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Attentive mothers versus minimally invested/ neglectful mothers : the development of new neurons in the hippocampus specifically activated by foster pup exposure

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Title: Attentive Mothers versus Minimally-Invested/Neglectful Mothers: The Development of New Neurons in the Hippocampus Specifically Activated by Foster Pup Exposure

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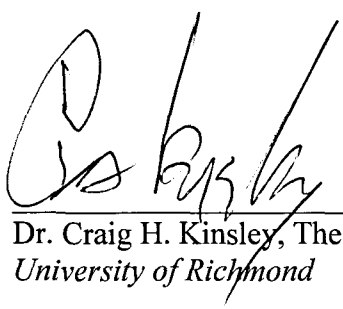

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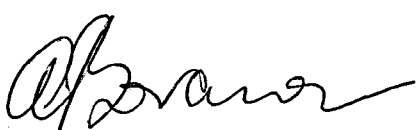

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ATTENTIVE MOTHERS VERSUS MINIMALLY INVESTED/NEGLECTFUL MOTHERS: THE DEVELOPMENT OF NEW NEURONS IN THE HIPPOCAMPUS SPECIFICALLY ACTIVATED BY FOSTER PUP EXPOSURE

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Abstract

As pregnancy progresses, the female is transformed from an animal that actively avoids pup-related cues (Kinsley, 1994) to one highly motivated to build nests, and retrieve, group, groom, and crouch over a set of pups. In the vast majority of events, motherhood progresses normally; in a striking subset, however, it does not. This study seeks to evaluate neurological differences in the dentate gyrus between primiparous females that respond maternally and those that do not when exposed to foster pups. It was hypothesized that the attentive mothers which perform the expected maternal behaviors have a different number of triple labeled BrdU(measuring new neurons)/Fos (showing activated neurons)/NeuN (showing mature neurons) neurons than the un-responsive neglectful mothers which do not show maternal behaviors. It was concluded that mothers who display maternal behavior have more mature neurons showing both c-fos activation and BRDU incorporation than mothers who do not respond maternally.

Attentive Mothers versus Minimally-Invested/Neglectful Mothers: The Development of New Neurons in the Hippocampus Specifically Activated by Foster Pup Exposure

Perhaps no other developmental milestone is exemplified by a more sudden and dramatic change in behavior than that observed in the maternal mammal. As pregnancy progresses, the female is transformed from an animal that actively avoids pup-related cues (Kinsley, 1994) to one highly motivated to build nests, and retrieve, group, groom, and crouch over a set of pups. The experiences of pregnancy imprint on the female an enhancement of her recognition of her young that transfers to other offspring, known as maternal memory. Previous research shows that there is an important window of time immediately following parturition involving the development of the maternal memory (Li & Fleming, 2003). Even just an hour of interaction with pups during this window of time is enough to enhance maternal memory more than a week later (Lee, Li, & Fleming, 1999). The development that takes place during the first twenty-four hours following parturition is key to the female animals' ability to respond quickly and efficiently to her pups in the future (Byrnes & Bridges, 2000).

When the female mammal reproduces, she encounters a great number of demands that must be fulfilled in order to reap the benefits of her genetic investment (Kinsley et al., 1999). She must protect, provide for, and shelter her young, and in many cases, must teach her progeny about the world. Completion of her various responsibilities depends, for a large part, upon a honing of her cognitive abilities (Kinsley et al., 1999). The maternal cognitive processes that are especially instrumental in carrying out these

responsibilities include learning/memory systems. "Neural activity brought about by pregnancy and the presence of pups may literally reshape the brain, fashioning a more complex organ that can accommodate an increasingly demanding environment," one that performs better in learning and memory tasks that will help to ensure her pups survival (Kinsley et al., 1999).

The neurobiological landscape in the maternal brain and its shifts in behavioral responses appear to be an ideal model of natural, neural and behavioral plasticity. Neuroplasticity also refers to how the brain manufactures new cells. There are data that suggest that individual neuronal maternal responsiveness may underlie this intensive maternal motivation. This work addresses a fundamental question regarding the development of the maternal brain. Previous research has documented some alterations of neurogenesis and cell proliferation that play a role in the display of maternal behavior (Shingo et al., 2003; Furuta and Bridges, 2005).

Shingo et al. (2003) discusses the importance of the olfactory system on the females' ability to recognize and raise young. They found that in female mice pregnancy stimulates the production of neural progenitors in the subventricular zone of the forebrain, which goes on to generate new olfactory neurons. Based on these findings they suggest that this neurogenesis in the forebrain could be important for the female's ability to respond maternally.

