Sucrose Preference Testing as a Measure of Anhedonia in “Postpartum” Rats

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Fall 2008

Honors Thesis

PASS WITH DISTINCTION

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TO THE UNIVERSITY HONORS COLLEGE:

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I have read this paper and find it satisfactory.

Rebecca Craft
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Sept. 29, 2008
Date
Précis

Postpartum depression is a serious illness that affects 10-15% of all women who give birth. Although the exact causes of this mood disorder are still unknown, one theory suggests that it may be related to the rapid decline in ovarian hormones that occurs after childbirth. Further studies investigating the specific role of withdrawal from ovarian hormones on the development of depression are necessary in order to confirm or reject their role in the development of postpartum depression.

Because hormone manipulation in humans is potentially harmful, animal models provide the best means for studying the role of hormone withdrawal on the development of postpartum depression. For studies involving rats, the hormone-simulated pregnancy regimen can be used to simulate the natural increase in ovarian hormones, such as estradiol, during pregnancy and their subsequent decline (which occurs at birth). Previous studies using this model have shown that rats experiencing estradiol withdrawal tend to show more depression-like behavior (i.e., "behavioral despair") than those who are not. This method for studying postpartum depression has advantages over studies using actual pregnant rats because it allows for the investigation of specific hormones (since these are controlled by the experimenter), and it eliminates the complications that the weight gain of real pregnancy causes in behavioral analysis.

Because the hormone-simulated pregnancy is potentially a valuable tool for studying postpartum depression, we wanted to further test its validity. Although there is already strong evidence to suggest that estradiol withdrawal from a hormone-simulated pregnancy produces "behavioral despair", the model would be more convincing if it could also produce additional symptoms associated with postpartum depression, such as anhedonia, the decreased ability to experience pleasure. In addition, we wanted to determine how closely the hormone withdrawal
period of the hormone-simulated pregnancy modeled an actual rat pregnancy in terms of postpartum depression-like symptoms. To test this, we administered a sucrose preference test to rats which had undergone a hormone-simulated pregnancy, and to rats that had been previously pregnant, and compared them to their appropriate controls (rats that did not experience hormone withdrawal). Sucrose solution is highly palatable to rats and is typically preferred over water. Thus, we reasoned that results showing decreased sucrose preference in rats undergoing estradiol withdrawal, compared to controls, would suggest the development of anhedonia.

The results of our experiments suggested that rats that had undergone hormone-simulated pregnancy did develop anhedonia in the early hormone withdrawal (“postpartum”) period. However, we were surprised to find that the same did not hold true for actual previously pregnant rats. These results led us to conclude that the dramatic decline in estradiol that occurs when women give birth may be one of the major contributing factors to postpartum depression, but that the hormone-simulated pregnancy may not be an accurate model for real pregnancy in rats. This is not surprising since the postpartum period of actual pregnancy involves marked changes in hormones besides estradiol that are not modeled in the hormone-simulated pregnancy.

Such results indicate a need to investigate additional factors of pregnancy that might contribute to postpartum depression. For example, the fact that actual pregnant rats did not develop anhedonia gives reason to determine the role that other hormones such as prolactin and oxytocin – which increase during pregnancy and the early postpartum period – might play in protecting against the development of this illness. In addition, it might be important to determine how environmental factors such as stress affect the development of postpartum depression. Future investigation of these possibilities will ultimately improve our understanding of and ability to treat this debilitating mood disorder in women.
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1. Introduction

Characterized by symptoms mimicking major depression, postpartum depression (PPD) is a debilitating illness that affects 10-15% of new mothers (Halbreich 2005). Symptoms tend to arise within the first 5-6 weeks following birth, and may persist as long as one year (Cox et al. 1993). The devastating consequences of PPD have been reported in a number of studies. For example, children of depressed mothers tend to show abnormal cognitive, motor and social development (Spinelli 2005), and are more likely to experience depression or anxiety later in life (Nomura et al. 2002). In addition, untreated PPD has been linked to a number of child abuse and infanticide cases (Chrousos et al. 1998). Although the mechanism for PPD onset is highly debated, there is evidence suggesting that the rapid decline in reproductive hormones immediately following childbirth plays a major role. For example, postpartum estradiol treatment reduced depressive symptoms in women with PPD (Sichel et al. 1995). Conversely, pharmacological withdrawal from estradiol and progesterone produced depressive symptoms in women with a history of PPD (Josefsson et al. 2002). Such studies indicate that continued research on the effects of reproductive hormone withdrawal during the postpartum period are important for determining what the mechanisms are and how to best treat PPD.

