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Biological Basis of Motherhood and Effect of Stress on Maternal Psychophysiology

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Outline and table of contents

1. Introduction

From a Darwinian perspective, the success of an individual is determined by its ability to pass on its genes to the following generation. After courtship, copulation, and fetal development, successful reproduction hinges upon whether the offspring will ever reach reproductive age. The evolution of maternal behaviors revolutionized reproduction. Sustained maternal protection and nurturing of offspring until they were able to fend for themselves allowed for a much higher rate of offspring survival. Parental behavior has evolved in many species, especially among the mammals and birds. It has been proposed that parental behavior arose in evolution as a means to supplement the physiological mechanisms of reproduction [1]. In other words, when fertilized eggs did not develop, hatch, or result in enough survivable offspring to ensure the survival of the species. The level of parental care provided by an animal is largely dependent upon two factors, the first being reproductive strategy. Some animals, mainly fish, amphibians, and some reptiles lay many more eggs than will hatch and leave them without parental care. When they hatch the offspring have to defend for themselves. Other animals, such as many mammals and birds, spend energy to produce only a handful of offspring and then provide extensive protection and nourishment. The second factor that determines level of parental care provided is the developmental condition of the offspring. For example, less parental care is necessary for offspring that are relatively well developed at birth and are able to fend for themselves. However, more extensive care is necessary in species, such as humans, that give birth to largely immobile altricial offspring.

Reproduction is costly. Individuals are limited in the degree to which they can devote time and resources to producing and raising their young. While this energetic

expenditure may be detrimental to the condition of the parent, such expenditure is typically beneficial to the offspring, enhancing their condition, survival, and reproductive success. These differences may lead to parent-offspring conflict; however, parents are naturally selected to maximize the difference between the benefits and the costs, and parental care will exist when the benefits are greater than the costs. For the most part, an animal's life is spent preserving itself; however, when an animal becomes a parent, what was once a largely self-directed organism concerned primarily with its own needs and survival suddenly becomes one focused on the needs and survival of another individual – its offspring. Parents sometimes go to extremes to ensure the survival of their offspring. One extreme example of this is the harp seal *Phoca groenlandica.* Harp seal mothers nurse their pups on 48% fat seal milk continuously for 12 days without eating [2]. During this time, the pup gains an average of 5 lbs per day, while the mother loses about 7 lbs per day [2]. This sacrifice made by the seal mother ensures that the seal pup will be able to survive the bitter cold of the arctic. This altruistic behavior does not necessarily benefit the individual, but the species as a whole.

Behaviors are a consequence of motivational states. For example, hunger is a motivational state that elicits a specific set of behaviors – food seeking and /or eating. By the same token, an animal in heat displays behaviors associated with reproduction, like seeking a mate, displaying courtship behaviors, etc. There are two kinds of motivations: motivations for the survival of the self and motivations that promote the survival of the species. For example, the motivation to self-groom is a motivation that is beneficial to and promotes survival of the individual. While the reproductive motivation observed in the male brown antechinus *Antechinus stuartii* is an extreme example of a

motivation that promotes survival of the species. Once males of this species reach sexual maturity, they will continuously mate often foregoing eating, leading to their death [3].

Motivational states are influenced by hormones, such as testosterone, progesterone, estrogen, oxytocin, etc. Hormones are secreted from endocrine glands into the bloodstream and are transported throughout the body. Due to their method of transport, hormones produce systemic changes leading to the marshalling of resources of the body to produce motivationally salient, goal directed behaviors. Hormones acting at the level of the brain extensively modulate behaviors. For example, estrogen has been shown to drive female reproductive motivations in rats. Females that lack estrogen fail to exhibit mate seeking, courtship, and lordosing behaviors [1]. Diverse and intricate hormonal interactions occur during pregnancy to promote fetal development. Therefore, hormonal action during pregnancy may promote maternal motivation and behavior. The first part of my thesis will examine the biological mechanisms by which maternal motivations and behaviors are modulated.

In the presence of competing motivations, an animal has to choose which motivations to attend to. For example, a hungry animal may initially forego eating if there is a risk to its survival. However, prolonged hunger to the point of starvation may drive an animal to risk searching for and consuming food. This thesis will examine how the presence of a stressor influences the maternal motivations for providing care and/or protection to offspring.

The body responds to stressors via activation of the hypothalamic-pituitary-adrenal (HPA) axis, part of the endocrine system [4]. In response to a stressor,

activation of the HPA axis leads to rapid secretion of corticotropin-releasing hormone (CRH) in the hypothalamus. CRH binds to CRH receptors on the anterior pituitary gland, promoting the release of adrenocorticopropic hormone (ACTH). ACTH binds to receptors on the adrenal cortex and stimulates adrenal release of adrenal glucocorticoids (CORT). These hormones have a system-wide effect and work to alter metabolic pathways involved in ATP production and divert energy from physiological processes nonessential for immediate survival. This thesis will examine how induction of the HPA axis in response to stressors influences the biological modulators of maternal behaviors, and thus influences the quality of maternal care.

Behavioral characteristics associated with maternity

Maternal behaviors are defined as any responses or behaviors displayed by the mother that support the development and growth of the offspring [1]. Maternal behaviors are classified into two categories – those directed towards the offspring and those indirectly related to the offspring, but may not be directed towards the offspring. Offspring-directed responses generally involve direct physical contact with the young and include licking and/or grooming, nursing, maximizing contact with young, and providing warmth and safety. Non-directed parental behaviors typically include protection of young from other individuals, increased food consumption, lactation, and diminished anxiety that is associated with exploratory activities [1]. Although all mammals exhibit similar parental behaviors, it is important to recognize that exact parental responses should be set within the behavioral framework of the given species. For example, while offspring retrieval is a common indicator of maternal behavior in

rodents [2-4], cats and dogs, in sheep, maternal bleats and proximity to young are used as measures of maternal responsiveness [5]. Although characteristic behaviors may vary, it is important to note that these behaviors serve the same basic purposes – providing the offspring with protection, comfort, and nourishment. These differences in maternal behaviors may be due to biological constraints. For example, a female sheep cannot physically retrieve lambs as cats retrieve kittens due to the size of the lamb; therefore, sheep use bleating as a way to direct the lamb [5]. Both of these behaviors however achieve the same purpose – offspring retrieval and ultimately protection from harm. Since maternal behaviors appear to be conserved across many species, it can be assumed that similar biological mechanisms may be regulating these behaviors.

Rodents are the most widely used model for maternal behavioral studies, therefore, it is useful to briefly discuss some common behaviors that are used for scoring parental responsiveness. Pup-directed maternal behaviors are typically studied by examining behavioral responses, such as: the latency to retrieve pups; number of pups retrieved; whether or not females group pups in the nest; whether or not females crouch in a nursing posture; liking/grooming of pups; and the percentage of time the female spends exhibiting each behavior [1, 4, 6, 7]. Other studies take advantage of the fact that lactating females often show aggression towards and/or attack intruders as a means to examine indirect-young associated maternal responses [8-10]. This behavior is termed maternal aggression. However, calling this behavior maternal aggression could be confusing, as it could also apply to infanticidal behaviors. Therefore, I will be referring to these specific behaviors as pup defense. Studies that use pup defense as a proxy for maternal responsiveness typically score defensive behavior by noting 1) the

latency for the mother to take interest in the intruder, 2) the number of agonistic behaviors (lunging, rearing on hind legs), 3) the number of times the mother attacks the intruder, and 4) the latency for the mother to attack after introduction of the intruder into the mother's home cage.

Research indicates that most female mammals appear to be instantaneously maternal at the time they give birth, or parturition. Moreover, many female mammals display an enhanced motivation to respond positively towards their offspring [1]. Early postpartum females voluntarily spend a vast majority of their time with their pups, bar press insatiably for access to pups and readily retrieve pups from anxiety provoking areas [11]. However, outside of the parturition and lactation period, the degree of parental responsiveness is highly variable among species, sexes, reproductive experience, and individuals. Seip and Morrell reported that maternal caregiving behaviors can be induced in both virgin, or nulliparous (females who have never been pregnant), female and male rats, but only following continuous exposure to pups [11]. Seip and Morrell examined the difference in maternal motivation between postpartum female rats and nulliparous female rats by noting the latency (in days) for the female to display full maternal behavior. The authors scored maternal behavior based on 1) the number of pups the female retrieved, 2) whether the female adopted a crouching or hovering posture, and 3) the quality of nest the female built (ranging from no nest to nest built using all of the material provided). There was a significant difference in latency to express full maternal behavior when comparing early postpartum females to nulliparous females. Early postpartum females displayed an immediate onset of full maternal behaviors. Nulliparous females, however, never displayed full maternal

behaviors unless they were exposed to pups over a prolonged period of time (21 days) prior to testing. Still, despite prolonged exposure to pups, the nulliparous females were significantly slower in expressing full maternal behaviors than early postpartum females. This research indicates that experience alone does not account for the dramatic behavioral and motivational changes that occur when females transition into motherhood. If experience alone is not responsible for these changes, then drastic and dynamic changes must be occurring at a biological level in association with gestation and parturition that result in these behavioral and motivational changes.