Furuta and Bridges (2005) found that as in mice, gestation stimulates neurogensis in rats as well. They marked proliferating cells in pregnancy rats with an injection of bromodeoxyuridine. They found changes in the amount of BrdU labeled cells throughout

pregnancy, with the greatest increase found in the subventricular zone on day 21. They did not find any changes in the BrdU labeled cells in the hippocampus and dentate gyrus due to gestation. It is possible that changes in this region of the brain might result from the initial contact with the pups and displays of maternal behaviors rather than pregnancy itself. The current study examines neuronal plasticity and neurogenesis in the dentate gyrus.

It is possible that how a brain responds to pups whether adequate numbers of new pregnancy/offspring-related neurons are created, or whether the ones that are born respond adequately in the mother may tell us about how a mother is formed, and about her maternal and affective responsiveness to young. The sensory experiences provided by the nursing pups have been studied carefully, and it has been found the nursing pups may re-organize the somatosensory cortex of the lactating mother (Xerri et al., 1994).

Xerri et al. (1994) describe how the nursing behavior involves powerful hormonal and reflexive changes in the rat. They found that the cortical representations of the skin surface are remodeled due to this life experience. The hormonal and reflexive changes cause physical changes which lead to the suckling behavior, that then causes experience based neurological changes. These experienced based neurological changes in the brain of the mother reinforce the importance of the interaction between the mother and the pups.

We will further pursue this interesting functional question of offspring-related neurogenesis. In the vast majority of events, motherhood progresses normally; in a striking subset, however, it does not. Some mothers do not respond maternally and can

be neglectful or even violent towards their young. In other situations the mother might simply not show interest in being maternal, or interacting with her young. This is not to say she would not provide the basic requirements of survival to her child, but she might be slow to respond to the infants needs or simply uninterested in her young. This form of maternal response might not be immediately detrimental to the well-being of the young but could potentially have long lasting developmental or behavioral effects on the young.

Bosch et al. (2007) evaluated the effects of prenatal stress on the female offspring in rats, and suggests a connection between this stress and the development of mood disorders particularly later in life in response to their pups. Although these females did not differ with controls on certain maternal behaviors such as maternal aggression or retrieval of pups, they did nurse their young significantly less than the control females. These animals also spent less time with their young, and showed an increased in levels of anxiety. Results also showed neuroendocrine alterations in the female offspring including corticotrophin-releasing hormone mRNA expression in the paraventricular nucleus among others. The neuroendocrine changes were primarily manifested in the female offspring during the lactation period.

In the mildest cases human mothers displaying these maternal responses or lack there of are referred as suffering from 'baby-blues', in more severe situations some mothers are hospitalized and treated for post-partum depression or psychosis for the safety of themselves and their young. Brummelte, Pawluski, and Galea (2006) suggest that post-partum stress and depression are associated with hypercortilism in humans. They found that heightened levels of corticosterone post-partum in mothers, which is

related to stress and anxiety, had a depressive effect compared to controls. The investigators found that pups raised by these mothers exhibited decreased postnatal cell proliferation in the dentate gyrus.

The evidence that the results of even mild forms of maternal neglect are detrimental and long lasting on the young gives us a strong incentive to seek an understanding of the neurological processes involved. A better understanding of the maternal brain will thus shed light on this phenomenon on the brains cognitive, emotional and plastic nature.

Maternal Behavior

For most mammals, motherhood is not simply providing life to young, but also involves great amounts of time and care that are invested to the offspring. New born rodents are entirely dependent on their mothers. They are nearly immobile, can not feed themselves, or control their body temperature. Their survival is contingent upon the care provided by their mother. Maternal care in rats consists of nest building, pup retrieval and grouping, crouching behavior to allow pups to nurse, and licking and smelling the pups (Leckman & Herman, 2002). In the majority of primiparous females these behaviors come about naturally. These behaviors are cued by numerous interrelated factors including hormonal, environmental, and neurological changes.

Leckman and Herman (2002) used gene knockout technology to identify at least nine genes involved in the display of maternal behaviors. The genes they identified encode for various neurobiological factors of maternal behavior including three

transcription factors for the enzymes dopamine, beta hydroxylase, and neuronal nitric oxide synthase; prolactin and estrogen receptors, and the neuropeptide oxytocin. Each of these are important components of maternal behavior, and Leckman and Herman's (2002) research demonstrates genetics as one of the many influences on maternal behavior.