Proper investigation of the physiological factors involved in the development of postpartum depression requires the use of animal models. This is because numerous social factors, which may also contribute to PPD in humans, make it difficult to pinpoint the specific role of hormone withdrawal in the development of this illness. The hormone-simulated pregnancy (HSP) regimen may provide a valid animal model for testing a “depression-like” state in “postpartum” female rats. This model works by simulating the natural increases in estradiol and progesterone that occur during normal pregnancy, then after terminating hormone treatment,
testing females for depression-like behavior in the forced swim test (Galea et al. 2001; Molina-Hernandez and Tellez-Alcantara 2001; Stoffel and Craft 2004). In this test, immobility (the behavior observed when the rat no longer attempts to escape the water tank) is considered a measure of "behavioral despair." The weakness of this model arises from its ability to only demonstrate one of several aspects of PPD (apathy or "behavioral despair"). Clearly, a single test demonstrating just one symptom of PPD is not sufficient to conclude that estradiol withdrawal contributes significantly to the development of PPD. Therefore, additional "symptom tests" are necessary to validate the HSP-hormone withdrawal model of PPD.

Anhedonia, or the decreased ability to experience pleasure, represents one of the core symptoms of depression (including PPD). Anhedonia can be measured in rats by administering a sucrose preference test, a test which essentially measures a rat’s appetite for a highly palatable, rewarding substance. This test has been used extensively to measure anhedonia in chronic mild stress models of depression (Willner et al. 1987; Papp et al. 1991), and is a good candidate test for measuring anhedonia that may be associated with postpartum estradiol withdrawal. It is possible that by measuring sucrose preference in rats during the "postpartum" period of the HSP regimen, we can establish that a second symptom of PPD is associated specifically with hormone withdrawal.

The sucrose preference test may be additionally important for validating the HSP regimen as an accurate model of pregnancy in rats. Preference results can be directly compared between HSP and actual pregnant females since the characteristics of actual pregnancy, such as weight increase, are unlikely to affect sucrose preference (although they may affect total consumption). In contrast, the HSP model and actual pregnant females cannot be accurately compared using the forced swim test, since the increased weight gain of pregnancy may itself increase immobility.
In this regard, the sucrose preference test is preferable to the forced swim test, although the sucrose preference test has some drawbacks of its own.

The major weakness of the sucrose preference test is that rats may initially be neophobic to sucrose solution, a factor which may decrease consumption, and thus make it difficult to distinguish anhedonia from fear of an unfamiliar substance. The “learned safety theory” described by Rozin and Kalat (1971) explains this phenomenon as an evolutionary protective mechanism in which rats must first learn that a taste is safe before consuming it freely. This theory stems from earlier studies showing that rats developed an aversion more quickly when a poison was associated with a novel substance compared to a familiar substance (Revusky and Bedarf 1967). The relevance of the “learned safety” theory to the sucrose preference measure of anhedonia is that exposure to the sucrose solution prior to testing is important for interpreting preference results. Without examining neophobia in each treatment group prior to testing, results showing decreased sucrose preference during the postpartum period can be interpreted two ways: 1) hormone withdrawal causes anhedonia or 2) hormone withdrawal exaggerates pre-existing neophobia. By including experiments in which rats are habituated to the sucrose solution prior to the actual preference test, we can differentiate between these two interpretations.

The current study has two main purposes: 1) to support previous forced swim test results which suggest that withdrawal from pregnancy levels of estradiol contributes to the development of PPD symptoms (e.g., Stoffel and Craft 2004), and 2) to validate the HSP regimen as an accurate model for pregnancy, by comparing sucrose preference between HSP-treated and actual pregnant rats during the postpartum period. Such results are ultimately important for determining the significance of hormone withdrawal in the onset of this psychological disorder, as well as directing future studies aimed towards the development of effective treatments.
2. Hypotheses

- Females that have undergone HSP or actual pregnancy will show less preference for 1% sucrose solution compared to control females, during the early postpartum period.

- The sucrose preference concentration-effect curve will be shifted to the right in HSP and previously pregnant females compared to control females, during the early postpartum period. That is, HSP and previously pregnant females will still show a preference for sucrose, but at higher sucrose concentrations than controls.

