Hormone Exposure During Pregnancy and Maternal Care

by

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Abstract

It is suspected that prenatal hormones can affect maternal care, which has important implications for the neurological development of pups. To determine if the altered hormone levels affected maternal care, pregnant dams were exposed to sex hormones and maternal care behavior was quantified and analyzed. Pregnant dams were exposed to corn oil (solvent control), 50ug/kg of estradiol benzoate, or 8mg/kg dihydrotestosterone propionate from gestational day 15.5 to 17.5 which corresponds with a specific developmental window of neurological development. Maternal care during postnatal days 7 and 14 was recorded using JWatcher software and analyzed using R statistical software. No statistically significant differences were found among the treatment groups; however, there was an interesting variation seen in the results. There are many different possibilities for why I saw no significant effects. For example, I only dosed animals for three days. The variation in response to sex hormone exposure suggests that females respond differently to hormone exposure and this idea would be interesting to study further.

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Introduction

Maternal behavior is defined as the ability of individuals to raise healthy offspring (Rees, et. al., 2004); rat maternal care has been studied for many years as well (Weisner and Sheard, 1933). Intrauterine reproductive hormones help nurture the pups in the womb; however they also put together a set of neural systems that ensure the proper maternal care for the pup once born (Rilling et. al., 2014). Therefore if the hormones are significantly altered, maternal care will in turn be altered. Maternal care affects more than just their offspring's physical well being, it also affects their emotional and neurological development. Fetuses are affected by minor changes in hormone levels, and the fetal environment plays an important role in organizing (or programming) the brain properly (Vandenbergh 2003). If the hormones alter the pups, the dam's behavior towards the pups could then be altered or the dam exposed to the hormones could just provide less maternal care in general (Johnson et. al., 2015)

In males, fetal exposure to estrogen induces physiological and morphological changes that organize their neural circuitry such that they act masculinized later in life (McCarthy et al 2008). The extreme male brain theory of autism hypothesizes that over-exposure to testosterone can induce autistic traits (Baron-Cohen 2002; Sparks et al. 2002; Crespi and Badcock 2008); however, maternal care has also been proven to affect this kind of brain development. Brains are considered hyper-masculinized when regions that are normally larger in males relative to females are enlarged even more than expected (Goldstein et al. 2001, Knickmeyer et al. 2006). Altered levels of prenatal hormones, however, could also produce similar yet not as extreme results by changing the levels of maternal care.

In this study, we tested our theory by exposing pregnant rat dams to either an androgen that cannot be converted to estradiol or an estrogen, then measured whether or not either hormone induced a difference in maternal care. Hormone levels of females, such as estrogen, can change maternal behavior, and offspring can be affected by extrinsic environmental factors, such as maternal care or endocrine disrupting compounds (Dulac et. al., 2014; Johnson, 2015). In rodents, differences in maternal care affect social, emotional, and cognitive development (Johnson, 2015; Rilling et. al., 2014; Birnie et. al., 2013). My study tests the hypothesis that maternal care was altered by hormone exposure during pregnancy.

Objectives

This research will determine if prenatal exposure to dihydrotestosterone propionate or estradiol benzoate alters maternal care whether it be the hormones affecting the pups therefore affecting the levels of maternal care, the hormones affecting the mother therefore affecting the levels of maternal care, or both causing maternal care to be altered.

Hypotheses

If a mother's prenatal hormones are adjusted to non-normal levels, then maternal care will be altered.

Methods

Study System

The rat is an appropriate model for this work because maternal behavior in rats has been exclusively studied for many, many years (Weisner and Sheard, 1933). Therefore it is known which behaviors are significant and which ones to watch for so when comparing the control to the hormone treated groups it'll be more effective.