Other researchers have looked at these components individually to understand their specific roles in maternal behavior. Grattan et al. (2001) studied prolactin receptors during pregnancy and lactation to identify their implications on behavior. They observed changes in expression of prolactin receptors during gestation and lactation in certain hypothalamic nuclei. The changes in expression show important implications on the neuronal functions regulated by prolactin which may influence maternal behavior, feeding and appetite, and stress responses. They conclude that because of the elevated levels of prolactin present during pregnancy and lactation in addition to the increase in prolaction receptors in the hypothalamus these activities are likely to be enhanced in the brain of a new mother. These enhancements alter the mothers' brain in preparation for her young, and allow her to feed and nurture her offspring.

Lucas et al. (1998) studied the importance of the prolactin receptor gene, and found that a mutation of this gene produces a defect in maternal behavior. They found that nulliparous females carrying either one or both copies of the mutant gene showed a lack of pup-induced maternal behaviors, and even primiparous females with one copy of the gene showed a significant deficiency in maternal care when presented with foster pups. The mutation did not affect the rats' olfactory functions, appetite, motor abilities,

or exploration. In accordance with related research, the authors concluded that the prolactin receptor gene is an important regulator of maternal behavior, and negative interference at this point is detrimental to an animals' ability to display maternal behavior.

In addition to lactation and feeding young, another specific area of maternal behavior studied is the mother's ability to retrieve her young. Numan et. al. (2005) evaluated the importance of dopamine receptors in the nucleus accumbens on this maternal behavior. They found that injecting a D1 dopamine receptor antagonist into the nucleus accumbens disrupted the mothers' retrieval of her pups. They also noted that this decrease in retrieving behaviors was not due to impaired motor abilities, emphasizing the importance that dopamine receptors play in the display of maternal behavior.

A third component of maternal behaviors widely studied is the role of estrogen and oxytocin receptors. Although the basic maternal behaviors displayed are the same from one mother to the next, crouching, retrieving, licking, grooming, etc; there are individual differences in the amount and quality of the way these behaviors are displayed. It is suggested that these individual differences affect neural development and are passed from mother to female offspring. Champagne, Diorio, Sharma, and Meaney (2001) studied the link between estrogen and and oxytocin receptions and how they are associated in these individual differences in the expression of maternal behavior. Specifically, they looked at maternal behaviors of licking and grooming the pups, that are crucial to pup development.

Their results showed that female offspring exposed to high amounts of licking and grooming as pups, displayed greater maternal responsivity to pups later in life than the offspring of mothers who displayed low levels of licking and grooming behavior. They also found that the mothers who were quicker to display maternal behavior and more responsive to the pups had significantly higher levels of oxytocin receptors. When mothers were injected with an oxytocin receptor anatagonist the differences in the amount of grooming and licking were removed. Champagne et al. (2001) conclude that oxytocin receptor levels are functionally related to maternal behavior.

They also found that oxytocin receptor binding increased when female offspring of high licking mothers were treated with estrogen, but not when the female offspring of low licking mothers were given the same treatment. It is suggested that this demonstrates how the care a pup receives from its mother influences the pup's sensitivity to estrogen, and eventually their display of maternal behavior towards their young.

Thus, previous research demonstrates the complexity of the range of maternal behaviors and the various influences from genetics, to neurological, to environmental and how they are all interconnected. Rosenblatt (1994) combines these multifaceted regulators of maternal behavior into two basic phases. Although the majority of research on maternal behaviors is based in non-primate rodent models, he suggests that the general conclusion can aid us in our understanding of human maternal behavior as well.

He describes the first phase which takes place during pregnancy as the hormonal priming phase. Rosenblatt states that this phase is based on the presence of prolactin, estrogen, and progesterone. He labels the second phase, which occurs at the end of

pregnancy, as the triggering phase dependent on the decline in progesterone and increase in estrogen, prolactin, and oxytocin.

Rosenblatt notes that although the onset of maternally behavior is triggered hormonally, postpartum maternal behaviors require the stimuli the mother receives from the presence of the young. He discusses the transition period between these phases and notes that contact with the young is critical to the onset and maintenance of maternal behaviors. Rosenblatt suggests that when females become mothers they face a conflict between approach and withdrawal responses to their young. He postulates that the onset and display of maternal behavior is the resolution to this conflict. This conflict is an aspect of the current work.

Behavioral Changes Due to Reproductive Experience

In addition to behaviors directly associated with maternal behavior, mothers must also be able to complete additional behavioral tasks to ensure the survival of their young. She must be aggressive and able to protect against any potential threats. She must be able to forage and hunt for food. She also will need to remember the location of water and shelter, and be able to quickly return to her nest.

Although non-maternal rats must also be able to complete these behaviors, improvements in these behaviors have been shown to result from reproductive experience in females (Lambert et al, 2005). In laboratory settings primiparous females show better spatial memory task performance, better foraging and hunting ability. Reproductive experience has also been shown to affect the animals stress response (da Costa, Ingram,

Lightman, & Aguilera, 2001) and reduced anxiety like behaviors (Byrnes & Bridges, 2006), levels of aggression (Lonstein & Gammie, 2002).