- Females that have been habituated to sucrose will consume more sucrose solution than non-habituated females; however, relative differences between HSP and actual pregnant females vs. controls will be unchanged by habituation.

3. Methods and Materials

For each of the experiments described below, 8-10 female rats (70-100 days old)/group were used. The vivarium was maintained on a 12:12 h light-dark cycle; lights on at 0600 h, off at 1800 h.

3.1. Hormone Simulated Pregnancy (HSP) Regimen

Each female was ovariectomized. Pre-surgery, 0.5 mg/kg s.c. morphine (an analgesic), and a 3.0 ml/kg i.p. Equithesin (a general anesthetic: active ingredients pentobarbital and chloral hydrate) were administered. Both dorsal sides of each rat were shaved and cleaned with clorhexadine. A small incision was made on each rat’s right side, through the skin, connective tissue, and muscle layer. The ovary was exteriorized and the blood supply tied off with 5-0 silk
suture; then the ovary was removed along with the associated fat pad, fallopian tube and upper uterine horn. The muscle wall was closed with a single chromic suture and the skin layer closed with Nexaband wound adhesive and a single 4-0 nylon suture. The same procedure was then performed on the left side. Following surgery, a 2.0 mg/kg s.c. injection of morphine (postsurgical analgesia) was administered. Rats were allowed to recover for approximately 24 h before beginning hormone treatments.

The HSP regimen began with daily s.c. injections of progesterone (4.0 mg/0.3 ml/rat) and estradiol benzoate (2.5 μg/0.1 ml/rat), administered between 1100 h and 1300 h on days 1-16 following surgery. On days 17-22, daily s.c. injections of a higher dose of estradiol benzoate (50μg/0.1 ml/rat) were given. Vehicle-treated (control) rats received daily injections of safflower oil in the same volumes. A nesting behavior test was conducted on days 21 and 22 following surgery to confirm the effects of the hormone treatments on maternal behavior (Bridges 1984). Hormone and vehicle treatments ceased after day 22, initiating the hormone withdrawal period (Stoffel and Craft 2004).

3.2. Pregnancy/Timed Pregnancy Regimens

An equal number of female rats were assigned to either a pregnancy (mated) or control (unmated) group. Females assigned to the pregnancy group were paired with adult males for approximately 14 days or until they appeared definitively pregnant, as evidenced by abdominal swelling. The males were removed from the cages at this time. Any females that did not appear pregnant after 21 days were re-paired with a different male. Females assigned to the control group were paired together for the same amount of time as pregnant females, i.e. pregnant and control females were separated on the same day and tested at the same postpartum time point.
Thus, at testing, females in the previously pregnant and control groups had been caged alone for equal amounts of time.

Females undergoing the sucrose habituation procedure followed a timed pregnancy regimen so that habituation could be conducted on the same day of pregnancy in every rat. Daily vaginal smears were performed prior to pairing so that sexual receptivity could be determined (indicated by a large number of nucleated cells characteristic of the proestrous phase of the estrous cycle). Females determined to be sexually receptive were then paired with males for at least 24 h. The presence of sperm in the vaginal smear the following day provided the most reliable indicator of pregnancy. If the vaginal smear did not show sperm the following day, the male was left with the female until a sample with sperm was observed. The first day that sperm were observed was designated as the first day of pregnancy. Females that never got pregnant were assigned to the control group.

In both pregnancy procedures, each female was picked up by the base of the tail daily between 1100 h and 1300 h to simulate the handling that occurred when HSP females received daily injections (and to accustom females to handling). Once nesting behavior was observed, pregnant females were checked three times daily so that date and time of pup birth could be accurately determined. Pups remained with the female until just prior to sucrose preference testing. The discovery of pups marked the beginning of the postpartum period, or postpartum day 0. The postpartum testing dates were then calculated as follows:

- Postpartum Day 1 = 20-28 hrs after pup birth
- Postpartum Day 2 = 40-56 hrs after pup birth
- Postpartum Day 4 = 3.5-4.5 days after pup birth
- Postpartum Day 7 = 6.5-7.5 days after pup birth
- Postpartum Day 14 = 13.5-14.5 days after pup birth
3.3. Sucrose Preference Test

Sucrose preference testing was conducted from 1800-1900 h. Prior to each experiment, rats were weighed and placed into separate cages. Two pre-weighed bottles, one containing 0% (tap water) and the other containing either 0.5%, 1%, 2% or 8% sucrose solution, were placed on each cage. Bottle order (left-right placement of water vs. sucrose bottles) was counterbalanced among rats in each group. After 1 h, bottles were removed and re-weighed to determine consumption in g. Vaginal smears were taken at this time to determine estrous cycle phase.