Animals and Hormone Treatments

All work reported herein was approved by East Carolina University's Institutional Animal Use and Care Committee (AUP # D300). Male and female Sprague-Dawley rats (Charles River Laboratories, NC) were housed in the same colony room with 12 hours of light and dark (L:D cycle), and standard rodent chow and water were available ad libitum. Female rats were paired with stud males overnight. The next morning they were examined for the presence of a copulatory plug or sperm in the vaginal smear; females showing either of these indicators were confirmed to be pregnant and defined as being on embryonic day (E) 0.5. Pregnant rats were randomly assigned to a treatment and co-housed until treatment began. From E 15.5-17.5, pregnant rats received subcutaneous injections of either 8 mg/kg dihydrotestosterone propionate (DHTP, N=5), 50 μg/kg estradiol benzoate (EB, N=5), or corn oil vehicle alone (CO, N=4). Injection volume was standardized across treatments by animal weight as 0.5 ml/kg, and administered 4 to 5 hours after the start of the light phase of the L:D cycle. After the dosing period ended, pregnant rats were left undisturbed until parturition.

Litters were culled to eight pups consisting of four males and four females based on anogenital distance, and returned to the dam. One litter of control animals yielded only three males, so five females were kept in the cage to maintain a litter size of eight pups. One litter of EB animals yielded only five pups (three females and two males), so this litter was not culled.

Maternal Care Data Collection

On postnatal days (PND) 7 and 14, the pups and mother were transported in their home cage to a separate room and any bedding or objects obstructing the cameras view was removed as well as the wire lid. A 10-minute period of habituation allowed the animals to adjust to the mild stress due to cage movement; it is crucial the mothers be calm to allow our data to be as accurate as possible. Then, 20 minutes of video was recorded using a camcorder for further analysis on a later date. After each recording session, any removed objects were replaced, the video file was copied onto a computer, and the cage was placed back in the homeroom. Once all the videos had been recorded, the first 15 minutes of the videos were coded for behaviors using software called JWatcher. Behaviors of interest that were to be quantified included: licking and grooming, arched-back nursing, passive nursing, and total maternal care. Licking and grooming, defined as a mother licking her pups in a manner of cleaning them, is important for the stimulation of neurological and emotional development. Nursing in an arched-back manner vs. passive nursing has many advantages; arched-back nursing, defined as nursing while the mother's back is arched allowing her to multitask, gives the pups access to milk, regulates their temperature, and also allows their mother to protect them from the environment. The quantified data was then analyzed using R statistical programing environment and compared across treatments to determine significance.

Statistical Analyses

All data were analyzed using statistical software program R v. 3.1.232. Maternal care data were analyzed using a generalized linear model with a quasipoisson distribution. Models included the response variable, predictors of interest, and their interaction. All models contained a random effect to account for dam effects, and sample sizes reflect the number of dams per treatment rather than offspring. Count data was analyzed using a generalized linear mixed effect regression with a Poisson distribution. Q-Q plots were used to assess whether model assumptions were met.

Results

Data was collected for the behaviors of licking and grooming, arched nursing, passive nursing, carries, exploration, and total maternal care. For licking and grooming, there were no significant effects shown for either week one or week two, but there was a larger difference in the variation between the DHTP treatment group than the EB treatment group (Figure 1, $p > 0.05$).