Lambert et. al (2005) found that individual maternal experiences including pregnancy, parturition, lactation, and pup exposure come together to produce behavioral changes resulting in improved foraging abilities which allows the mother to better care for her young. In addition to this, nulliparous animals were tested after receiving exposure to pups and the results showed that they performed better on the foraging task than the nulliparous animals that were not exposed to pups.

The work of Lambert et. al (2005) suggests that the exposure to pups could be more important than the actual experience of pregnancy on maternal behaviors that are not directly related to care of the young. The importance of the exposure to pups has also been shown to have a protective quality against maternal depression. Boccia et. al (2006) found that long separations from pups in rat mothers elicited depressive like symptoms in the mothers.

Another ancillary behavior related to motherhood is maternal aggression. Maternal aggression is regulated by several components including sensory, hormonal, neuroanatomical, and neurochemical mechanisms (Lonstein & Gammie, 2002). Bosch et. al (2005) links oxytocin in particular to the regulation of maternal aggression, and also links maternal aggression with anxiety. They found that animals bred for high-anxiety behaviors displayed more maternal aggression against an intruder than the animals bred for low-anxiety behaviors.

Bosch's results showed that the levels of maternal aggression were related not to the number of oxytocin receptors, but to the actual release patterns of oxytocin itself. Oxytocin release was higher in the high-anxiety behavior animals. In these animals maternal aggression could be decreased using an infusion of oxytocin receptor antagonists. In the low-anxiety behavior females infusions of synthetic oxytocin increased their aggression towards an intruder.

Conversely, other research indicates that higher levels of oxytocin is related to lower levels of anxiety-like behaviors on an elevated plus maze task, and lower levels of oxytocin were related to higher levels of anxiety (Mantella, Vollmer, & Amico, 2003). However, this study did not include maternal experience in its evaluation of the relationship between oxytocin and anxiety. It is likely that the maternal experience alters then brain in a way that might change how levels of oxytocin effect anxiety.

This interaction is demonstrated by Neumann, Torner, and Wigger (2003). They found that infusions of oxytocin receptor antagonists increased the release of stress related secretions of corticotrophin and corticosterone in virgin animals, but not in pregnant or lactating animals. Treatment with oxytocin antagonists decreased anxiety related behaviors in pregnant and lactating animals, but not in virgin animals. The work by Numann, Tomer, and Wigger (2003) indicated that the maternal experience modulates the response of the oxytocin and stress hormone systems.

In addition to these studies on anxiety-related behaviors, numerous researchers have evaluated the stress response during pregnancy using a variety of approaches. Research has shown that female pregnant and lactating mice and rats show

hyporesponsiveness to stress, due to a variety of different factors. Douglas et. al (2003) found that inhibition by opioids and intracerebral oxytocin does not seem to be involved in the hypothalamo-pituitary-adrenal hyporesponsiveness in late pregnancy.

Ma, Shipston, Morilak, and Russell (2005) concluded that the attenuated adrenocorticotropin stress response in late pregnant rats is due to vasopressin release by parvocellular paraventricular nuclei neurons. Another study evaluated the suppressive effects of neurosteroids on pituitary-adrenal response to emotional stress (Patchev, Hassan, Holsboer, & Almeida, 1996). Yet another study investigated changes in noradrenaline neurotransmission, and concluded that reduced noradrenergic input to the paraventricular nucleus may contribuate to the reduced responsiveness of the HPA axis during pregnancy (Douglas et. al, 2005). In a chapter on the reduction in responsiveness of the HPA axis, Neumann (2001) indicates that much of the stress response depends on the innate level of emotionality of the rat.

Despite the variety of potential influences on the stress response and anxiety related behaviors, it is clear that reproductive experience does alter the animals' levels of anxiety like behavior. Byrnes and Bridges (2006) found that on an elevated plus maze and in an open field task primiparous animals displayed fewer anxiety like behaviors than their nulliparous counterparts. However, as animals aged parity was associated with anxiety-related behaviors.

In addition to the experience of pregnancy, the presence of pups has also been shown to elicit a resistance to stress in rats. Leuner and Shors (2007) found that virgin females who were exposed to pups for several days and responded maternally displayed a

resistance to stress. This indicates that the presence of young and the participation in the nurturing and care-giving activities protects the female animals against the deleterious effects of stress.