Biological mediators of maternal behaviors

At the biological level, behavior is regulated by three interacting systems - genes, hormones, and the nervous system. It is important to also note that while each of these interact and influence each other, external environment can influence each of them, and the manner the three systems interact with each other, and thus influence behaviors. One way to envisage behavior is that behaviors tend to maintain homeostasis by responding to internal and/or external stimuli. For example, an organism would respond to the hunger by searching for food and consuming food. However, not all behaviors can be attributed to maintenance of homeostasis, as most social behaviors, including sex are not critical for the survival of individuals. These behaviors however are critical for the propagation of one's genes, and thus, the survival of species. Parental behaviors fall in the latter category. One of the contemporary theories of the regulation of maternal behaviors posits two phases of control [1]. The first phase is the onset of maternal behavior and is proposed to be strictly under hormonal control. This phase takes place

during pregnancy and parturition as intricate hormonal fluxuations that prepare the mother to exhibit nurturing behaviors immediately after giving birth [12, 13]. The second phase is the maintenance of maternal behaviors and is thought to be driven by some hormones in addition to reinforcing interactions between offsprings and mothers [14].

Hormonal Regulation of Maternal Behaviors

Hormones are a class of signaling molecules, produced by endocrine glands, that travel via the bloodstream to produce system-wide regulating effects. Hormones, perhaps due to their preexisting role in sexual physiology and behavior, are reported to plan an important role in the induction, maintenance, and retention of parental behaviors. Interestingly, among mammals, many of the different hormones that are required to maintain pregnancy and nursing also seem to play an integral role in mediating maternal behaviors. This section briefly discusses how various hormones (like estrogen, progesterone, and different lactogens) modulate maternal psychophysiology required for providing maternal care.

Pregnancy: preparing for motherhood

From a physiological vantage point, pregnancy and maternity lie on a continuum, with pregnancy leading to and facilitating maternity. Moreover, from an evolutionary vantage point, mammalian females' investment in offspring starts with pregnancy. Therefore, it is important to briefly discuss some physiological characteristics of pregnancy, many of which will gradually enable females' transition into motherhood, and induction of maternal psychophysiology. The onset of pregnancy is characterized by a

shift in the circulating hormonal profile of pregnant females. Hormonal changes concomitant with pregnancy play critical roles in initiating the process of implantation of the fertilized ova and suspension of the ovarian cycle. The ovarian hormone, progesterone is responsible for these events. Progesterone is often referred to as the “pregnancy hormone” due to its essential role in preparing the uterus for implantation of the fertilized egg and maintaining pregnancy [13]. Shortly after fertilization, there is a sharp increase in progesterone levels. Circulating progesterone continues to be maintained at high levels throughout pregnancy, until a short period before parturition, which is marked by a crash in progesterone levels. Another hormone that plays a critical role in pregnancy is estrogen. The role of estrogen is well-known in regulating female sexual psychology. After facilitating ovulation, circulating levels of estrogen gradually increase and remain high throughout pregnancy, parturition and lactation. Hormone prolactin is a critical lactogen – it regulates milk secretion. As pregnancy progresses, under the influence of prolactin, the mammary glands begin to proliferate secretory tissue that will produce milk and ducts that will carry the milk to the newly-enlarged nipples. Prolactin also plays a critical role in facilitating pregnancy. During the first half of pregnancy, prolactin is secreted by the anterior pituitary gland, following a cyclical pattern, that corresponds with its role in regulating progesterone secretion [13]. Progesterone feeds back on prolactin secreting cells and suppresses prolactin release from cells in the anterior pituitary. At this time, regulation of progesterone secretion is taken over by placental lactogens. A few days before parturition, the placenta stops secretion of lactogens, leading to a sharp decrease in progesterone levels. At this time, prolactin secretion, no longer suppressed by progesterone, rapidly increases to prepare

for milk production [13]. Throughout most of the pregnancy, oxytocin remains suppressed and only increases in the last few days to induce uterine contractions.

Interestingly, the same hormones responsible for mediating different stages of pregnancy, also mediate the initiation of maternal behaviors. In one of the earliest studies that investigated the role of hormones in maternal behaviors, Rosenblatt and Siegel [15] hystertomized female rats in various stages of pregnancy to terminate their pregnancy and study its effect on the onset of maternal behaviors. Following hysterectomy, the authors quantified the number of days the females need to be exposed to pups to display maternal behaviors (pup retrieval, adopting a nursing posture, grooming pups, and grouping pups). They found that the numbers of days of pup-exposure needed before the display of maternal behaviors was significantly fewer when the hysterectomy was performed later in the pregnancy, ranging from seven days in day 8 pregnant females to two days in day 19 pregnant females. The authors furthermore reported that hysterectomized females displayed full maternal behavior sooner than intact pregnant females at the same stage of pregnancy, demonstrating that the termination of pregnancy leads to a rapid onset of maternal behaviors. The finding that the latency to exhibit maternal behaviors decreased the later in the pregnancy the hysterectomy was performed indicates that intricate fluctuations of hormones during pregnancy play an integral role in preparing females for the immediate expression of maternal behavior at parturition. The authors dubbed this hormonal effect on subsequent maternal behaviors as “hormonal priming”. The roles of these individual hormones on maternal behaviors are briefly discussed below.

The role of estrogen in regulating maternal psychophysiology

Estrogen is a steroidal sex hormone produced primarily by the ovaries in females. In females estrogen is essential for modulating reproductive processes, including menstruation, pregnancy, and specific reproductive behaviors. In addition to its role in modulating reproductive processes, estrogen has been shown to be required for initiation of maternal behaviors at parturition. In the same study discussed above, Rosenblatt and Siegel [15] investigated the role of estrogen in mediating maternal behaviors in female rats. Pregnant female rats at different stages of pregnancy were either hysterectomized or ovariectomized + hysterectomized and tested for the latency to display maternal behaviors. In all stages of pregnancy, the ovariectomized + hysterectomized females took significantly longer than the hysterectomized females to display full maternal behaviors. This indicates that ovaries must be present for female rats to exhibit maternal behaviors. These results also suggest a critical role of estrogen in modulating maternal behaviors.

Further studies have shown that estrogen can stimulate maternal behaviors even in non-pregnant hysterectomized and ovariectomized female rats [15, 16]. After being hysterectomized or hysterectomized + ovariectomized, non-pregnent rats were tested to determine the onset of maternal behaviors - complete pup retrieval and adopting a nursing posture. These females were continuously exposed to pups of a period of five days until they displayed full maternal behaviors, a process previously referred to as pup sensitization. Additionally, some of the hysterectomized + ovariectomized individuals received doses of 100μg/kg of estrogen at surgery and 0.5 mg of progesterone 44 hours later, 100μg/kg of estrogen at surgery, or 20μg/kg of estrogen at

surgery. The authors reported that neither hysterectomy nor hysterectomy + ovariectomy alone accelerated the onset of maternal behaviors in virgin rats. While 20μg/kg of estrogen was able to stimulate maternal behaviors in pregnancy-terminated females in previous studies, a higher dose of 100μg/kg given with or without progesterone was required to produce the same initiation of maternal behaviors in non-pregnant females. The results of this study demonstrate that estrogen is able to induce maternal behaviors, even in non-pregnant female rats. However, when the results of non-pregnant and pregnancy-terminated females are compared, non-pregnant females require a higher dosage of estrogen to display the same onset of maternal behaviors. This likely occurs because the pregnancy terminated females have been stimulated by estrogen and progesterone during pregnancy, whereas non-pregnant females have not received this hormonal stimulation. These results suggest that the rise in estrogen at the end of pregnancy [17] is able to stimulate maternal behavior at parturition because the female has been hormonally primed throughout pregnancy. Another study by Stolezenberg et al. [18] provides further support to the concept that estrogen is required for priming of maternal behaviors. In this study, authors measured the onset of maternal behaviors in pregnancy terminated and virgin rats. Pregnancy terminated and virgin rats were hysterectomized + ovariectomized. On the day of pup presentation, the females were given100μg/kg of estrogen. A single dose of 100μg/kg estrogen was able to stimulate maternal behaviors in the pregnancy terminated females, but not in the virgin females, thus providing further supporting evidence that estrogen is involved in producing a “priming” effect in mothers.

The role of progesterone in regulating maternal psychophysiology

Circulating progesterone levels are elevated throughout pregnancy and decline shortly before birth. Progesterone secretion by the corpus luteum is essential for establishing and maintaining pregnancy. In addition to its role in maintaining pregnancy, progesterone also has been shown to play an important role in regulating both the expression of maternal behaviors and well as lactogenesis [1] by inhibiting the action of other hormones (prolactin and oxytocin). It has been proposed that progesterone is involved in two behavioral functions [19].The first behavioral function progesterone has is to prime females brains during pregnancy in order that the female can provide maternal care to pups at parturition. The second is to control the timing of increased responsivity to pups. In rats, as the duration of progesterone exposure in the gestating female lengthens, the female becomes more primed to respond maternally to foster pups once circulating progesterone levels decline [1]. However, cumulative priming is produced only once progesterone levels decline. Siegel and Rosenblatt [15], hyterectomized + ovariectomized virgin female rats and injected them with 100µg/kg of estrogen immediately following surgery. Following surgery, rats were injected with either 5.0 mg or 0.5 mg of progesterone, 24 hours, or 44 hours after estrogen injection. Female rats were then tested for latency to exhibit full maternal behaviors. Injection of progesterone at the highest dose, 5.0mg, completely abolished estrogen-induced maternal behaviors, regardless of injection time. These individuals showed behavior patterns that resembled hysterectomized + ovariectomized virgins that received no estrogen. However, at a lower dose, 0.5 mg, progesterone's effects on estrogen-induced maternal behavior are time dependent. When administered immediately after

estrogen injection, 0.5 mg of progesterone increased the latency to exhibit maternal behaviors. Additionally, progesterone was most effective at inhibiting maternal behaviors when given 24 hours after the estrogen injection; however, by 44 hours after estrogen injection, progesterone did not appear to interfere with maternal behaviors. Therefore, this study concludes that the effect of progesterone on estrogen-induced maternal behaviors in nonpregnant hysterectomized + ovariectomized females is both dose and time dependent. Additionally, the results of this study suggest that high progesterone levels interfere only with the initiation not maintenance of maternal behaviors. These findings suggest that elevated levels of progesterone during pregnancy may inhibit maternal behaviors, and that the decline in progesterone just prior to parturition may be necessary for the onset of maternal behaviors at parturition.

