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# Maternal care and affective behavior in female offspring: Implication of the neurosteroid/GABAergic system

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ABSTRACT

In female rats, the proestrus phase of the estrous cycle is associated with decreased levels of anxiety-like and depressive-like behavior relative to the metestrus phase. Progesterone likely modulate these behaviors, in part through the influence of its metabolite, allopregnanolone (THP) on hippocampal GABA$_A$R subunit expression. As natural variations in maternal care have been found to influence both progesterone levels at proestrus and anxiety-like behavior in female offspring, we sought to investigate the importance of maternal care and the estrous cycle on affective behavior in female rats that had received Low or High levels of licking/grooming (LG) during early life. Subjects were tested for anxiety-like behavior in the elevated plus maze at proestrus or metestrus or for estrous cycle-dependent changes in depressive-like anhedonic behavior with a saccharin preference test. GABA$_A$R subunit expression, and THP levels in the dorsal hippocampus and in plasma were also evaluated. Estrous cycle phase influenced saccharine preference and hippocampal THP level in both phenotypes. Low LG animals showed higher levels of hedonic behavior and anxiety-like behavior, irrespective of estrous cycle phase, as well as lower THP levels within the dorsal hippocampus when compared to High LG animals. Only High LG animals showed positive correlations between hippocampal THP levels and GABA$_A$R subunit expression, suggesting a relative insensitivity to THP's modulation of these receptor subunits in Low LG offspring. These findings suggest that natural variations in maternal care influence anxiety-like and hedonic behavior through the modulation of the neurosteroid/GABAergic system.

## 1. Introduction

Variations in parenting style during early life have a profound effect on offspring mental wellbeing. Children exposed to harsh parenting show an increased likelihood of developing affective disorders such as anxiety and depression in adulthood (Rapee, 1997), and may be more resistant to treatment for these disorders (Chambers et al., 2004; Sakado et al., 1999). Similarly, in the rodent, exposure during early life to relatively low levels of maternal licking/grooming (Low LG) results in increased anxiety-like behavior relative to High LG offspring (Francis et al., 1999; Pedersen et al., 2011; Uriarte et al., 2007). By cross fostering neonatal rats to dams of a different LG phenotype, it has been demonstrated that LG behavior received, and not the LG phenotype of a rat's birth mother, appears to be a critical determinant of anxiety-like state in adulthood (Francis et al., 1999). To date, the effects of LG on depressive-like behavior have not been investigated in a rat model.

One potential source of differences in anxiety-like behavior between Low and High LG females is the hormone progesterone, which shows a higher peak during the proestrus phase of the estrus cycle in Low LG than High LG offspring (Cameron et al., 2008). Although few animal studies have investigated the effects of endogenous gonadal hormone levels on mood, exogenous administration of progesterone has anxiolytic and antidepressive effects (Koonce and Frye, 2013), while progesterone withdrawal increases anxiety-like and depressive-like behavior (Gulinello et al., 2002, 2003; Li et al., 2012). Progesterone influences these behaviors through the actions of its metabolite, allopregnanolone (THP). Levels of THP parallel that of progesterone, with increases in both plasma and brain tissue at proestrus relative to diestrus (Frye et al., 2000; Zimmerberg et al., 2005). In the few studies that have looked at cycle effects, brain and plasma THP levels negatively correlate with changes in both anxiety- like and depressive-like behavior across the estrous cycle, with a decrease reported at proestrus rel-

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ative to diestrus (Frye and Walf, 2002; Marcondes et al., 2001). THP administered to the dorsal hippocampus (Mòdol et al., 2011; Rodriguez-Landa et al., 2009) or peripherally (Brot et al., 1997; Rodriguez-Landa et al., 2007) has decreased both anxiety-like and depressive-like behavior in the rodent. In addition, intra- hippocampal or peripheral treatment with finasteride, a 5α-reductase inhibitor that prevents the synthesis of THP from progesterone, attenuates anxiogenic and depressive effects (Frye and Walf, 2002). These data demonstrate that increased hippocampal or peripheral THP alters rodent affective behavior.

THP is believed to influence affect through its actions on the γ-aminobutyric acid (GABA)-ergic system, which is associated with affective state in both humans and rodents (Kalueff and Nutt, 2007). Specifically, THP acts as a positive modulator of extrasynaptic GABAA receptors ($GABA_AR$) comprised of α4βd subunits (Belelli et al., 2002). During late diestrus/early proestrus, the phases of the estrous cycle associated with increasing/peaking levels of progesterone and THP, $GABA_AR$ α4 and δ levels are elevated in the hippocampus (Sabaliauskas et al., 2014). Exogenous administration of THP also increases both α4 and δ levels within the female rat hippocampus (Shen et al., 2005). THP's actions on $GABA_AR$ located in brain regions such as the hippocampus that are implicated in the expression of anxiety-like behavior may explain the anxiolytic effects of this neurosteroid.