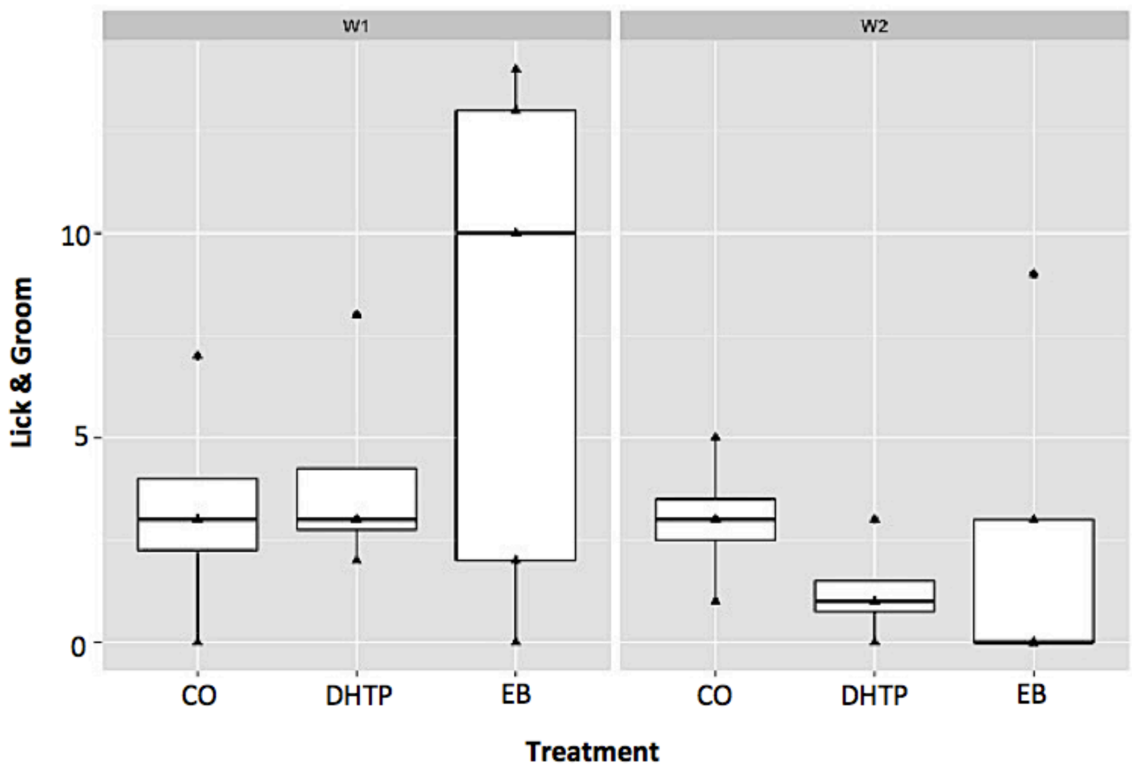

Figure 1. Licking and grooming box plot for week interactions

Arched nursing is defined as the mother nursing while her back is in an arched position, usually a sign of multi-tasking, had quite varied results. From week one to week two, there were still no significant effects found but the control treatment group increased greatly in variation whereas both the DHTP and EB treatment groups decreased in variation (Figure 2, $p > 0.05$). In week two, the EB treatment group's value is practically non-existent.