3.4. Data Analysis

In each of the experiments described below, sucrose preference (which is theoretically independent of body weight) was calculated as: \((\text{sucrose consumption (g) / total fluid consumption (g)}) \times 100\). Sucrose preference data from each experiment were analyzed by two-way, between-subjects analysis of variance (ANOVA): treatment group (2) X time (5) (Experiment 1), treatment group (2) X sucrose concentration (4) (Experiment 2), or treatment group (2) X habituation condition (2) (Experiment 3). In addition, body weight-adjusted intake (sucrose intake (g)/ body weight (kg)) was calculated for the 24-h sucrose habituation period (Experiment 3). Body weight-adjusted intake data were analyzed by two-way, between-subjects ANOVA: hormone level (2) X gonadal state (2). Significance level in each analysis was set at \(P \leq 0.05\). Independent sample t-tests with Bonferroni corrections were performed in the event of a main effect or interaction in order to identify the time point or concentration at which the effect occurred (Experiments 1 and 2).
3.5. Experiments

For each of the experiments described below, HSP/vehicle and pregnant/control groups were tested over the course of a semester, approximately. For the purpose of comparison, postpartum day in previously pregnant females corresponds to hormone withdrawal day for HSP females (postpartum day 1 = hormone withdrawal day 1).

3.5.1. Experiment 1: 1% Sucrose Preference Time Course

The sucrose preference test was conducted on postpartum days 1, 2, 4, 7 or 14. At each time point, consumption of 0% (tap water) vs. 1% sucrose solution was measured in separate rats (see Table 1)

<table>
<thead>
<tr>
<th>Treatment (N=8-14/group)</th>
<th>Procedure (Day 0)</th>
<th>“Pregnancy Period” Days 1-16</th>
<th>Days 17-22</th>
<th>“Postpartum Period” Postpartum Day 0 Injections Stop</th>
<th>Sucrose Test Postpartum Day 1, 2, 4, 7 or 14</th>
<th>[Sucrose Solution] 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Ovariectomy</td>
<td>Vehicle injections</td>
<td>Vehicle injection</td>
<td>Injections Stop</td>
<td>Postpartum Day 1, 2, 4, 7 or 14</td>
<td>1%</td>
</tr>
<tr>
<td>HSP</td>
<td>Ovariectomy</td>
<td>Estradiol &amp; Progesterone injections</td>
<td>Estradiol injection</td>
<td>Injections Stop</td>
<td>“”</td>
<td>“”</td>
</tr>
<tr>
<td>Control</td>
<td>Female/Female Paired</td>
<td></td>
<td>“”</td>
<td>“”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>Male/Female Paired</td>
<td>Gestation</td>
<td>Pups born</td>
<td>“”</td>
<td>“”</td>
<td></td>
</tr>
</tbody>
</table>

3.5.2. Experiment 2: Sucrose Concentration-Effect Function

On day 2 of the postpartum period, sucrose consumption was measured for either 0.5%, 1%, 2%, or 8% sucrose solutions. A different group of rats was tested at each sucrose concentration (see Table 2).
TABLE 2

<table>
<thead>
<tr>
<th>Treatment (N=8-12/group)</th>
<th>Procedure (Day 0)</th>
<th>“Pregnancy Period”</th>
<th>“Postpartum Period”</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days 1-16</td>
<td>Days 17-22</td>
</tr>
<tr>
<td>Vehicle</td>
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<td>HSP</td>
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<td>Estradiol injection</td>
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<td>Control</td>
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<td>Pups born</td>
</tr>
<tr>
<td>Pregnant</td>
<td>Male/Female</td>
<td>Gestation</td>
<td>“”</td>
</tr>
</tbody>
</table>

3.5.3. Experiment 3: Effect of Sucrose Habituation on Sucrose Preference

On day 17 of the HSP/pregnancy regimen, in half of the rats in each group, the regular water bottle was removed from each rat’s home cage, and a pre-weighed bottle containing 1% sucrose solution was placed on each cage for 24 h. The bottle then was removed and re-weighed so that consumption (in g) during the habituation period could be determined. On day 2 of the postpartum period, a 1 h sucrose preference test was conducted with 1% sucrose (see Table 3).
4. Results

4.1 1% Sucrose Preference Time Course

Figure 1 (below) shows 1% sucrose preference for hormone-simulated pregnant (HSP) and vehicle-treated rats on “postpartum” (hormone withdrawal) days 1, 2, 4, 7 and 14. HSP rats showed significantly less preference for 1% sucrose solution than vehicle-treated rats (F(1,104)=10.80, P=0.001).