Interestingly, the GABAergic system is also influenced by early life environment. Male offspring of Low LG mothers show higher levels of α4 in the amygdala and prefrontal cortex relative to offspring of High LG dams, and a decreased sensitivity to acute treatment with diazepam, a $GABA_AR$ agonist (Caldji et al., 2003). It is thought that the difference in α4 subunit expression between Low and High LG male offspring contributes to behavioral differences in stress reactivity, as α4 knockout mice show increased anxiety-like behavior (Loria et al., 2013). It is unknown whether the differences in subunit expression between male offspring of Low and High LG mothers are also observable in female offspring.

The present experiments sought to characterize the influence of the estrous cycle on affective behavior and on the relation between THP and GABA in Low and High LG female offspring. Subjects were tested in a saccharin preference test, which assesses anhedonia, one of the diagnostic criteria for depression (American Psychiatric Association, 2013). This test shows high validity in modeling depressive-like behavior (Moreau, 2002). Behavioral assays were selected for their demonstrated vulnerability to estrous cycle phase effects (Clarke and Ossenkopp, 1998; Frye et al., 2000; Kanaka et al., 1979; Marcondes et al., 2001). THP levels were also investigated in plasma and in the dorsal hippocampus, a region through which THP appeared to influence both $GABA_AR$ subunit expression and anxiety- and depressive-like behavior (Frye and Walf, 2002; Mòdol et al., 2011). Given the greater fluctuations in progesterone in Low LG females (Cameron et al., 2008), we hypothesized that these animals would show estrous cycle-dependent changes in anxiety and depressive-like behaviors that would reflect changes in THP levels. Furthermore, we predicted that Low LG female offspring would show globally lower levels of $GABA_AR$ α4 and δ subunits in the dorsal hippocampus. Finally, we predicted that levels of THP and $GABA_AR$ α4 and δ subunits would be positively correlated within the hippocampus.

## 2. Methods

### 2.1. Animals

The subjects in all experiments were adult female offspring of animals bred within our colony. Long Evans male and female rats purchased from Charles River Laboratories (Wilmington, DA, USA) were pair-housed at Binghamton University in standard polypropylene cages (22 × 23 × 45 cm) and provided *ad libitum* access to water and rat chow (Purina 5001). No environmental enrichment was provided 22other than social interaction, as enrichment has been shown to alter the effects of LG on offspring outcome (Bredy et al., 2004). Following one week of breeding (2 females: 1 male p/cage), females were again paired with same-sex cagemates, then were isolate-housed during the last week of pregnancy. Litters were not culled so as not to disturb the dams. During pregnancy up through weaning, animals were housed on a 10:14 h light/dark cycle (lights on at 0900 h to allow for maternal observation). Following weaning, animals were housed on a 12:12 cycle (lights on at 0000) to permit behavioral testing during the dark cycle. Subjects utilized for behavioral testing were only exposed to one behavioral test. All animals were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of Binghamton University.

### 2.1.1. Maternal characterization

Pregnant dams were allowed to give birth (postnatal day or PND0) and were observed from PND1 to PND6 as described previously by our laboratory (Popoola et al., 2015). Briefly, dams were observed for LG behavior five times a day (0700, 1030, 1300, 1800, and 2000 h) every three minutes during 75 min sessions. Observations during the dark cycle (at 0700 and 2000 h) were performed under red light conditions. The mean percentage of LG was calculated per dam, and dams with a LG frequency of 1SD or more above the cohort mean were classified as High LG dams, whereas dams with a LG frequency of 1SD or more below the cohort mean were classified as Low LG dams. Offspring of Low and High LG mothers were weaned at PND22, housed two/cage and tested in adulthood at 3–5 months of age. No more than two animals *per* litter were used in each test.

### 2.1.2. Determination of estrous cycle phase

Vaginal secretions were collected daily to confirm day of estrous cycle as our laboratory previously described (Borrow et al., 2013; Cameron et al., 2011). Animals were lavaged each morning at approximately 1100 h with a glass pipette filled with normal saline (NaCl 0.9%). Samples were placed on glass slides and visualized using light microscopy (Axiostar Plus, Zeiss) as described by Marcondes et al. (Marcondes et al., 2002). Estrous cycles were tracked for two weeks prior to behavioral assessment, both to reliably establish cyclicity and to habituate the animals to the procedure. Only animals displaying normal estrous cyclicity (4–5 days) were used in experiments.

### 2.2. Saccharin preference test

Anhedonic-like behavior in Low (n = 17) and High (n = 21) LG offspring was assessed using a two- bottle choice test over a one-hour period daily for ten days. To prevent alterations in drinking behavior in response to the novelty of the paradigm, subjects were exposed to bottles of saccharin and tap water and to a mesh divider for three days prior to testing. On each day of testing, water bottles were removed from each cage two hours prior to the test, and the body weight of each subject was determined. At approximately 1300 h, a wire mesh divider was inserted into each cage, isolating subjects from their cagemates, and subjects were provided access to two bottles- one with tap water and one with a 0.05% saccharin solution- which were positioned randomly to prevent the development of a side preference. Consumption was evaluated by measuring changes in bottle weight, and preference was calculated as [(saccharin consumption in g/kg)/(total consumption in g/kg) X 100]. In addition, mean saccharin preference and mean total