The study of the reduction of the stress response in pregnant females has been studied in human females as well (de Weerth & Buitelaar, 2005). Kammerer (2002) exposed pregnant women and new mothers to a cold hand stressor test, and measured salivary levels of cortisol. The results shows that the control females had a significantly strong response to the test, but the pregnant group showed no response to the natural stressor. The postpartum group did show a response, but it was not significant. Kammerer concluded that the reduced stress response acts as a protective mechanism to shield the fetus from the negative effects of prenatal stress.

Nierop et. al (2006) conducted a similar study with human females but used a psychosocial stressor rather than a physiological stressor. They suggest that human pregnancy does not result in the reduction of the HPA axis in response to psychosocial stress. Conversely, Kammerer, Taylor, and Glover (2006) suggest that the HPA axis is linked to two different forms of maternal depression, melancholic and atypical.

When mothers do encounter high levels of stress during pregnancy the result in often depressive like symptons (O'Mahony et. al, 2006). Nierop, Bratsikas, Zimmermann, and Ehlert (2006) suggest that heightened levels of stress related cortisol might actually be useful in identifying mothers at risk for post-partum depression. The association between stress hormone levels and post-partum depressive is confirmed by additional research as well (Carr et. al, 1981; Magiakou et. al, 1996). Despite the varied

approaches and results, the common thread throughout these changes in behavior is that all seem to improve the female animals' ability to better care for her young.

Brain Plasticity, the Maternal Circuit and Neurogenesis

These changes in behavior must be linked to changes in the brain. Previous research supports this concept, and shows that environmental and developmental factors are constantly working to change the neural circuitry of the brain. Kolb, Gibb, and Robinson (2003) and Smith et. al (2006) discussed the variety of influences that affect brain plasticity including everything from genetics, dietary factors, growth factors, disease, stress, brain injury, psychoactive drugs, and gonadal hormones to simply stated 'experiences.' Motherhood involves changes involving many of those factors, and it is logical then that we would see a great amount of change in the female brain as she becomes a mother.

Kolb and Wishaw (1998) define brain plasticity as the brain's ability to change structure and function. Kolb states that life experiences stimulate these changes in brain function and structure in numerous species from insects to humans. Changes in the brain produced by experiences include increases in dendritic length, increases and/or decrease in dendritic spine density, formation of synapse, increases in glial activity, and changes in metabolic activity. These physiological changes then result in wide variety of behavioral changes. There are numerous experiences and components that influence these changes, and numerous areas of the brain affected by these different factors.

Foy (2002) discusses plasticity of the hippocampus specifically, and the role that estrogen plays in the synaptic plasticity of this region. The hippocampus is an important structure involved in learning and memory. Foy's work connects estrogen's effect on the synaptic plasticity in the hippocampus with long-term potentiation and long-term depression, showing the lasting effects of alterations in brain plasticity.

Other research is geared specifically towards brain plasticity and motherhood. Brunton and Russell (2008) gives an extensive review of the changes that take place in the brain as a female adapts for motherhood. Their review highlights the maternal networks that are activated during pregnancy and parturition that allow the mother to perform the necessary behaviors to care for pups, such as nest building, nursing young, and gathering pups. They conclude that different neural networks are responsible for each of the different behaviors, but collectively they for the neural circuitry.

Brunton and Russell list the medial preoptic area as a crucial component in the regulation of these behaviors. Various factors including progesterone and oestrogen receptors, GABA receptors, oxytocin neurons, peptide hormones unique to pregnancy, prolactin, and others all effect the complex maternal circuitry. Roughly fifty-percent of women undergoing these dramatic changes face low mood around the time of birth, and between and 10-13 percent of women will develop major depression. Brunton and Russell (2008) relate this depression to the normalization of the various neurochemical changes in combination with certain genetic factors that take place during pregnancy and birth.

Jones, Robertson, Lendon, and Craddock (2000) evaluated the more severe mood disorder following birth known as puerperal psychosis. Their conclusions are in agreement with Brunton and Russell (2008) that genetic factors involving the serotonin transporter gene exercises substantial influences on a mother's susceptibility to an episode of this severe psychiatric disturbance within a few days following birth.

Numan (2006) developed a neural model that includes the medial preoptic area of the hypothalamus as a brain region that is involved in the regulations of a mother's responsiveness towards young. He proposes that there are two critical steps taking place involving the hormone primed MPOA. The first component involves the neural circuits between the amygdala, medial hypothalamus, and the midbrain. This part of the circuitry is involved in depressing the central aversion system, so that the mother will not respond to the pup stimuli with avoidance or aggression. The second component is the excitation of mesolimbic dopamine systems. This element promotes active and voluntary displays of maternal behavior. Numan also includes the effects of oxytocin and actual maternal experiences into this model of maternal circuitry as well.