While progesterone may interfere with the initiation of maternal behaviors at parturition, progesterone my actually facilitate some maternal behaviors during lactation. From the sharp decline in progesterone levels just prior to parturition until day 3 of lactation, circulating levels of progesterone are minimal. However, serum progesterone levels gradually increase from day 3 of lactation until day 10 of lactation, and do not appear to interfere with maternal behaviors at this point [19]. This increase in progesterone levels following the onset of lactation may facilitate maternal defensive behaviors [19]. Lactating female rats were either ovariectomized or sham operated and subsequently tested for pup directed behaviors (grooming, nursing posture, retrieval, nest building) and maternal defense in response to a male intruder. After behavioral testing, hormonal profiles were assessed for each female. Compared to sham operated females, ovariectomized females spent a significantly less amount of time grooming

their pups and retrieve pups less. There was no difference between the two groups in exhibiting nursing and nest building behaviors. During the maternal defense test, the ovariectomized females initiated significantly less attacks and bites towards the intruder than the sham operated females. Results of the hormonal profile showed that ovariectomized females had significantly lower levels of progesterone and significantly higher levels of prolactin in comparison to the sham operated females. The results of the maternal defense test and hormonal profiled showed a correlation between lowered progesterone levels and decreased maternal defensive behaviors. This suggests a potential facilitative role of progesterone in mediating maternal defensive behavior.

The role of prolactin in regulating maternal psychophysiology

Lactation is critical to mammalian offspring survival. Plasma prolactin levels increase at the end of pregnancy [13] and appears to play a critical role in stimulating lactation and initiating maternal behaviors. Prolactin is a polypeptide hormone that is synthesized and secreted by lactotropic cells of the anterior pituitary gland [20]. Prolactin receptors or binding sites have been identified in numerous cells and tissues in mammals [21]. In mice and rats, two forms of the prolactin receptor exist, generated by alternative splicing of a single gene [22]. Mice carrying a germ line deletion of the prolactin gene were produced via homologous recombination [21]. The development of a mouse line that is deficient in the prolactin receptor provides an ideal means to better understand the role of prolactin in mediating maternal behaviors. When compared to wild-type female mice, both homozygous (-/-) and heterozygous (+/-) females exhibit profound deficits in maternal care when challenged with foster pups. Heterozygous (+/-)

and homozygous (-/-) females exhibit a significantly longer latency to begin retrieving pups to the nest when compared to wild type (+/+) females. Whereas wild-type females typically took 1-2 days to start retrieving pups, heterozygous females took 4-5 days, and homozygous females took 6 days [7]. When virgin females were tested for full onset of maternal behaviors (retrieving all 3 pups to the nest and crouching over them), wild-type mice were fully maternal within 1-2 days, heterozygous mice were fully maternal within 4 days, and homozygous mice were never fully maternal within the 6 day testing period[7]. When virgin females were tested for maternal behaviors, heterozygous females displayed altered maternal behaviors when compared to wild-type females. In wild-type virgin mice, normal components of nurturing behavior include nest building, retrieving and grouping of pups in the nest, anogenital licking, and crouching over pups in the nest to provide warmth and nursing [7]. Heterozygous mothers were observed to leave pups scattered throughout the cage, a behavior never observed along wild-type females.

Intracerebroventricular (i.c.v.) infusions of prolactin can induce maternal behaviors in virgin female rats [23]. Prior to receiving central infusions of prolactin, the female rats were ovariectomized and hormonally primed with estrogen and progesterone to mimic hormonal changes that naturally occur during pregnancy. One week after the ovariectomy, the females received i.c.v. infusions of either 10μg, 50μg of prolactin, or a control vehicle twice daily. Beginning the day following prolactin injections, all animals were observed in their home cages for 1 hour to determine maternal behaviors (pup retrieval, grouping of pups in nest, latency to respond to pups, and adopting a nursing posture). Infusions of both 50μg and 10μg of prolactin

stimulated a rapid onset of maternal behaviors when compared with controls. Prolactin appeared to affect maternal behaviors in a dosage-dependent manner. Significantly more females were fully parental on test day 4 in the 50μg prolactin group than in the 10μg prolactin group. The same pattern was seen when comparing the 10μg prolactin group with the control group. Results from these studies suggest that the presence and proper functionality of prolactin is essential for the initiation of maternal behavior in the female rat.

The role of oxytocin in regulating maternal behaviors

In addition to estrogen, progesterone, and prolactin, oxytocin is another hormone that appears to be critical for the induction of maternal behaviors. However, secretion of oxytocin occurs only at the very end of pregnancy. During labor, oxytocin stimulates contractions and is required for the milk-ejection reflex [24]. Oxytocin is a nonapeptide hormone that is synthesized within the hypothalamic paraventricular nuclei (PVN) and supraoptic nuclei (SON) [2]. Oxytocin has been implicated in various social behaviors, such as reproductive and parental behaviors [2, 25]. Administration of an oxytocin antagonist inhibits spontaneous maternal behaviors in adult female prairie voles [25]. Additionally, epidural anesthesia prevents both central oxytocin release and maternal behaviors in parturient ewes, whereas i.c.v. infusions of oxytocin reverse the behavioral effects of epidural anesthesia [26]. OT has been implicated in various social behaviors, such as reproductive and parental behaviors [2, 25]. Oxytocin acts by binding to a single receptor subtype [25]. Olazabal et al. (2006) [25] examined oxytocin receptor (OTR) density throughout the brain and its correlation with parenting behavior in adult female

prairie voles. Authors found that there was significant variability in the OTR binding in the nucleus accumbens (NAc) and lateral septum (LS) correlated with maternal response. Maternal females had higher OTR densities in the NAc than non-maternal females. Administration of an oxytocin antagonist into the NAc resulted in an inhibition of “spontaneous” maternal behaviors. An additional study by Rich et al (2014) examined the role of Oxytocin in regulating maternal behaviors using post-parturient rats with an OTR knockout[27]. Both wild-type and OTR mutants were tested for maternal behaviors (pup retrieval and maternal defense). 10 out of 15 OTR mutant females had abandoned their litters within one day of giving birth. In this case, all of the pups were found either cannibalized or dead in the cages. In contrast, only 2 of the 11 wild-type females had abandoned their litters within one day of giving birth. Although OTR mutants have increased abandonment, those who initiated maternal behavior did not differ from the wild-type in expression of maternal behaviors. From the results of this study, authors indicate that while oxytocin is important for the initiation of maternal behaviors, it may not be important for the maintenance of maternal behaviors. However, this interpretation conflicts with other studies, as oxytocin has a crucial role in lactation [28].

Oxytocin, prolactin and lactation

Both prolactin and oxytocin secretion are present at high levels during the postpartum period and are necessary for lactation. Recently, oxytocin has emerged as a primary prolactin-releasing factor. Oxytocin binds to oxytocin receptors found on the cell membrane of lactotropic cells [28]. Additionally, experiments performed in vitro have shown that administration of oxytocin to lactotropic cells initiates prolactin release that is

preceded by intracellular Ca^{2+} release, suggesting that oxytocin stimulates prolactin secretion via a Ca^{2+} dependent mechanism [28]. Several studies have shown a relationship between oxytocin and prolactin secretion in response to suckling. In response to suckling, a rise in peripheral oxytocin levels precedes the increase in suckling-induced prolactin secretion [106, 107]. Administration of an oxytocin antagonist effectively blocked the suckling-induced prolactin secretion. Interestingly, prolactin secretion stimulates oxytocin secretion during lactation. Oxytocin neurons in the PVN were shown to increase during lactation [28]. Additionally, these oxytocin neurons express the prolactin receptor. Therefore, this suggests that oxytocin and prolactin may be coupled in a positive-feedback loop that leads to increased release of oxytocin, which can then act at the lactotropic cell to stimulate prolactin secretion.

During periods of non-stimulation, lactotropic cells release prolactin in a constitutive manner. Therefore, prolactin release is tonically inhibited by dopaminergic neurons. Administration of a dopamine receptor antagonist induced prolactin release from the pituitary gland, while administration of a dopamine receptor agonist inhibited prolactin secretion [108]. In response to suckling, dopamine arriving at the anterior pituitary is decreased and prolactin secretion is elevated before negatively feeding back to stimulate dopamine release by binding to prolactin receptors on dopaminergic neurons [28]. Therefore, this creates a negative feedback loop where prolactin controls its own secretion via dopaminergic action. From evidence, it is thought that regulation of lactation by prolactin and oxytocin happens in the following manner: Suckling causes increased secretion of oxytocin, which activates prolactin, which in turn, feeds back and activates oxytocin in a positive feedback loop. Prolactin increase stimulates

dopaminergic neurons to feedback and inhibit prolactin secretion in a negative feedback mechanism [28].