4.2 Sucrose Concentration-Effect Function

Figure 2A (below, left) shows preference for sucrose concentrations of 0.5%, 1%, 2% and 8% in HSP and vehicle-treated rats on “postpartum” (hormone withdrawal) day 2. Sucrose preference increased as the sucrose concentration increased in both groups (F(3,71)=14.31, P<0.001), but there were no significant differences between the two groups at any particular concentration. Figure 2B (below, right) shows preference for the same sucrose concentrations in previously pregnant and control rats on postpartum day 2. Again, sucrose preference increased in both treatment groups as sucrose concentration increased (F(3,78)=3.840, P=0.013), but there
was no significant difference in sucrose preference between the two groups at any particular concentration.

**A.**

Figure 2. Preference (mean ± S.E.M.) for 0.5%, 1%, 2% and 8% sucrose solutions. (A) Sucrose preference on hormone withdrawal day 2 in HSP and vehicle-treated rats; N= 8-12/treatment group/[sucrose]. (B) Sucrose preference on postpartum day 2 in previously pregnant and control rats; N=9-11/treatment group/[sucrose].

**4.3 Effect of Sucrose Habituation on 1% Sucrose Preference**

Figure 3A (below, left) shows preference for 1% sucrose in HSP and vehicle-treated rats on “postpartum” (hormone withdrawal) day 2. Habituated rats were exposed to 1% sucrose solution approximately one week prior to testing, while non-habituated rats had no prior exposure to sucrose solution. HSP-treated rats showed less preference for 1% sucrose than vehicle-treated rats on “postpartum” day 2 (F(1,31)=9.24, P=0.005). Post-hoc analysis showed that this decrease in preference was significant only for the non-habituated condition (F(1,14)=0.49, P=0.003); however, neither treatment group showed a significant difference in preference between the habituated and non-habituated conditions. Figure 3B (below, right) shows preference for 1% sucrose in previously pregnant and control rats on postpartum day 2. Habituation conditions were the same as those described for HSP/vehicle-treated rats. Habituated rats showed greater preference for 1% sucrose than non-habituated rats.
(F(1,28)=5.69, P=0.025). There were no significant differences in sucrose preference between the two treatment groups under either habituation condition. Analysis of body weight-adjusted sucrose intake on the habituation day (injection/pregnancy day 17) revealed that HSP, vehicle, pregnant and control rats sampled similar amounts of 1% sucrose solution during the 24-h exposure period (data not shown).

Figure 3. 1% sucrose preference (mean + S.E.M) in rats previously habituated to 1% sucrose (habituated) vs. those that had not been habituated to sucrose (non-habituated). (A) HSP and vehicle-treated rats; N=8/treatment group/habituation condition. (B) Previously pregnant and control rats; N=6-9/treatment group/habituation condition. *Significantly different than non-habituated, vehicle-treated rats, P< 0.05.

5. Discussion

5.1 Estradiol Withdrawal Leads to the Development of Anhedonia in the Hormone-Simulated Model of Pregnancy

In the time course experiment, HSP rats showed significantly less preference for 1% sucrose than did vehicle-treated rats. Since the “postpartum” period of the HSP regimen mainly simulates estradiol withdrawal, this result suggests that withdrawal from pregnancy levels of estradiol leads to the development of anhedonia in the early postpartum period. Such results agree with previous findings by Stoffel and Craft (2004), who demonstrated increased...
immobility ("behavioral despair") on "postpartum" days 2 and 4 in HSP rats exposed to the forced swim test. A correlation between postpartum estradiol and the negative mood effects associated with PPD has been reported in several studies in women (Abou-Saleh et al. 1998; Bloch et al. 2003; Josefsson et al. 2002). In addition, treatment with estradiol has been shown to reverse the negative mood symptoms experienced by women with PPD (Ahokas et al. 2001; Gregoire et al. 1996; Sichel et al. 1995), and to reverse depression-like behavior in an animal model of PPD (Galea et al. 2001). Thus, the present results using the sucrose preference test provide additional evidence that estradiol withdrawal during the postpartum period contributes to negative mood changes such as those observed in PPD.