Sheehan and Numan (2002) also includes the ventral bed nucleus of the stria terminalis and the dorsal and intermediate lateral septum as parts of the maternal circuit along with the medial preoptic area. They found that estrogen stimulation promoted maternal behavior by enhancing activity in these areas, and that progesterone reduced maternal behavior by inhibiting activity in some of these areas involved in the maternal circuitry.

Other research has evaluated areas of the brain that appear to inhibit the onset of maternal behavior. Bridges, Mann, and Coppeta (1999) investigated the involvement of the ventromedial hypothalamus, the dorsal hypothalamus, and the anterior hypothalamus on maternal behavior in rats. They found that in steroid treated animals, stimulation of the ventromedial hypothalamus resulted in the rapid onset of maternal behaviors. Results also showed that lesions in the dorsal hypothalamus and the anterior hypothalamus also caused an onset of maternal behaviors in steroid treated rats. Their results show that these three areas are involved in steroid dependent inhibition of the onset of maternal behavior.

There is a variety of other research outlining other changes taking place in the brain during pregnancy and parturition. Meddle et. al (2000) investigated oxytocin and vasopressin neurons within the supraoptic nucleus and found that they are activated by the brainstem and olfactory bulb at parturition. Catheline et. al (2006) noted that during parturition and lactation the hypothalamic oxytocin sytem displays morphological plasticity involving the surface of the neurons becoming juxtaposed and therefore contacted by an increased number of synapses. However, they prevented this remodeling in female rats, and found that parturition and lactation continued normally. They suggest that although this remodeling effects the modulation of oxytocin neuronal activity it is not fundamental to parturition and lactation.

Leng, Dye, and Bicknell (1997) and Douglas et. al (2002) also evaluated the effects of the oxytocin neuron activity and the supratoptic nucleus. Both of these article

found evidence connected these factors to brain plasticity involved with pregnancy, parturition, and lactation as well.

Altemus et. al (2004) approached changes in the brain due to pregnancy by evaluating differences in the cerebral spinal fluid neurochemistry in pregnant women. They found a reduction in g-aminobutyric acid (GABA) in pregnant in the cerebral spinal fluid of pregnant women, and a large increase in the levels of cerebral spinal fluid prolactin. They also noted an increase in cerebral spinal fluid oxytocin, but not at a significant level. They suggest that the changes in these levels during pregnancy is involved in the vulnerability pregnant women face towards depression during pregnancy and the postpartum period.

In addition to studies on neurochemical changes in the brain, several studies focus on changes to the cell bodies and arrangement of cells as well. Rosenzweig and Bennett (1996) found that experience and learning is related to changes in cortical thickness, the size of synaptic contacts, the number of dendritic spines, and dendritic branching. They also found the experience early in life improved performance on later tests of learning. They suggest that enriched environments and training induce a cascade of neurochemical events that cause plastic changes in the brain.

Keyser-Marcus et. al (2001) evaluated alterations to neurons to the medial preoptic area following pregnancy in rats. They evaluated changes in the number of dendritic branches, cumulative length of the largest dendrite, the area of the cell bodies, and number of dendrites. They found the pregnancy does alter these factors, and that

some of the dendritic changes remain altered while the neuronal soma appears to return to a pre-pregnancy states during lactation.

Gould, Woolley, Frankfurt, and McEwen (1990) found that gonadal steroids are involved in the regulation of dendritic spine density in the hippocampal pyramidal cells. They suggest the the CA1 pyramidal cell may even fluctuate during less drastic changes than pregnancy such as the normal rat estrous cycle. Kinsley et. al (2006) confirmed these conclusions, and added that motherhood drastically increases the density of neuronal dendritic spines.

The restructuring of a female's brain as she enters motherhood also involves the proliferation and incorporation of new cells in the mother's brain. Darnaudery et. al (2007) investigated the relationship between the cell proliferation and cell survival two weeks later with spatial learning and hippocampal function. They found that after delivery rats displayed impaired spatial learning perfmorance, and a decrease in cell proliferation in the dentate gyrus. However, two weeks later they noticed the new cells were not decreased, and their spatial abilities were improved. These results show that the period following parturition is highly associated with a modification of hippocampal function and demonstrates the plasticity of the maternal brain.

Leuner, Mirescu, Noiman, and Gould (2007) show results that coincide with Daunaudery et. al (2007). In addition they found that the presence of the young during the period following parturition is involved in the decrease in cell proliferation in the dentate gyrus. They suggest that changes in the adrenal steroid are one way that the

presence of young suppresses the adult cell proliferation initially following birth, without causing a decrease in the number of new cells two weeks later.