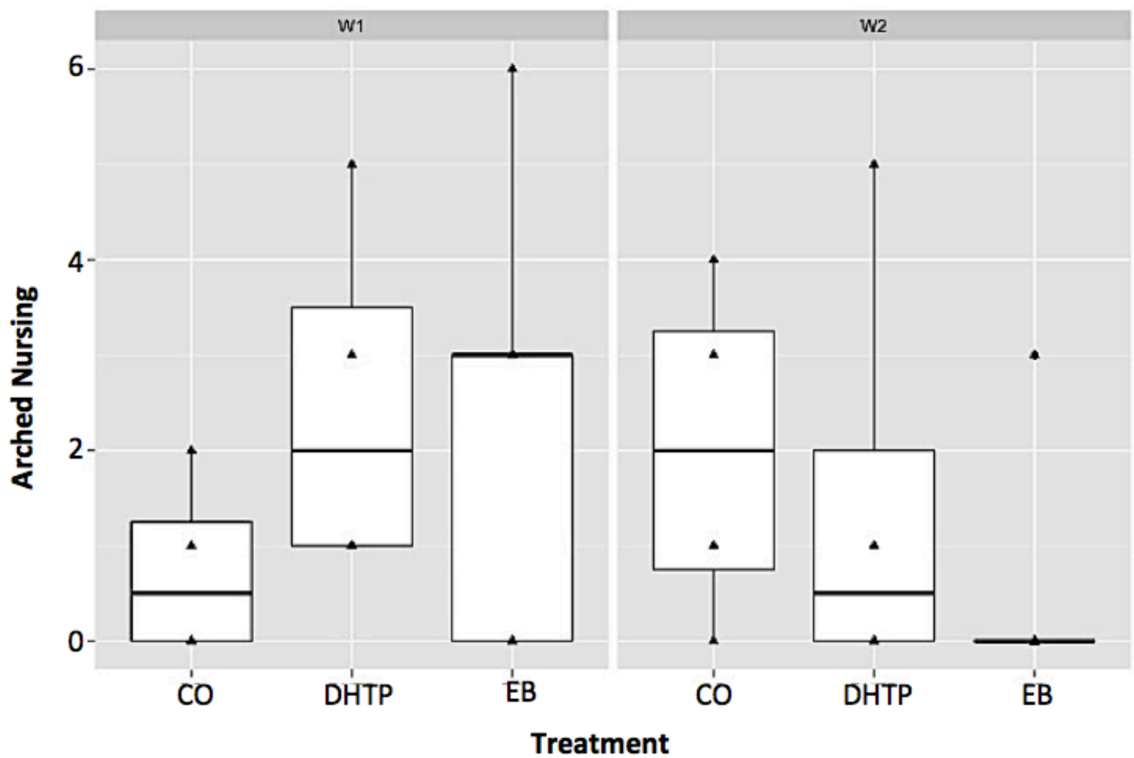

Figure 2. Arched nursing box plot for week interactions

Passive nursing is defined as the mother laying on her back or side while nursing the pups or lying over the pups like a blanket while nursing. From week one to week two, there were no significant effects found but the control treatment group had a dramatic increase in its variation, the DHTP treatment group slightly decreased in its variation (Figure 3, $p > 0.05$). The EB treatment group remained the same from week one to week two; it's mean value being zero.

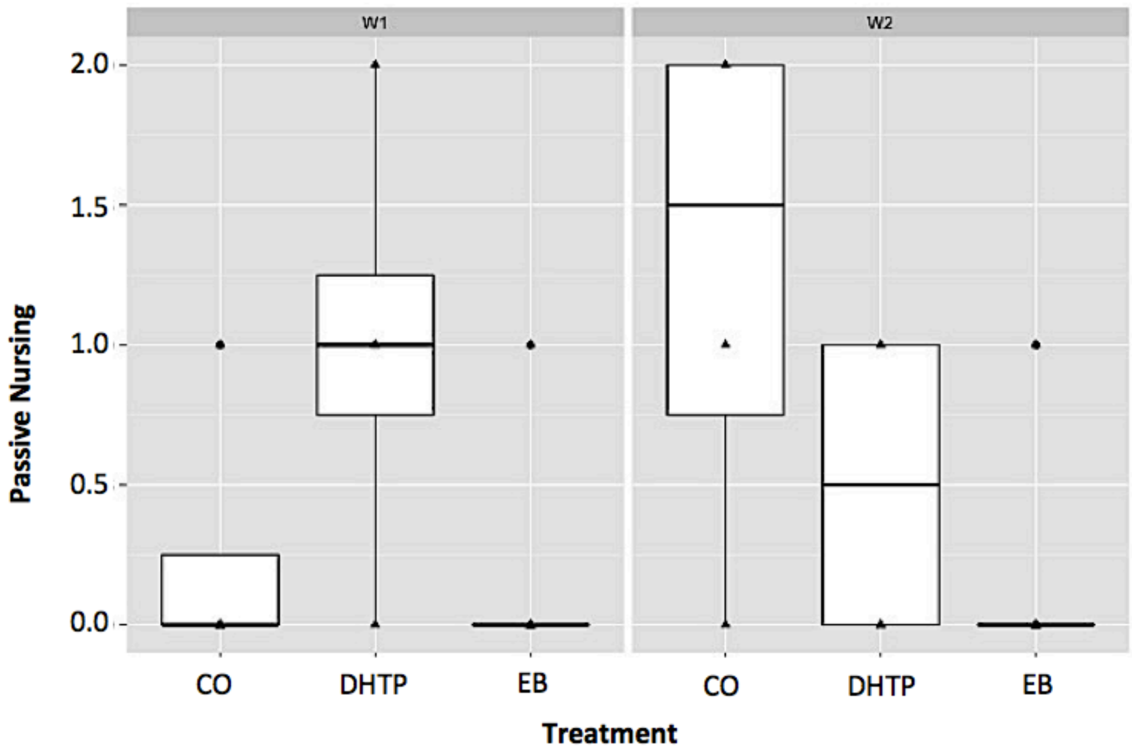

Figure 3. Passive nursing box plot for week interactions

Total maternal care was defined as a sum of all the maternal care behaviors. From week one to week two, there were no significant effects found but the control treatment group had a very minor decrease in the variation yet it had an increase in the mean (Figure 4, $p > 0.05$). The DHTP treatment group decreased in variation from week one to week two and the EB treatment group decreased in variation by approximately half.