Neuroanatomical correlates maternal behaviors

Olfactory bulb/accessory olfactory bulb

Olfaction plays an important role in the control of maternal behaviors in many mammals [29]. Most mammalian females exhibit maternal behavior immediately after birth of their offspring, while both male and female virgin rats and mice show parental behaviors only after repeated exposure to pups, suggesting a role for pup-derived stimuli in mediating maternal behavior [29]. Multiple studies suggest that olfaction facilitates maternal behaviors that result from pup exposure [8, 9, 30, 31]. Pup exposure may influence behaviors via pheromonal signaling which is detected by the vomeronasal organ (VNO) [31]. Tachikawa et al. reported a direct relationship between pup exposure, parental behavior, and vomeronasal activity in male rats [31]. Males that were exposed to pups for a longer period of time displayed greater parental behaviors and decreased c-Fos expression in the AOB after pup exposure when compared to unexposed males. Males that exhibited pup-directed aggression exhibited high levels of c-Fos expression in the AOB following pup exposure. To confirm the importance of VNO activation for pup-directed aggression, VNO-ablated male mice were subjected to the pup retrieval assay. Males that received VNO ablation showed a significant increase in pup-directed behaviors and decreased c-Fos expression in the AOB and OB when compared to previously unexposed/control males. The results of this study suggest that pheromonal stimuli, generated by the pups, exerts an inhibitory effect on vomeronasal

nuclei involved in the induction of parental behaviors in virgin rats. Another study in female virgin rabbits supported the role of the AOB in inhibiting maternal behaviors [30]. In this study, virgin female rabbits were exposed to foster pups for 14 days and did not show maternal responsiveness. However, after surgical removal of the AOB, the females started displaying maternal behaviors towards the pups. The authors reported that the behavior appeared abruptly and appeared somewhere between days 3 and 13 of exposure to the pups. A study by Carretero et al. [32] further explored the inhibitory effect of pup pheromones in virgin female rats. Since previous studies reported that pheromonal activation of the VNO and AOB inhibited maternal behaviors, researchers infused a GABA antagonist directly into the AOB of virgin female rats and exposed them to pups. When compared with untreated virgin rats, virgin rats that received the GABA antagonist infusion exhibited significantly more maternal behaviors in a significantly shorter amount of time. Therefore, these findings suggest that the display of maternal behaviors requires a disinhibition of AOB neurons. From the VNO, pheromonal signals are relayed to the medial amygdala and BNST via the OB and ABO [31].

The amygdala

The amygdala is referred to as the “emotion center” of the brain and is widely believed to mediate fear and aggression in vertebrates [33]. Additionally, the amygdala is an important site of neural plasticity where associative memories are stored [33] and is a site that has been identified as one of the candidates involved in olfactory processing. Two subdivisions of the amygdala, the cortical nucleus of the amygdala (CoA) and the medial nucleus of the amygdala (mAMY) are directly associated with the

two olfactory systems in mammals [8]. The CoA is part of the main olfactory system and receives projections from the OB, while the mAMY is part of the accessory olfactory system as it receives projections mainly from the AOB [34]. Additionally, the mAMY appears to be a site for convergence of input from both the OB and AOB pathways [34]. Therefore, due to its intricate relationship with the olfactory system, the mAMY has been identified as a brain region that influences maternal recognition of offspring, maternal defense, and offspring avoidance [10, 34-36].

In sheep, the amygdala has a critical role in mediating maternal attachment and recognition of offspring [34]. At parturition, a maternal sheep will be responsive towards any newborn lamb; however, within 2 hours they restrict their care to their own offspring after learning olfactory cues allowing her to distinguish own lamb [34]. Keller et al. examined the role of the amygdala in maternal olfactory recognition by directly infusing lidocaine into either the medial, cortical, or basolateral amygdala during the first few hours after parturition, when lamb memory formation occurs [34]. After infusion, the ewes were tested for selective offspring response. The authors found that when given lidocaine perfusions into the medial and central amygdala, sheep failed to develop selective attachment towards their lamb, suggesting a role of the medial and central amygdala in olfactory learning in mothers.

In rats, adults and juveniles show differing responses to pups. For example, the odor of pups initially induces an aversive or neophobic response in adult rats that is overcome only after several days of exposure to pups, while juveniles do not avoid pups, but immediately approach them and display maternal behaviors [6]. Olazabal and Morrell examined *c-fos* immunoreactivity within the mAMY and CoA to determine the

role of the amygdala in producing this contrasting response to pups [6]. Olazabel and Morrell found that after exposure to pups, there was significantly greater c-fos expression in both the cortical and medial amygdala in the adults when compared to the juveniles. These findings suggest that c-fos-responsive neurons are behaviorally inhibitory and that the behavioral differences between the juvenile and adult rats are explained by increased inhibition in the adult. This increased inhibition at the level of the amygdala leads to pup avoidance behaviors. Oxytocin acts within the amygdala to mediate emotional responses to pup exposure. A radioligand binding assay was used to examine oxytocin binding in the amygdala in postpartum female rats [64]. In comparison to virgin females, postpartum females had increased amounts of ocytocin binding in the amygdala. Therefore, it is thought that oxytocin may function to mediate responses to pup exposure by reducing fear and anxiety responses in female rats. The amygdala sends projections to an area of the hypothalamus, the medial preoptic area - a site that appears to be essential for maternal behaviors.

Medial preoptic area: the key to maternal behaviors

The medial preoptic area (MPOA), a part of the hypothalamus, has been identified as one of the most crucial regions responsible for governing maternal nurturing behaviors because it is the site where both hormones and protein synthesis occurs in response to offspring. Lesions in the MPOA interrupt both hormonally and pup-induced maternal behaviors in adult female rats [37-39]. Additionally, studies have shown that damage to the MPOA also influences maternal behaviors, induced via pup sensitization, in juvenile female rats [39, 40]. Olazabel et al. [40] examined how lesions

in the MPOA interrupt maternal behaviors in juvenile rats. Authors induced cytotoxic lesions in the MPOA by injecting NMDA, an excitatory neurotoxin. Previous studies using NMDA as a neurotoxin showed the effectiveness of this drug to induce lesions in the MPOA [39]. After inducing cytotoxic lesions in the MPOA, Olazabel et al. examined maternal behavior in the juvenile females. Following a pup sensitization period, juvenile female rats typically exhibit pup retrieval, nest building, licking/grooming pups, and crouching over the pups [16]. Authors reported that all of the females with large lesions in the MPOA showed severe impairment of maternal behaviors and did not show any pup retrieval, nest building, crouching, or grooming behaviors. Of the females with small lesions in the MPOA, sixty-one percent displayed full maternal behaviors. The results of this experiment highlight the importance of the MPOA for the display of maternal behaviors. Additionally, this experiment shows that mild damage to the MPOA does not significantly affect the female's ability to display maternal behaviors, while large lesions in the MPOA significantly impair maternal behaviors.

When a rat or mouse takes care of pups, fos family genes are activated in a wide variety of adaptive neuronal responses [12, 36, 41-43]. The fos family consists of four homologous immediate early genes, c-fos, fos-B, fra-1, and fra-2, that encode transcription factors that induce late response genes to act as effectors of long term cellular responses [41]. To determine the role of fosB in parenting responses, Brown et al. (1996) [41] induced a mutation in the fosB gene of mice. FosB mutant (-/-) mothers and juveniles were tested for their ability to provide maternal behaviors (retrieval and nursing posture). Authors reported that pups of fosB (-/-) mothers were carried to term, but a majority of pups died 1-2 days after birth due to lack of maternal care. FosB

mutant mothers did not crouch over their pups at all and all of the pups were found scattered around the cage. When tested for pup retrieval, fosB mutants took fifty times as long as the wild-type mothers to retrieve the first pup to the nest. The authors also reported that the nurturing behavior of fosB mutant mothers was not improved after multiple pregnancies or after sharing a cage with a wild-type mother who gave birth and raised her pups in the presence of the mutant. These findings show that the deficiencies in nurturing behavior in fosB mutants cannot be overcome by learning and experience. The authors also reported that there were no gross hormonal abnormalities found in fosB mutants in comparison to wild-type mothers. Juvenile fosB mutant females and males also exhibited the same deficit in nurturing behaviors. These results suggest that fosB activation is essential for maternal behaviors. A further study by Kuroda et al. examined the location of fosB expression in response to exposure to pups. After subjecting female mice to nurturing tests, as described previously, the authors examined the expression of fosB mRNA by qRT-PCR [3]. FosB levels were significantly higher in the MPOA of animals that displayed parental behaviors then in the nonparental group.

C-fos, a homologue of fos B, is a transcription factor that is used as a proxy for neuronal activation [43]. Matsushita et al. (2015) compared c-fos expression in lactating and virgin mice to identify brain regions that are active during maternal behaviors [43]. Authors reported that high levels of c-fos-positive cells were found in the MPOA of both lactating and virgin mice after presenting them with pups. In both virgin and lactating females, pup presentation significantly increased the number of c-Fos-positive cells in the MPOA. These results along with data from previous lesion studies [40, 44, 45]

suggest that fosB is induced in MPOA neurons [12] and that these transcription factors induce the expression of other genes that are required to actuate parental behavior. Additionally, authors reported that there were high levels of c-Fos-positive cells in the MPOA of lactating females without pup presentation which was at a similar level as those found in virgin females with pup presentation. These findings indicate that the MPOA neurons may be constitutively active in lactating females, potentially due to previous hormonal priming during pregnancy.