The likely mechanism for this relationship involves estradiol regulation of central serotonergic neurotransmission. Disturbance in serotonergic activity has been linked to depression, and is the basis for successful treatment of PPD with selective serotonin reuptake inhibitors (SSRI's) such as Prozac® and Zoloft® (Flores and Hendrick 2002; Gjerdingen 2003; Wisner et al. 2002). Studies examining the interaction between estradiol and serotonergic neurotransmission reveal that estradiol downregulates the serotonin (5HT) autoreceptor and upregulates tryptophan hydroxylase, the enzyme responsible for converting tryptophan into 5HT (Pecins et al. 1996, 1999; Rubinow et al. 1998); each of these effects would tend to enhance serotonergic activity. Therefore, it likely that the period of estradiol withdrawal following parturition is accompanied by decreased serotonergic activity. This may increase vulnerability to PPD symptoms such as anhedonia. To confirm that the interaction between serotonin and estradiol is the mechanism by which anhedonia developed in our HSP model, future studies could be conducted to determine whether SSRI treatment (which increases brain serotonergic
activity) during the hormone withdrawal period eliminates differences in sucrose preference between HSP and vehicle-treated rats.

An alternative explanation for decreased sucrose preference in HSP female rats relates to the involvement of estradiol in post-ingestive control of feeding. Previous studies in ovariectomized rats suggest that estradiol may suppress food intake (i.e. sucrose intake) as a result of its interaction with cholecystokinin-mediated signals in the brain (Blaustein et al. 1976; Butera et al. 1993; Geary et al. 1995; Hrupka et al. 1996). Cholecystokinin is a satiety-promoting hormone released in the stomach and duodenum in response to food stimuli. The release of cholecystokinin initiates an afferent signaling pathway which targets areas of the brain such as the paraventricular nucleus of the hypothalamus. Although the exact mechanism remains to be worked out, it has been postulated that estradiol may enhance this afferent input to the paraventricular nucleus, resulting in the suppression of feeding behavior (Akesson et al. 1986; Butera et al. 1989 and 1992; Wade and Schneider 1992). If HSP rats still had higher estradiol levels than vehicle-treated rats during the early “postpartum” period, then the difference in sucrose preference between HSP and vehicle groups may have resulted from differences in post-ingestive satiety signals rather than anhedonia. Plasma hormone levels determined in HSP and vehicle-treated rats from our lab indicate that HSP rats maintain elevated estradiol levels compared to vehicle-treated rats on “postpartum” days 1-4, but by day 7 estradiol levels are not different between the groups (data not shown). Since HSP rats still showed less preference for 1% sucrose compared to vehicle-treated rats on “postpartum” days 7 and 14, group differences in satiety signals probably do not account for the results, at least not at all postpartum time points.

We predicted that neophobia might also influence sucrose preference, perhaps differentially in HSP vs. vehicle-treated rats. For example, if HSP rats were more neophobic to
sucrose solution than vehicle-treated rats, one might expect the HSP rats to show less preference for 1% sucrose than vehicle-treated rats. However, the habituation experiment showed that previous exposure to sucrose (habituation) failed to significantly increase sucrose preference in either HSP or vehicle-treated rats (Fig. 3A), which suggests that either neophobia is not limiting rats’ consumption of sucrose, or that the habituation procedure we used was inadequate for familiarizing rats to the sucrose solution. In regard to the latter possibility, perhaps HSP and vehicle-treated groups did not sample as much of the sucrose solution as the previously pregnant and control rats on the habituation day, and thus could not habituate to the sucrose. However, we found that sucrose intake on the habituation day (“pregnancy” day 17) was similar in all treatment groups (HSP, vehicle, pregnant and non-pregnant) suggesting that at least exposure to sucrose was the same in all groups. Finally, although habituation slightly decreased the size of the group difference between HSP and vehicle-treated rats, the fact that there was no significant difference in preference between habituated and non-habituated rats in each group suggests that the group difference in sucrose preference is not due to a group difference in neophobia.