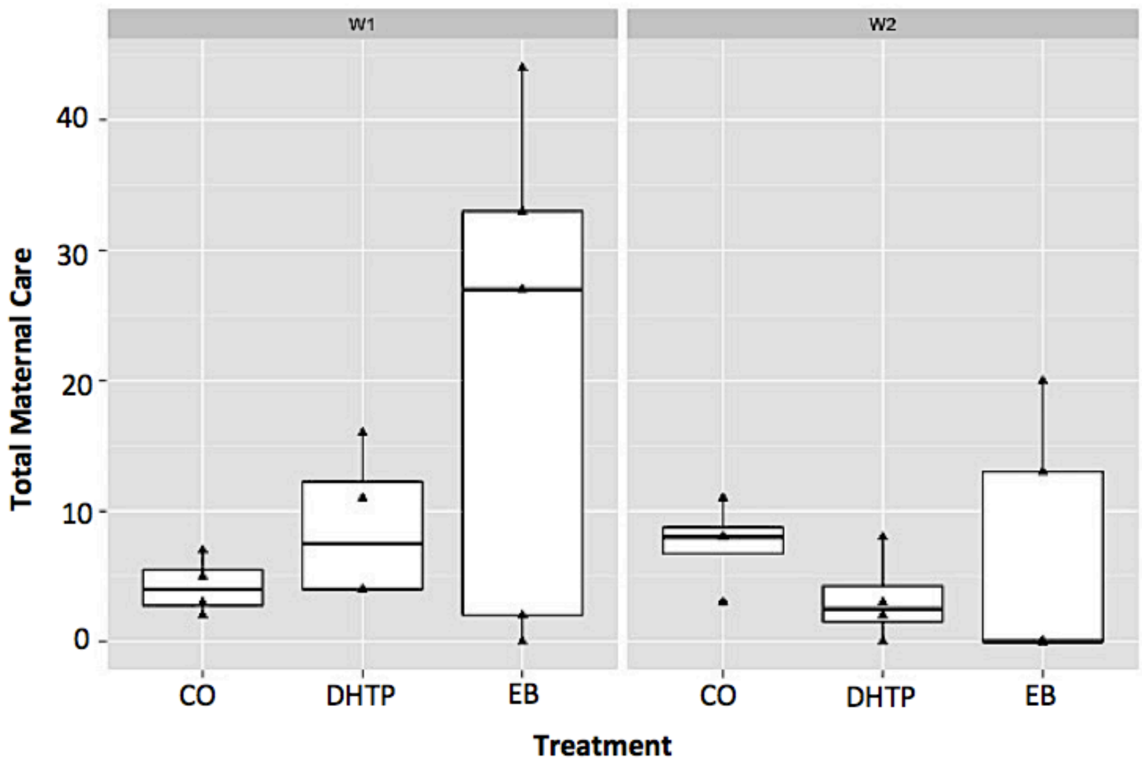

Figure 4. Total maternal care box plot for week interactions

Discussion

I found that prenatal sex hormone exposure during gestational days 15.5 to 17.5 did not affect mean amount of parental care provided by rat dams in the first and second weeks of their offspring's life. This data suggests that behavioral differences detected in offspring prenatally exposed to prenatal hormones was due to exposure rather than differences in maternal care.

For licking and grooming, there was a large variation in the EB treatment group and similar variations for the control and DHTP treatment groups. This tells us that in week one, the EB treated mothers were more variable in how often they licked and groomed their pups but on average did not lick and groom more than the other treatment groups. However, from week one to week two, the variation in the EB treatment group decreased and becomes similar to both the control and DHTP treatment groups (Figure 1). For arched nursing, there was a small amount of variation found in all three of the treatment groups (Figure 2). From week one to week two, the variation in the control treatment group increases and the variation in the DHTP and EB treatment groups decreases (Figure 2); the EB treatment group actually decreases to zero. This could signify different needs in this treatment groups' pups as they get older.