The MPOA appears to be the key site in the maternal network for integration of inputs. The MPOA receives olfactory inputs via projections from the medial amygdala, sensory cues and tactile inputs, cortical inputs, and is site of hormone action [1]. Matsushita et al. [43] used a retrograde tracer, Flurorgold, and c-fos expression to investigate MPOA projections in association with maternal behaviors [43]. Authors found that GABAergic neurons in the MPOA project to the bed nucleus of the stria terminalis (BNST). Data from this study suggests that GABAergic modulation by MPOA neurons my suppress activation of BNST neurons in lactating females. The BNST is involved in responses to negative affective states such as fear, anxiety and aversions; however these negative affective states appear to be lessened in lactating females [46]. The decreased fear and anxiety in lactating females may be due to GABAergic projections from the MPOA to the BNST. Additionally, hormonal priming of the BNST and MPOA may be required for BNST suppression via MPOA GABAergic projections [43]. MPOA neurons also project to the ventral tegmental area (VTA) [1], a region reported to stimulate the mesolimbic dopaminergic projections to reward centers.

Stimulation of the VTA and reward pathway leads to maternal behaviors, while stimulation of the BNST and fear pathways lead to pup avoidance behaviors [1].

Hormonal signals are integrated in the medial preoptic area to regulate maternal behaviors

The medial preoptic area (MPOA) has been implicated as a prime location for hormone action associated with maternal behaviors due to the abundance of hormone receptors [47-50]. The maternal hormones (oxytocin, progesterone, prolactin, estrogen) have been found within the MPOA [1]. Wang et al. compared regional density of cells containing oxytocin, vasopressin, and estrogen receptor-α in Chinese striped hamsters (*Cricetulus barabensis*) and Mongolian gerbils (*Meriones unguiculatus*) using immunoreactive labeling [48]. Estrogen receptor–α and Oxytocin were found in high densities in the MPOA of females of both species. Other studies have shown that progesterone and prolactin receptors also are found in high densities within the MPOA [7, 15, 49]. Moreover, in studies that target oxytocin, progesterone receptors, prolactin receptors, and estrogen receptor-α for knock out, both virgin females and females who have given birth exhibit disrupted nurturing behaviors when presented with pups [7, 27, 47]. Ribeiro et al. [47] used siRNA to specifically silence estrogen receptor- α in the MPOA [47]. Authors reported a deficiency in maternal behaviors in these female rats associated with estrogen receptor- α silencing in the MPOA. These studies provide support to the thought that the MPOA is the major site of hormonal control of maternal behaviors. The presence of progesterone receptors, prolactin receptors, and estrogen receptors combined with the finding that basal numbers of C-fos-positive cells in the

MPOA are significantly higher in lactating females than in virgin females [43], suggest a role of the MPOA as a site for hormonal priming.

Activation of the maternal reward circuit

Neurobiological reward systems have evolved to reinforce behaviors that are necessary for survival and reproductive success. The most important reward system within the brain is the mesolimbic dopamine system, which is comprised of the ventral tegmental area (VTA) and NAc. This system has been recognized for its central role in motivated behaviors and various types of reward. The mesolimbic areas are activated during natural reward behaviors, including food, social behaviors, sexual behavior [51, 52] and aggression [53]; and in response to conditioned cues predicting natural rewards [54, 55], and drugs of abuse [56, 57]. This circuit is also activated in maternal response [58]. Systemic injection of haloperidol, a dopamine licking, in rats. When these maternal behaviors are prevented via muzzling, female rats persistently attempt to make snout contact with pups by pushing them with the muzzle, indicative of intense maternal motivation. However, when given haloperidol, the time the female spent contact-seeking by muzzle-pushing was severely and dose-dependently reduced. It appears that a threshold level of dopamine is necessary for efficient handling and maternal motivation. Therefore, maternal motivation appears to be dopamine dependent [59].

The VTA is the origin of mesolimbic dopaminergic neurons and activation of these neurons stimulates the mesolimbic reward pathway. Dopaminergic neurons from the VTA project to the NAc, hippocampus, amygdala, ventral pallidum, and various cortical projections [53]. The VTA has been found to play a role in maternal behaviors

[58]. Suppression of dopaminergic neurons via lesions or applying dopaminergic antagonists significantly reduces the reward pathway [60]. Additionally, suppression of dopaminergic neurons in the VTA of lactating rats via a dopaminergic antagonist resulted in a disruption of maternal behaviors [61]. Varying doses of muscimol hydrobromide, a dopamine antagonist were injected into the VTA of lactating rats. Following muscimol administration, females were tested for ability to provide maternal care to pups (crouch duration, latency to retrieve all pups, nursing duration, number of pups retrieved). Muscimol inhibited maternal behaviors in a dose dependent fashion. Rats that received no muscimol performed significantly higher in all tests of maternal behavior (latency to retrieve pups, number of pups retrieved, nursing and crouching duration) than those that received muscimol. Of those that received muscimol, those who received the lower dose performed slightly better in all tests of maternal behavior than those that received the higher dose. A similar pattern was found when another drug, baclofen, was administered. Both drugs work by inhibiting dopaminergic neurons, and thus prevent dopamine release into the NAc. Therefore, the findings of this study suggest that the VTA and dopaminergic neurons of the VTA are involved in promoting maternal response to offspring. Oxytocin increase at parturition and throughout the peripartum period is involved in the activation of maternal behaviors. Studies have shown that activation of oxytocin neurons thought to target the VTA stimulates dopaminergic neurons [62]. Oxytocin receptors and their corresponding mRNA have been localized in the VTA [63]. High levels of bound oxytocin have been found in the VTA and MPOA during lactation [64]. When compared to pregnant female rats, bound oxytocin was significantly higher in the MPOA and VTA of lactating females.

Ovariectomized females had similar levels of bound oxytocin to pregnant females. Postpartum Females were tested for maternal behaviors after receiving an infusion of oxytocin antagonist into the VTA or MPOA. When compared to control females, those who received infusion of oxytocin antagonist into the VTA exhibited significantly prolonged latency to retrieve four pups. Infusion of an oxytocin antagonist robustly blocked pup retrieval. Latencies to retrieve pups were very significantly greater in antagonist treated females compared with control females. Another study reported that infusion of an oxytocin antagonist into the VTA blocks many maternal behaviors in parturient rats, who then leave the pups scattered and 'neglected' [64]. Additional studies have confirmed that oxytocin neurons project to both the VTA and MPOA [36, 58]. The localization of oxytocin effects in the VTA strongly suggests that there may be an oxytocinergic projection from the MPOA to the VTA, and infusion of an oxytocin receptor antagonist into the VTA disrupts maternal behavior.

From the VTA, dopaminergic projections are sent to the NAc. Studies employing electrochemical methods have revealed that rapid dopamine release in the NAc is observed within seconds of goal-directed actions for cocaine and other natural rewards [65]. Critically, these changes in dopamine signaling occur on a timescale that is similar to NAc phasic cell firing. Additionally, similarity in temporal dynamics between NAc cell firing and rapid dopamine release support the idea that dopamine functions to modulate NAc firing that encodes and ultimately influences goal directed actions [65]. To study the neural basis of positive reinforcement, intracranial self-stimulation is often used, in which the subjects perform an action in order to obtain exogenous stimulation of a specific brain area [66]. Using intracranial self-stimulation, it has been found that

optogenetic activation of dopaminergic terminals innervating the NAc was sufficient to drive intracranial self-stimulation. Administration of dopamine receptor antagonists into the NAc significantly attenuated intracranial self-stimulation in rats. This data demonstrates that the NAc is a critical target for dopamine action that drives the neural circuitry mediating positive reinforcement.

Research indicates that the NAc is critical for pup-directed behavior in females [37, 67, 68]. Electrolytic lesions of the NAc before pregnancy resulted in poor lactational performance, inferior maternal behavior (indexed by high pup mortality), and cannibalism [69]. Another study reported that lesions of the NAc during the immediate postpartum period significantly disrupted pup-retrieval in rats [68]. Bilateral injections of a dopamine receptor antagonist, pimozide, into the nucleus accumbens of lactating rats interfered with maternal behaviors [70]. After bilateral pimozide injection into the nucleus accumbens, females were tested for aspects of maternal behaviors (latency to retrieve pups, latency to group pups, nursing latency, nest building, licking and grooming of pups). Females receiving the highest dosage of dopamine receptor antagonist showed increased latencies for all aspects of maternal behaviors in comparison to very low doses of antagonist or no antagonist. A further study indicates that variations in nucleus accumbens dopamine receptors is associated with individual differences in maternal behavior in the rat [67]. In vivo voltammetry was used to monitor changes in extracellular dopamine in the nucleus accumbens of lactating rats. Subsequently, Champagne and colleagues reported that dopamine signal increased significantly with pup licking/grooming. The magnitude and duration of the increase in the dopamine signal were significantly correlated with the duration of the licking/ grooming bout. In

high-licking mothers, the magnitude of the increase in the dopamine signal associated with licking/grooming was significantly higher than in low-licking mothers. In addition, it was found that dopamine transporter binding was increased in low-licking compared to high-licking females. Injection of a selective dopamine uptake inhibitor was administered to low-licking mothers, and was found to increase the dopamine signal in the NAc to a level, comparable to high-licking mothers. The results of these studies indicate that functional availability of dopamine within the NAc is necessary for the display of maternal behaviors. In addition, decreasing the functional availability of dopamine results in attenuated maternal behaviors; increasing the functional availability of dopamine increases maternal behaviors in lactating rats.

