to her.

When females are administered just some of their own pups, the trend indicates that their minds are put at ease and they are grooming themselves less. Again, it would be interesting to see at which point the female reaches the brink of anxiety and how many of her own young it would take to keep that level subdued. Similarly to the results for interaction time, it must be noted that the current results have not yet indicated a significant difference between the groups, but an increase in subjects within each condition appears to be promising.

Experiment 2

Method

Participants

Female, 7-10 week-old, sexually naive, out-bred Sprague-Dawley rats (Harlan, Madison, WI), were singly-housed in polypropylene plastic cages with *ad lib* access to tap water and breeder chow (Harlan). All animals were housed on a 14:10 light/dark cycle with lights on at 0500.

Animals were mated and allowed to deliver their pups. On day 5-7 of lactation they were prepared for behavioral testing and designated with a random order in which the conditions would be presented for the within-subject variable (see below; n = 7/group).

Behavioral Testing

Approximately 16 hours prior to behavioral testing, all rats and pups were moved

from their opaque homecage to a clear testing cage with the same dimensions and new bedding in order to prevent a display of neophobia during testing. A sheet of paper towel was also introduced at this time to serve as a simple measure of maternal behavior if the female were to shred and use it for nesting at the time of testing. The females had their pups removed for 30-min, whereupon pups were identified for gender. Each female was exposed to three conditions in random order: MALE, FEMALE, and MIXED. In each condition, the female was introduced to the center of the testing cage with one pup in a 2-inch diameter glass "pup cup" on both ends of the cage. In the MIXED condition, one male and one female pup were marked and placed at either end, while the MALE and FEMALE condition included two males or two females occupying either ends of the cage, respectively. All pups were marked with numerals "1" or "2" with two different color markers (randomly assigned between animals) previous to exposure regardless of condition. Pups were marked with their respective number in four areas across their back; one in the anterior and posterior portions of their back and one on each side of the pup. Females were exposed to each condition for 5-min with 5-min intervals between each behavioral test. Behavioral data was recorded and collected for further analysis.

Behavioral encoding included: The total time the female spent interacting with each pup*, and the total time the female spent grooming *herself* as a measure of possible anxiety, the first pup that she approached, and whether she retrieved each pup during the 5-min exposure.

*Interaction time was operationally defined as any direct touching or clear attention given by the female to a pup including retrieving, licking, grooming, sniffing, or stepping on. If the mother was touching a pup, but clearly searching for something else, this time was

not included.

Results

Interaction Time with Pups: A one-way ANOVA did not indicate a significant difference between the amount of time females spent interacting with male or females pups combined across all three conditions, $F = (1, 40) = 0.003$, $p > .05$ (Male $M = 20.2$ sec, $SD = 13.3$; Female $M = 20.0$ sec, $SD = 13.0$). In addition, there was not a significant difference indicated in the amount of time the females interacted with male or female pups when presented simultaneously in the Mixed condition $F = (1, 12) = 0.22$, $p > .05$ (Male $M = 19.9$ sec, $SD = 12.0$; Female $M = 22.9$ sec, $SD = 11.9$).

There was an interesting trend that manifested relating to the total amount of time a female interacted with pups and the trial number of behavioral exposure: No matter the condition, females on average interacted with pups the least during the 3rd trial than the first two trials (1st trial $M = 22.4$ sec, $SD = 13.8$; 2nd trial $M = 23.7$ sec, $SD = 12.7$; 3rd trial $M = 15.9$ sec, $SD = 11.9$). This trend did not reach significance, however, $F = (2, 39) = 1.48$, $p > .05$.

Time Grooming Herself: Females spent the least amount of time grooming themselves when presented with two male pups in comparison of all three conditions (Male $M = 2.4$ sec $SD = 3.3$; Female $M = 5.3$ sec, $SD = 8.6$; Mixed $M = 6.0$ sec, $SD = 8.2$), however this interesting finding was not significant according to a one-way ANOVA, $F = (2, 18) = 0.50$, $p > .05$.

Discussion

Despite the inconclusive results of this experiment, it would be worthwhile to modify the design or specify the current dependent variables measured even further. The variable "interaction time" served as an umbrella for several maternal behaviors that may be significantly different between animals when isolated in observation; the attention a female administers when she is grooming a pup may be very different than the attention she is giving in stepping on a pup. It might prove valuable to separate just these two variables as there may be a difference in the intention behind the female's action and may be more readily administered to a male or female pup. It is highly likely that the combined measures of "interaction time" were too generalized to accurately evaluate maternal behavior. Indeed, the umbrella term might have even included some behaviors that actually indicate rejection. The collected behavioral data could be analyzed with these further details and questions in mind in future research.