For passive nursing, the variation in the DHTP treatment group is increased in comparison to the control treatment group and the EB treatment group (Figure 3); the EB treatment group is at zero again for this behavior. This could signify that the DHTP mothers tend to do more passive nursing during week one in comparison to the other treatment groups. For week one to week two, the control treatment group's variation increases, the DHTP treatment group's variation decreases, and the EB treatment group's variation stays the same (Figure 3). This could signify that some control treatment group mothers increase passive nursing as the pups get older and that some DHTP treatment group mothers decrease passive nursing as the pups get older. For total maternal care, the EB treatment group showed large variation in comparison to the control treatment group and the DHTP treatment group is slightly increased (Figure 4). This shows that some that the mothers in the EB treatment group provided an increased amount of maternal care than others. For week one to week two, the control and DHTP treatment groups remained approximately the same but the EB treatment group slightly decreased in variation (Figure 4). This shows that mothers converged in their behavior in the second week.

When starting the larger experiment, it was a necessary assumption that the prenatal hormones were only going to affect the pups' later social behavior, if that. However, there were three alternative affects that these hormones could have: one, that the hormones could affect the mother, which in turn affects the way she cares for her pups therefore affecting the social development of the pups; two, the hormones could affect the pups, which in turn affects the way the mother takes cares of them therefore affecting the social development of the pups; or three, both could happen. In doing this maternal care experiment, it was known that it would determine whether maternal care was affected or not. If maternal care was affected, abnormal social behavior of the pups we could be a result of maternal care or hormone treatment; however, mean maternal care was not affected, so social behavior of the pups is most likely due to pre-natal hormones affecting the pups.

During week one, the pups were barely capable of moving and taking care of themselves and therefore appeared to required more care in comparison to week two. The amount of pup independence in week two might have influenced the variation in maternal care therefore it was quite interesting to find that there were no significant effects. Due to this, we placed more emphasis on the week one data in order to examine the maternal care more closely without that added interaction of the mother and pups.

Although there was no statistical significance found across the treatment groups, the variation that was seen in the data was very interesting in week one alone as well as between week one and week two. There are many possible reasons for this variation, and although it can't be determined from the data I collected, it calls for further investigation of prenatal hormonal influence on maternal care; the published experiments found on the subject seem to find significant results in response to prenatal hormones. One reason for this variation could be due to the small sample size (N=5-6); one mother alone might have influenced results if it had an extremely elevated or lowered value. Also, in response to sample size, each mother responds in her own way to this experiment and this small sample size may not show the true range of responses for each treatment. A second possibility could be that it is just naturally occurring in our experiment due to a mother's individual responses. The third reason describing why we found no significant effects and saw interesting variation could be that we only examined count data versus duration data; count data examines how many times a behavior occurs where as duration examines the length of each behavior. This could be important because one mother could be performing a certain behavior 20 times which would make her look like she was taking better care of her pups than a mother performing a certain behavior 5 times; however, duration could reveal to us that the mother who performs that behavior 20 times could only be doing it for an average of 30 seconds per count (10 total minutes) whereas the mother who only does that behavior 5 times could be doing it for an average of 5 minutes per count (25 total minutes). Thus interpreting both types of data would allow us to better interpret each mothers' behavior.

In the larger experiment as a whole, there was significant data found in the social behavior of the sex hormone treated pups. The fact that in this experiment there were no significant effects found from maternal care increases the powerfulness of that result because we know that the maternal care did not significantly affect this outcome.

Why did we find no significant effects when other similar experiments did? It can't be known for sure. Indeed, other studies have found significant differences in maternal care induced by prenatal hormone exposure. One of the differences in studies could be sample size; having a larger sample size would increase our confidence in our estimation of the mean effect. A second difference is the length of the dosage window; our dosing window was only three days during development. This time period was long enough to induce behavioral changes in the pups but did not affect maternal behavior. Previous studies on hormonal effects on maternal care expose mother for longer. In future maternal care experiments I would recommend increasing the sample size in order to see if that variation remained or not. It would also be interesting to record video on more days over the first two weeks of life to further examine the progression of maternal care as the pups mature.

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