Oxytocin receptors in the NAc have been shown to promote maternal behaviors [25]. Female prarie voles were tested for maternal behavior (time spent licking and grooming, active crouching, pup retrieval). Oxytocin receptor density was determined using radioligand receptor autoradiography in the central palladium (CP), lateral septum (LS), MPOA, and NAc. There was significant variability in oxytocin receptor binding in the NAc. When maternal and non-maternal females were compared, maternal females showed higher oxytocin receptor density in the NAc than females that did not show any maternal response. The time spent adopting a crouching behavior also varied in these females. Oxytocin receptor binding in the NAc was positively correlated with the time spent adopting a crouching behavior. Injection of oxytocin antagonist into the NAc was shown to block spontaneous maternal behavior. Maternal females that received an oxytocin antagonist infusion were significantly slower to approach pups than maternal females that received no treatment. Therefore, these results suggest that the NAc is

critical for the expression of spontaneous maternal responses and that oxytocin facilitates maternal responses. In addition, oxytocin receptors in the NAc have also been implicated to play a role in the consolidation of 'maternal memory' in postpartum rats [71]. Maternal memory is the long-term retention of maternal responsiveness as a result of prior experience with offspring. Postpartum female rats were infused with either a low or high dose of oxytocin antagonist into the NAc and administered a maternal defense test, after 10 days of isolation from pups. Females that were given a higher dose of the antagonist had a significantly longer latency to exhibit full maternal behavior after the isolation period compared to those that received a lower dose. This study is consistent with the idea that oxytocin and dopamine receptors facilitate the formation of social bonds [72]. Access to both oxytocin and dopamine is essential for pair bond formation, as blockade of either type of receptor prevented pair bond formation. In the NAc, blockade of oxytocin receptor prevented partner preference induced by dopamine agonist, whereas blockade of dopamine receptors blocked oxytocin –induced partner preferences. Therefore, data from this study suggests that concurrent activation of oxytocin and dopamine receptors in the NAc is necessary for pair bond formation. Mother-offspring attachment may be mediated in a similar manner; however, the role of the NAc in mediating this bond is unclear. It is known, however, that the presence of both dopamine and oxytocin receptors are necessary [25] [70] [5, 67].

Stressors-induced activation of the HPA axis interferes with maternal behaviors

Stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis during the gestation period can result in preterm labor and premature birth, which threatens the survival of offspring. Exposure to chronic stress during pregnancy and the postpartum period can negatively impact maternal health and behaviors. Activation of the HPA axis during the last part of pregnancy and postpartum period has been shown to alter the display of maternal behaviors [73-76]. In a study by Hillerer et al. [73], pregnant female rats were used to test for the effects of chronic stress on maternal behaviors. Pregnant females were stressed by daily restraint and being placed in an overcrowded cage. After parturition, these females were tested for maternal behaviors (nursing, licking/grooming pups, pup carrying, and self-grooming), anxiety-related behaviors (using the elevated plus-maze), and depression-related behaviors (forced swim test). Levels of adrenal corticotropic hormone (ACTH) and corticosterone (CORT) were assessed in the females as a measure of stress. Chronic stress was shown to significantly reduce body-weight gain in both stressed virgin and peripartum females compared with their respective non-stressed controls. ACTH and CORT levels were significantly higher in stressed females. In addition to having higher ACTH and CORT levels, chronically stressed females also spent significantly less amount of time nursing their pups compared to non-stressed females. These findings indicate that exposure to chronic stress during pregnancy lead to increased stress related hormones and altered maternal response to pups.

Restraint stress during the lactation period has been shown to lead to increased nest building and pup cleaning activities in lactating female rats [77]. Additionally chronic social stress in the postpartum period leads to altered maternal behaviors [74].

Lactating female rats were tested for maternal behaviors (nursing, pup retrieval, nesting, and pup grooming) and exposed to a male intruder daily [74]. Maternal behaviors were reassessed after 2, 9, and 16 days. On day 9 of lactation, the latency to initiate nursing was longer in the chronically stressed females. The durations of pup grooming and total maternal care were significantly lower in the chronically stressed females. Locomotor activity was elevated in the chronically stressed females. Stressed females had significantly reduced weight gain in comparison to non-stressed females. Offspring of stressed females had significantly reduced weight gain in comparison to offspring of non-stressed females. These findings show that exposure to a chronic social stressor during lactation attenuates maternal care and both mother and pup weigh gain. Exposure to a chronic stressor also increases anxiety-related behavior.

Postpartum stress has also been found to interfere with maternal defense in lactating female mice [74, 78]. When lactating females were chronically stressed and tested for maternal defense, they displayed more overall aggressive behaviors towards intruders [74]. Stressed females had shorter aggression latencies towards intruders and longer average aggressive bouts. On day 16, total aggression and self-grooming was elevated in the chronically stressed females. Bridges and Nephew reported that exposure to a chronic social stressor during the postpartum period leads to increased maternal defense and anxiety-related behaviors, with decreased pup care behaviors. These findings, however seemingly contradict findings of other work that shows stress during the postpartum period leads to decreased defensive behaviors [78-80]. When exposed to acute stress and challenged by an intruder, stressed females spent significantly less time engaging in defensive behaviors, attacked fewer time, and were

significantly slower to initiate attacks against the intruder in comparison to non-stressed lactating females [78]. These findings are consistent with work showing that central injections of corticotropin-releasing hormone (CRH) impair maternal defense [79].

CRH is a neuropeptide that mediates stress-induced behaviors, including elevating fear and anxiety. CRH plays a critical role in the HPA axis. High CRH levels during lactation interfere with maternal defense [79]. At six to seven days postpartum, adult lactating female mice were given i.c.v. injections of 0.02, 0.2, or 1.0 μg of CRH. After CRH injections, females were tested for maternal aggression behaviors (latency for the mother to attack, number of attacks, and total duration of attacks) towards a male intruder. CRH injections significantly inhibited expression of maternal aggression in a dose-dependent manner. Mothers given 0.02 μg CRH had the same effect as mothers given saline. Mothers injected with 0.2 μg of CRH had a significant decrease in the duration of aggressive behaviors towards intruders. Mothers given the highest dose of CRH, 1.0 μg, did not exhibit aggressive behaviors. Additionally, females given the highest dosage of CRH did not actively follow or sniff at the intruder. Infusion of a CRH antagonist did not increase maternal aggression when compared to control females. These results indicate that reduced CRH is necessary for normal defense behaviors that occur during lactation. In addition to interfering with maternal defense behaviors, CRH has also been found to interfere with other maternal behaviors [81]. Infusion of a CRH agonist impaired maternal care in post-parturient female rats. Females that received CRH infusion spent significantly less amount of time nursing pups than control females. Females that received CRH infusion also spent significantly less time grooming their pups in comparison to control females. Therefore, the results of this

study indicate that CRH interferes with the display of both nurturing and defense behaviors in mothers.

Corticosterone (CORT) is the primary glucocorticoid of the HPA axis. Often called the “stress hormone,” CORT’s action is to provide the energy needed for combating physical or emotional stress. High levels of CORT have been associated with chronic stress during pregnancy and the postpartum period [80]. Female rats received either a 10 mg/kg or 40 mg/kg dose of CORT during pregnancy, postpartum, or both. Females were subsequently tested for maternal behaviors (licking, nursing behaviors, and time spent off pups). Females who received the highest dose of CORT during pregnancy, postpartum period, or both, spent significantly more amount of time off the nest than those that received a low dose of CORT or no CORT. Females who received low doses of CORT postpartum and during both pregnancy and postpartum and females who received high doses of CORT spent significantly less amount of time nursing compared to controls. Overall, only high CORT or prolonged exposure to low CORT was effective in reducing maternal care. Additionally, the authors reported that high levels of glucocorticoids during the postpartum period are more damaging to the females than high levels of glucocorticoids during gestation. Female rats in this study showed more sensitivity to high levels of CORT during the postpartum period than during gestation. Not only was maternal behavior significantly altered when the female was administered high CORT during the postpartum period, but these individuals also experienced significant loss of body weight. The weight of these individuals’ pups was also negatively impacted, which supports the finding that high CORT females spent significantly less amount of time nursing their pups when compared to controls.

CORT has also been shown to interfere with maternal behaviors in pup-sensitized virgin female rats [82]. Pup-sensitized virgin females were tested for maternal behaviors (pup retrieval, nest building, crouching over pups, anogenital licking). After maternal behavior testing, females were adrenalectomized and received CORT replacement in varying amounts: no CORT, 25 ug/mL (low), 100 ug/mL (medium), 300 ug/mL (high), 500 ug/mL (very high). After receiving CORT replacement, females were again tested for maternal behaviors. Rees et al. [82] reported that exogenous CORT decreased licking and crouching in the virgin rats, suggesting that CORT interferes with maternal behaviors. However, when females did not receive adrenalectomy and replacement CORT, endogenous CORT levels were positively related to elevated licking once rats had become maternal. Additionally, a study in testing the role of CORT in postpartum rats suggests that CORT may not interfere with maternal behaviors, but may promote them [83]. Postpartum rats were adrenalectomized and given CORT replacement. Removal of the adrenal gland decreased maternal licking and crouching in the postpartum rat, while the replacement with a high concentration of CORT increased these maternal behavior. Therefore, results from these studies indicate that the function of CORT in maternal behaviors is not clear.

It has been suggested that a possible explanation for these conflicting results lies in the state of the females [82]. The primiparous rat undergoes many changes during pregnancy and following parturition that are not experienced by the virgin rat. These include differences in underlying endocrine profile and hypothalamic-pituitary-gonadal function and differences in feedback sensitivities and responsiveness of the HPA axis [77, 84, 85]. Modulation of maternal behavior by the HPA axis may be dependent on the

actions of maternal hormones, such as estrogen, progesterone, oxytocin, and/or prolactin, that interact with the HPA axis and that differ between postpartum and virgin females. These interactions are discussed further in the following section.