The trend that was observed within females grooming themselves, albeit not significant, was interesting nonetheless. A female appeared to be less anxious during her time with male pups than the other two conditions. It may be interpreted that females are more complacent with two males than female pups, but future work should increase the number of subjects given within each condition in order to investigate the strength of this trend. It should also be investigated whether there are other means to measure anxiety or discomfort/displeasure in a mother.

Although it was not originally intended to examine a female's reaction to pups as they are presented over time, it was an intriguing finding that mother's tended to spend less time interacting with pups as time progressed. This may be a novel attribute about

maternal behavior that could be interpreted in many different ways. It would be valuable to investigate what these females are doing with their time otherwise. This may give some insight as to whether there is a generalizable behavior for mothers towards her pups as time progresses through an experiment and as the time since she last interacted with her *entire* litter increases.

Lastly, it was observed that the retrieval of pups, or the neglect to do so, seemed to be more a variable according to the female observed than the condition. Females who did not retrieve either pup in one trial often did not retrieve any pups in the remaining trials. It is possible that these females chose to allocate their time to other maternal investigations in the first few minutes of exposure and females are habitual in their behavior regardless of the gender of the pup. It's possible that this behavior would have changed over time and with more trials and may give insight into a habitual nature of a female.

General Conclusions

It was found that mothers do indeed retrieve foreign young more slowly than own or mixed groups of offspring. However, there was not a measureable difference found in the way a female will discriminate between a group of pups that is partially her own or fully her own. A significant difference in interaction time and self-grooming has not yet been identified in this experiment, however the trend may reach significance in the future given an increase of subjects within each condition. The current finding indicates that alien mothers spend less time interacting with the given pups and more time grooming themselves than the own or mixed conditions. Future work will include full analysis of the collected stained tissue and will serve to contribute to the current findings with an

examination of neural c-fos expression. This will measure brain activity in the hippocampus and medial pre-optic area.

In addition, there was not a significant difference observed in the maternal discrimination of male and female pups as found in previous research (Moore, 1982; Clark et al., 1989). It is possible that the current work did not adequately quantify maternal behavior and future research should be done to modify the current design or measures used in this experiment. Once a behavioral difference is observed, it would be valuable to begin collecting tissue samples in order to examine differences in neuronal activation between females exposed to only males, only females, or both.

This research is important to further the present body available and evaluate the strength of those findings. Animal models may be insightful to natural human tendencies. And the ultimate hope, of course, is to extrapolate the findings made here, and in other research, to useful and practical knowledge that can be applied in various other realms. This work contributes but a piece of the puzzle that is maternal behavior and future endeavors to modify and refine this body of research would prove valuable to clarifying the picture even further.

References

Beach , F. A. Jaynes , J. (1956). Studies of maternal retrieving in rats: I. Recognition of young. *Journal of Mammalogy, 37,* 177-180.

Clark , M.M. Bone , S. Bennett , G. Galef , B.G. , Jr. (1989). Uterine positions and schedules of urination: Correlates of differential maternal anogenital stimulation. *Developmental Psychobiology, 22*, 389-400.

Francis , D. D. Meaney , M. J. 1999. Maternal care and the development of stress responses. *Current Opinion Neurobiology, 9,* 128-134.

Lambert, K.G., Kinsley, C. H. (2012). Brain and behavioral modifications that accompany the onset of motherhood. *Parenting, Science and Practice, 12,* 74.

Hirschfeld DR, Biederman J, Brody L, Faraone SV, Rosenbaum JF. 1997a. Associations between expressed emotion and child behavioral inhibition and psychopathology: a pilot study. *J. Am. Acad. Child Adolesc. Psychiatry* 36, 205–213

Hirschfeld DR, Biederman J, Brody L, Faraone SV, Rosenbaum JF. 1997b. Expressed emotion towards children with behavioral inhibition: association with maternal anxiety disorder. *J. Am. Acad. Child Adolesc. Psychiatry* 36, 910–917

Meaney , M.J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review Neuroscience, 24*, 1161-1192.

Moore, C. (1982). Maternal behavior of rats is affected by hormonal condition of pups. *Journal of Comparative and Physiological Psychology, 96*, 123-129.