Ruscio et. al (2008) exposed parental prairie voles to foster pups, and investigated the levels of cell proliferation in the dentate gyrus in response to the presence of the pups. They also evaluated how the level of parental response correlated to the levels of cell proliferation. Their results showed that there was an increase in cell proliferation in the dentate gyrus in the animals that were exposed to foster pups. They also found that animals that did not respond parentally had more BrdU labeled cells in the dentate gyrus than the animals that displayed maternal behavior.

The results of these three studies suggest that the presence of young has a direct effect on levels of cell proliferation in adult animals. However there appears to be additional moderators connected with pregnancy that temporarily reverse the direction of these effects, and allows changes in hippocampal functioning resulting in what might be a more efficient maternal brain.

Duration of Behavioral and Neural Modification

The next question then is how long do these changes in the brain last? Does the re-modeling that takes place as the mother goes through pregnancy and interacts with her pups remain a lasting change? Research has shown that these changes in memory and behavior do persist (Love et al., 2005). In this study, the question is not simply do the behaviors and neurological changes of motherhood persist, but are there changes in the

maternal circuitry resulting in neurons that are specifically active to the stimuli received by exposure to new born foster pups four weeks after the mother delivered her own pups.

Love et. al (2005) used a dry land maze and elevated plus maze to evaluate the cognitive and emotional responses of nulliparous, primiparous, and multiparous females every four months between the ages of 5 months and 22 months. On average the nulliparous females took longer on the dry land maze task. Beginning at ten months of age, the parous animals began to spend more time on the open arms of the elevated plus maze than the nulliparous animals. However, on the neuronal measures reproductive experience did not seem to have an effect.

A different approach to evaluate long-term plasticity is the study of specific receptors. Brussard and Herbison (2000) and Concas et al. (1998) investigated the plasticity of GABA –receptors in pregnant rats. Brussard and Herbison (2000) suggest that hypothalamic oxytocin neurons that exhibit plasticity through the reproductive cycle provide a model for the long-term plasticity of GABA-mediated transmission. They discuss the importance the GABA receptor functions have on the oxytocin neuron and therefore the neural control of pregnancy and lactation.

Engert and Bonhoeffer (1999) discuss the long-term enhancement of synaptic efficacy in the hippocampus as a model of the cellular mechanism of neural plasticity, circuit reorganization, learning, and memory. After inducing long-lasting functional enhancements in the CA1 they found new spines on the postsynaptic dendrite, which were not seen in the control regions.

Kee, Teixeira, Wang, and Frankland (2007) evaluated neurogensis and brain plasticity in the hippocampus as well, and found that new neurons added to the dentate gyrus are more likely than existing granule cells to be incorporated into circuits supporting memory after they reach four or more weeks of age. At this point the new cells are mature and make a unique contribution to memory processing in the dentate gryus.

Kee, Teixiera, Wang, and Frankland (2007) measured the incorporation of new cells into the spatial memory circuitry by staining for c-fos, BrdU, and NeuN. Animals were injected with BrdU, and test on a spatial maze task. The animals' performances on the spatial maze task and the number of cells showing staining were quantified at various points in time. Results showed that new cells are incorporated into the spatial memory circuitry as early as two weeks, but maximal inclusion of the new cells doesn't occur until four weeks of development. The current study will focus on cells just over four weeks of age.

Current Study

The current study addressed how the primiparous rat undergoes a fundamental re-modeling of her brain as a consequence of experiencing the normal and natural events of pregnancy and the offspring, and also evaluated behavioral consequences of a brain that does not fully achieve this re-modeling. Compelling data support the hypothesis that maternal experience produces neurobiological modifications in the female rat that affect maternal states, behaviors, affective states, and the underlying neurobiology of the

female, but in some cases the rats do not express the typical maternal states, and behaviors.

Pilot work over the past year has indicated that when primiparous females are exposed to pups a week following weaning, neurons born during their initial experience with pups at parturition are selectively reactivated. This previous work was encouraging, and the current study expanded on this early work and focused primarily on the behavioral and neurological differences between maximally attentive and minimally invested or neglectful mothers. This study aimed to evaluate the quality and selective activity of the neurons that develop immediately following parturition in both responsive and non-attentive mothers.

In addition to the basic maternal behaviors, mother also involves changes to non-reproductive behaviors such as learning, memory, and stress and anxiety regulation. The current study evaluates the hippocampal brain region since these functions have been associated with neurogenesis in these areas (Leuner, Mirescu, Noiman, & Gould, 2007). We will evaluate the relationship of changes in the denate gyrus with the display of maternal behavior.