Activation of HPA axis interferes with endocrine mediators of maternal care

Stress and, stress-induced activation of the HPA axis during pregnancy and/or the postpartum period can negatively affect maternal care and offspring growth and development [76, 79, 81, 86, 87]. However, it appears that mothers are equipped with adaptive biological mechanisms that provide a buffer against stress and promote offspring care. It is well documented that the HPA-axis is blunted during late pregnancy, parturition, and early lactation [87-89]. In rodents, ACTH and CORT secretion from the anterior pituitary and adrenal cortex, as well as CRH expression in the PVN is suppressed the day of parturition, and the hormone concentrations decline to their lowest levels during labor and delivery [76]. These adaptations buffer the impact of stress by reducing fetal exposure to maternal glucocorticoid, thus minimizing the risk of detrimental glucocorticoid programming while promoting anabolic adaptations in the mother necessary for successful pregnancy and subsequent care of offspring [90]. Maternal hormones have been shown to interact with the HPA axis and lead to its attenuation during pregnancy, parturition, and lactation [87, 91, 92].

Female sex steroids alter stress-induced HPA response during pregnancy

In female rats, estrogen and progesterone levels peak during the last week of pregnancy, making them prime candidates for inducing pregnancy-related adaptations

in HPA axis activity. However, estrogen and progesterone are not directly involved in suppressed HPA axis responses to stress in late pregnancy. In contrast, the progesterone metabolite, allopregnanolone does play a crucial role[90]. Allopregnanolone is a neuroactive steroid that secreted from the adrenal cortex and increases in response to stress [93]. During pregnancy, both circulating and brain levels of allopregnanolone increase, as a consequence of increasing progesterone secretion. The most elevated levels of allopregnanolone levels are found at the end of pregnancy [91], which corresponds with attenuated stress. Allopregnanolone is formed when the enzyme 5α- reductase converts progesterone to 5α-dihydroprogesterone, which in turn is converted into allopregnanolone by 3α- hydroxysteroid dehydrogenase [94]. In pregnant rats, it has been reported that administration of 5α-reductase inhibitor blocks allopregnanolone production and restores HPA axis responses to physical and emotional stressors [95] , while allopregnanolone administration attenuates HPA axis responses in non-pregnant females. Additionally, treatment of virgin rats with estrogen alone, combined estrogen and progesterone, progesterone alone, or dihydroprogesterone alone [95] are all ineffective in mimicking the suppressed HPA axis responses to stress as seen in late pregnancy. Therefore, the ineffectiveness of allopregnanolone precursors, progesterone and dihydroprogesterone, even in the presence of estrogen, further emphasizes the importance of the upregulation of allopregnanolone synthesizing enzymes in the brain during late pregnancy[95] as a mechanism for inhibiting oxytocin and preventing early labor.

Allopregnanolone acts as a neuroactive steroid and a potent allosteric modifier at synaptic and extasynaptic $GABA_A$ receptors [93]. It modulates the effect of GABA by

binding to the $GABA_A$ receptor complex and prolonging the opening time of chloride ion channels within $GABA_A$ receptors, thus enhancing inhibitory neurotransmission. Parvocellular CRH neurons in the PVN are under direct inhibitory GABAergic control [96]. GABAergic neuronal circuits play a critical role in the regulation of neuroendocrine stress responses evidenced by the fact that more than one third of inputs to CRH neurons in the PVN are GABAergic [96]. Removal of tonic GABA inhibition by administration of a $GABA_A$ receptor antagonist strongly stimulates the secretion of ACTH, with peak concentrations in plasma not different between virgin and late pregnant rats [92]. Additionally, allopregnanolone appears to upregulate opioid peptides produced by NTS neurons [95]. These opioids act presynaptically on noradrenergic terminals in the PVN to inhibit noradrenaline release to IL-1β in late pregnancy [92]. Noradrenaline release in the PVN excites CRH neurons, leading to an increase in CRH. Therefore, it appears that increased levels of allopregnanolone in the brain may act to suppress HPA axis by inhibiting CRH release in two ways: 1) by inhibiting the release of noradrenaline, and 2) by enhancing the action of GABAergic neurons synapsing on PVN CRH neurons. These two mechanisms enhance inhibitory neurotransmission in PCN CRH neurons to prevent a rise in CRH levels.

Oxytocin blunts the HPA axis

It has been postulated that oxytocin may mediate blunted HPA activity during late pregnancy and lactation [85]. In rats, the attenuation of stress responses observed during late pregnancy and lactation is associated with increases in hypothalamic oxytocin [97], with an augmentation of release into the limbic system and other areas of

the brain involved in HPA axis regulation [85]. The presence of a stressor has been shown to activate fos expression, a proxy for neuronal activity, in oxytocin-immunoreactive neurons in the PVN [98]. Additionally, oxytocin receptor binding and mRNA levels, and neuronal sensitivity to oxytocin are increased [85]. It has also been shown that central administration of oxytocin decreases CORT responses to stress in ovariectomized female rats receiving estrogen replacement [77]. These findings indicate that oxytocin may inhibit the HPA axis. However, other studies have reported that administration of oxytocin may have no effect, or may actually stimulate the HPA axis. For example, in male rats, central administration of an oxytocin antagonist did not modify ACTH responses to acute restraint stress, but augmented responses to repeated stress [75]. Blockade of endogenous oxytocin by i.c.v. administration of an oxytocin antagonist has failed to reverse the inhibition of the HPA axis activity [99]. In ovariectomized female rats, i.c.v. oxytocin failed to inhibit HPA axis responsiveness in the presence of low circulating levels of estrogen [85]. There was a lack of significant effects of central oxytocin on CRH mRNA and plasma ACTH responses to restraint stress. However, estrogen levels were below physiological levels in this experiment, suggesting that the central effects of oxytocin on HPA axis activity are modulated by ovarian steroids. When plasma estrogen levels were increased, i.c.v. oxytocin infusion progressively resulted in inhibition of basal and stress-stimulated CRH mRNA and ACTH responses. The disparity of oxytocin's role in the HPA axis in different studies may be attributed to differing physiological or experimental conditions. It appears that oxytocin does have an ability to attenuate the HPA axis. However, its ability to do so is dependent upon the presence of estrogen.

Moreover, the role of oxytocin in mediating the HPA axis may be dependent upon female physiological condition. For example, oxytocin may have little to no effect on the HPA axis during pregnancy, while becoming a prime mediator during lactation. Increased secretion of oxytocin in late pregnancy as a result of stress may prematurely stimulate uterine contractions and lead to preterm labor (provided oxytocin receptor expression is upregulated) [100]. In late pregnancy, the oxytocin system is less responsive to stress and non-reproductive related stimuli [100]. Therefore, during pregnancy, oxytocin may play less of a role in mediating the HPA axis because it itself is being suppressed in a similar manner to suppression of the HPA axis. In pregnancy, an allopregnanolone-induced endogenous opioid mechanism, similar to that which controls HPA axis activity, restrains the basal activity of oxytocin and secretory responses to stimulation [101]. Late pregnant female rats who received an allopregnanolone inhibitor showed significantly higher plasma oxytocin responses to immune stress (IL-1β) than untreated pregnant females[100]. Additionally, virgin rats given allopregnanolone and subjected to immune stress had lower oxytocin levels than control females. Oxytocin secretion after immune challenge was significantly lower in late pregnant rats compared to virgin rats. Blocking allopregnanolone generation with a 5α-reductase inhibitor reduces allopregnanolone by approximately 90%. In late pregnant rats, it was reported that treatment with a 5α-reductase inhibitor produced an exaggerated oxytocin secretory response to IL-1β, which was prevented with simultaneous allopregnanolone treatment. Together, these findings indicate that allopregnanolone restrains the oxytocin response to IL-1β. The mechanism by which allopregnanolone acts to suppress oxytocin response may involve an interaction with

$GABA_A$ receptors. About one in three synapses on oxytocin neurons have been found to contain GABA, and this number increases by the end of pregnancy, providing a strong base for potentiation of GABA actions on oxytocin neurons by allopregnanolone [90]. This finding explains a mechanism by which high levels of progesterone inhibit the onset of maternal behaviors [15].

Lactation acts as a buffer against stress

Shortly before parturition, progesterone levels decline and lead to decreased levels of allopregnanolone [92]. With oxytocin no longer suppressed by allopregnanolone, oxytocin secretion increases rapidly. While oxytocin does not block responses to stress during pregnancy, there is some evidence that it may act during the lactation period to decrease maternal sensitivity to stress via the HPA axis and promote maternal behaviors [85]. Additionally, at parturition there is a drastic increase of another neurohormone, prolactin, that has been suppressed during much of pregnancy. Prolactin is also involved in lactation and has oxytocin and prolactin release and decreases basal plasma levels of ACTH and CORT, suggesting been implicated in attenuation of the HPA axis during the peripartum period. Lactation is associated with diminished HPA activity. Therefore, it is thought that stress reactivity is buffered via oxytocin and prolactin. In lactating women as well as in lactating rats the suckling stimulus by the newborn increases an inhibitory influence of both peptides on stress-responsive neurohormonal systems [46]. Prolactin upregulates the expression of its own receptors and increased prolactin receptor has been reported during lactation [102]. Further evidence for anxiolytic action of the brain prolactin system was provided by

down-regulation of prolactin receptor expression in the brain by antisense targeting using an oligodeoxynucleotide probe. Reduction of prolactin availability by this treatment significantly increased anxiety-related behavior of lactating female rats [102].