5.2 The Hormone Simulated Pregnancy May Not Be a Valid Model for Actual Pregnancy

Another important question addressed in this study was how closely the hormone-simulated pregnancy modeled actual pregnancy in terms of the employed measure of depression. Although the concentration-effect functions in HSP vs. previously pregnant rats were similar – preference increased as sucrose concentration increased – the slope was somewhat steeper in the HSP group, suggesting that the two groups may not be comparable. Differences between HSP and actual pregnant females were even more pronounced in the habituation experiment. In contrast to HSP rats, previous exposure to the sucrose solution did significantly increase
preference in previously pregnant rats. Since HSP and pregnant rats sampled similar amounts of sucrose during the habituation period, this difference is unlikely to result from HSP rats failing to habituate to the sucrose solution during the 24-h exposure period. Thus, neophobia appears to limit sucrose consumption in previously pregnant but not HSP rats.

The lack of comparable group differences in sucrose preference between HSP and actual pregnant females may be due to the effects of other hormones that were not investigated in the present study. Of particular interest are hormones such as prolactin and oxytocin, which rise dramatically during pregnancy and the early postpartum period, and are not modeled in the HSP. The potential importance of these hormones is evident in recent studies suggesting that breastfeeding (an event accompanied by marked increases in prolactin and oxytocin) may decrease the risk of developing PPD (Carter et al. 2001; Mezzacappa and Katkin 2002). Thus, it may be that prolactin and/or oxytocin – which have been shown to possess positive mood effects in some recent animal studies (Arletti and Bertolini 1987; Neumann 2007) – buffer the negative mood symptoms caused by estradiol withdrawal. Such an effect would also account for the present results suggesting that HSP but not previously pregnant rats develop anhedonia during the postpartum period. Future studies aimed at determining the effects of additional hormones such as prolactin and oxytocin on depression-like behaviors in ovariectomized rats will be necessary to determine this possibility.

5.3 Conclusions and Future Directions

The present study provides valuable insight into the possible hormonal mechanisms underlying the development of PPD. Estradiol withdrawal suppresses sucrose preference suggesting the development of anhedonia, one of the core symptoms of PPD. This result, taken
together with previous results demonstrating increased immobility in the forced swim test in
HSP rats (Galea et al. 2001; Stoffel and Craft 2004), suggests that estradiol withdrawal occurring
in the early postpartum period – perhaps through its suppressive effect on the serotonergic
system – contributes to the development of PPD. However, this hypothesis would be more
convincing if future studies found that decreased sucrose preference in HSP rats could be
reversed with chronic or sub-chronic administration of an anti-depressant medication such as an
SSRI.

The validity of the HSP model remains somewhat uncertain, given that we did not find
decreased sucrose preference in any of our experiments using actual pregnant rats. On the other
hand, because PPD occurs in only 10-15% of mothers, perhaps it is not surprising that actual
pregnant rats did not show anhedonia. One way to increase the likelihood of observing
depression-like behavior in actual pregnant (and HSP) rats is to expose them to stress during the
gestation/hormone injection phase. The “chronic mild stress” procedure has been used
extensively as a model of depression in rodents (Willner 2005), and stress during gestation has
been shown to induce depression-like behavior in rodent studies (Siobhain et al. 2006; Smith et
al. 2003). Such a model is highly relevant to PPD in women since a number of studies also have
reported a strong correlation between major social and/or environmental stressors and the
occurrence of PPD (Nielsen et al. 2005; O’Hara et al. 1983; O’Hara and Swain 1996; Roberston
et al. 2004). Therefore it may be useful to add a gestational stress procedure to our current
model to determine if rats undergoing estradiol withdrawal are more sensitive to the
physiological effects of stress. Results showing that gestational stress decreases sucrose
preference in actual pregnant females during the early postpartum period might suggest a
combined role for estradiol withdrawal and gestational stress in the development of PPD.
Finally, the implications of this research are important not only for understanding the possible mechanisms of PPD onset, but also for determining the best course of treatment. Since it is likely that both environmental and physiological factors contribute to PPD, a combination of treatment regimens may produce the best results. For example, psychotherapy combined with estradiol replacement and/or SSRI treatment might address both environmental and physiological contributors to this illness. Continued research investigating the role of each of these potential factors will be necessary for the future development of successful treatment regimens.
6. References