Endogenous prolactin has been shown to inhibit HPA axis responses to a mild emotional stressor in the virgin female and lactating rat [102]. Chronic i.c.v. prolactin infusions enhanced brain prolactin receptor expression and caused a shift in HPA axis activity towards an attenuated ACTH secretory response following exposure to a stressor. In contrast, infusion of antisense oligonucleotides against the prolactin receptor and downregulation of prolactin receptor in the brain significantly affects ACTH secretion, causing further elevation of stress-induced ACTCH secretion [103]. Interestingly, ACTH responses in antisense-lactating rats were similar to those of virgin rats, indicating reversal of the attenuated ACTH response when prolactin receptor funtions are blocked [102]. Thus, the attenuated HPA axis responses seen in lactation are at least partly due to an inhibitory effect of brain and/or pituitary prolactin acting at brain prolactin receptors. Lactating rats received i.c.v. infusions of a prolactin receptor antisense probe, mixed base oligonucleotides, or a vehicle [102]. After 5 days of antisense treatment, basal levels of ACTH were not altered, whereas the stress-induced increase in ACTH secretion was significantly more pronounced compared to control rats, revealing a disinhibition of the HPA axis. These results clearly show an inhibitory effect of prolactin secretion on the HPA axis.

During lactation, oxytocin gene expression and OT immunoreactivity are increased in the magnocellular neurons of both the PVN and SON [104]. Multiple studies both in animals and women show that increased oxytocin secretion during

lactation serves to attenuate HPA axis activity and stress-related anxiety. Oxytocin release in hypothalamic as well as extrahypothalamic regions have been shown during suckling in both lactating rats and lactating sheep [46]. In women, both breast-feeding and holding the infant lead to a decreased state of anxiety [46]. Additionally, in lactating women, suppression of stress responses can be observed if breast-feeding starts 30-60 minutes before stress exposure. A study in women examined oxytocin and CORT levels in both breastfeeding and non-breastfeeding women [105]. Authors reported that both infant feeding method and maternal mood symptoms were associated with differences in response to a social stressor. Compared with women who had bottle fed their infants, women who had breast fed had lower CORT secretion. Oxytocin secretion was higher in women who had breast fed than those who bottle fed their infants. Of the women who breast fed, those with reported symptoms of anxiety and depression had higher levels of CORT. These individuals also had reduced oxytocin levels. These findings, along with previous animal studies [98, 99] [100] imply that oxytocin secretion during lactation produces a buffer against stress-induced CORT.

4. Discussion and Conclusion

The initiation and maintenance of maternal behavior is a critical aspect of maternal care and ultimately, offspring survival. As evidence reviewed in this thesis

suggests, maternal behaviors and motivations are posited to be under two phases of control: 1) initiation of maternal behavior and 2) maintenance. Initiation of maternal behavior appears to be highly dependent upon the action of intricate hormonal interactions during pregnancy. Estrogen was shown to be critical for the onset of maternal behaviors, in part due to its interactions with the other three hormones (prolactin, progesterone, estrogen), that have been shown to promote maternal behaviors. When estrogen is not present in high levels, the presence of any of the other three hormones does not compensate for the loss of estrogen, and thus, maternal behaviors do not occur [1]. At the same time, in order for maternal behavior to be initiated immediately upon parturition, the presence of both prolactin and oxytocin appear to be necessary [2, 3], as well as the absence of progesterone [4], which, via its metabolite, allopregnanolone, serves to inhibit the secretion of both of these hormones [5]. Estrogen is thought to produce a priming effect (producing a change in receptor synthesis) that facilitates increased oxytocin receptor binding. Estrogens have been shown to be required for oxytocin receptor expression and up-regulate oxytocin receptors within the brain [6]. In addition, receptors for both of these hormones have been found to be co-localized in neurons within areas of the brain that are critical for maternal behavior [6]. Estrogens are also required for normal responsiveness to prolactin. Ovariectomized mice showed a reduced number of prolactin-induced neurons [7]. When ovariectomized mice were treated with estrogen, they were significantly more responsive to prolactin.

These hormones act within specific brain areas to modulate maternal behaviors. Areas found to be involved in the regulation of maternal behaviors include: the OB/AOB,

mAMY, MPOA, BNST, VTA, and NAc. C-fos expression is enhanced in all of these areas following maternal behaviors [8] . When any of these areas were damaged, severe deficits in maternal behaviors arose. The MPOA, a region of the hypothalamus, appears to be the location where offspring related stimuli are integrated. The MPOA receives sensory cues, tactile inputs, cortical inputs, and projections from the mAMY [9].The MPOA also is the major site for maternal hormone interactions, as it has prolactin, progesterone, estrogen, and oxytocin receptors. The MPOA sends projections to the BNST and reward circuit (VTA and NAc).

Once maternal behaviors are initiated, equally important is the second phase of maternal behavior - its maintenance. The maintenance of maternal behaviors is thought to be under the control of both hormones and reinforcing offspring interactions. This includes lactation. Lactation requires an offspring related stimulus – suckling and/or vocalization [10]. This stimulus then initiates oxytocin release which influences prolactin release. Increased prolactin release promotes more oxytocin release and dopamine release. Dopamine eventually feeds back to inhibit prolactin release. When this occurs, if the stimulus is withdrawn, oxytocin and prolactin release will cease. For continued maternal care, there must be continued maternal motivation, and what better motivation than activation of the reward circuit? Lactation may promote maternal behaviors via activation of this circuit. During suckling, oxytocin is released. Oxytocin reactive neurons have been found to project from the MPOA to the VTA. Dopaminergic cells of the VTA project to the NAc. Activation of the NAc is critical for the continuance of maternal behaviors. Lactation appears to facilitate maternal-offspring attachment in sheep [11].

The olfactory system also plays a critical role in maternal-offspring attachment in sheep. Postpartum ewes preferentially care for their own offspring, while the violently reject other lambs. There is a period of a few hours after parturition where ewes will tend to other offspring. During this time period, they do not know the smell of their own offspring. However, after only a few hours, ewes are able to distinguish their own offspring from others. In some rats, there is a distinct difference in nurturing behaviors between post parturient females, and virgin females and males. In these rats, virgin females and males display aversive behavior towards pups or even attack pups [12]. Males and virgin females that exhibited heightened aggression towards pups had increased c-fos expression in the AOB when compared to nurturing animals and post parturient females. Surgical ablation of the AOB in virgin female rabbits resulted in a behavioral shift from pup avoidance and attack to nurturing behavior [12]. These results suggest that behaviors associated with maternal care may be the default due to a need for a biological inhibition mechanism. From an evolutionary perspective, inhibition of nurturing behaviors in non-maternal animals may be beneficial, as providing nurturing behaviors is energetically demanding.

The presence of a stressor, be it physiological or psychological, results in activation of the HPA axis. Activation of the HPA axis results in a system-wide response and the release of CRH, ACTH, and CORT. Activation of the HPA axis negatively impacts maternal care. When postpartum female rats were given injections of CRH, ACTH, or CORT, maternal care was disrupted. There was increased pup retrieval time, reduced nursing behaviors, reduced pup grooming, increased anxiety related behaviors, decreased pup grouping, and decreased pup defense [6, 13-15]. Pups of stressed

females weighed significantly less than those of non-stressed females. Additionally, survival rate of pups of stressed females was significantly lower than that of non-stressed females. Parvocellular CRH neurons in the PVN are under direct inhibitory GABAergic control [16], and therefore are inhibited in the absence of a stressor. However, when a stressor is present, there is noradrenaline release into the PVN that excites CRH neurons, leading to increased CRH release. Noradrenaline acts to focus attention towards a stimulus. Therefore, in a mother, where offspring demand attention and energy, the presence of a stressor may negatively impact maternal care as energy and attention is diverted away from maternal care to address the stressor. Many women experience subjective changes in their emotional states in the days after birth, with many experiencing increased anxiety or depression. Postpartum depression has been associated with increased HPA axis activity. Increased output from the HPA axis in depressed states is driven by hypersecretion of CRH [17]. Hypersecretion of CRH will lead to increased secretion of ACTH and CORT. Increased levels of ACTH were associated with postpartum depression in women.

Females appear to have evolved adaptive biological mechanisms that provide a buffer against stress and promote offspring care. HPA-axis activity is blunted during late pregnancy and early lactation. During pregnancy, the neuroactive steroid, allopregnanolone has been found to prevent CRH secretion by inhibiting noradrenaline release and increasing GABAergic suppression of CRH secreting parvocellular neurons. Oxytocin and prolactin release have been found to decrease basal levels of ACTH and CORT during the peripartum period. Additionally, in comparison to women who breast-feed, those who bottle feed have been found to have higher CORT secretion [18].

Further research is necessary to determine the exact impact that stress has in influencing maternal care. What is known is that stress may impact maternal care in a potentially negative way. Although females appear to have evolved specific mechanisms to lessen the effects of stress, it is still necessary to understand exactly how, on a biological level, stress is influencing the quality of maternal care provided. For example, it is not clear as to what CORT's role is in influencing maternal behaviors. It is also not clear yet what regions of the maternal brain are influenced by stress. However, to in order for this to occur, more research must be done to gain a better understanding of how different hormones, brain regions, and genetics influence and promote maternal motivations and behaviors.

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