Tashiro, Makino, and Gage (2007) suggest that there is a critical period during the first three weeks of development of new cells during which experiences can determine the survival of the new neurons. They suggest that this could be a mechanism by which the dentate gyrus in altered in a long term manner. In the current study the mothers will be housed in the presence of their young throughout the first three weeks following the BrdU injection marking the development of cells born immediately following parturition.

In order to evaluate the quality and selective activity of the neurons, we stained for bromodeoxyuridine (BrdU), c-fos, and Neuronal Nuclei (NeuN). BrdU is a thymidine analogue which is used to identify proliferating cells. Following injection, BrdU is incorporated into the DNA of the replicating cells. This will allowed us to detect only the new cells which were being generated during the period immediately following parturition. C-fos is an indicator of recently activated cells, and using this component in our staining allowed us to determine which cells were specifically activated in response to the presence of the pups. Finally including NeuN antibody, allowed us to confirm which cells were mature neurons, because the NeuN antibody specifically recognizes the DNA-binding neuron-specific protein NeuN present in most neuronal cells in the central and peripheral nervous systems.

Although all of the mothers in the study cared for their own pups until weaning, no distinction was made between the responsive and non-responsive females until exposure to foster pups one week after their pups were weaned. The attentive mothers were defined as those showing a full display of maternal behavior when exposed to foster pups. The neglectful or non-responsive mothers were classified as the mothers that did not exhibit maternal behavior when exposed to the foster pups.

It is hypothesized that the states of late-pregnancy and early lactation stimulate the production of a set of neurons that are integrated into a form of "maternal circuitry" in the mother's brain, and that the number of neurons with selective responsivity to offspring will differ between mothers with a strong maternal response to pup exposure after weaning and mothers with a weak maternal response to the secondary pup exposure.

Based on a previous study by Scanlan, Byrnes, and Bridges (2006) which found that primiparous females were more responsive to the presence of pups and had greater c-fos expression, it is expected that mature neurons (showing NeuN immunoreactivity) expressing both BrdU and c-fos will be more plentiful in the brains of the mothers that interacted with pups and displayed more maternal behavior. This research also found that the increase in c-fos expression was restricted to only certain primiparous females. In light of this, it is hypothesized that the brains of the minimally invested or neglectful mothers will not contain as many triple-labeled neurons as the maternally responsive mother group. Therefore, the restriction of the increase in c-fos expression might be connected to a more fine tuned measure of maternal behavior and the individual levels of motivation related to the displays of interest in the young.

Project Design and Methods

Subjects

We used twenty-five nulliparous adult female Sprague-Dawley rats weighing approximately 300 g in this experiment. Prior to mating the animals were housed in cages of two to three animals each. After mating each rat was housed individually in 20 x 45 x 25 cm clear polypropylene cages, the floors of which were covered with corn cob bedding (Harlan). The tops of the cages were wire lids which hold food and water. We limited human contact with these rats to delivery of the food (Harlan Teklad Rodent Diet) and water *ad libitum* and cleaning of the cages. All animals were housed in rooms controlled for both temperature and light for the duration of the work. All animal

maintenance and procedures used in this study were strictly conducted according to the standards set forth by the University of Richmond Institutional Animal Care and Use Committee and the National Institutes of Health (for which approval has been received: #06-05-6).

Materials

Behavioral apparatus. Animals were observed interacting with pups in a 20 x 45 x 25 cm clear polypropylene cage with corn cob bedding and wire lid. They were also observed with pups on a standard elevated plus maze. The maze was elevated 85 cm off of the floor and had two open arms (50.2 x 10.8 cm) and two closed arms (50.2 x 10.8 x 40.0 cm) with an intersection in the middle (10.8 x 10.8 cm). A small plastic box lid (3 x 5 x 1 in) was used to hold the pups which exposed the female to pup related cues (sounds, odors). The box lid was elevated to the level of the plus maze and was placed 15 cm from the edge of one of the open arms as in Scanlan, Byrnes, and Bridges (2006). This distance was used to prevent the animals from attempting to jump to the pups, but was close enough to draw the animals to the pups.

A video camera was set up directly in front of the clear cages and the elevated plus maze to record behavior for later analysis.

Neural assessments. In preparation for the bromodeoxyuridine (BrdU) injection, BrdU was dissolved in 0.1 M phosphate buffered saline (PBS), and heated to 50-60 °C. After testing, sodium pentobarbital was used to overdose the animals. Phosphate buffered saline (PBS) and 4% paraformaldehyde (PF) was used to perfuse the animal, and

brains were stored in 20% PBS sucrose. Sections were washed in 0.1 M Tris Buffer (TBS), TBS++, 2x SSC, and pretreated with 2 N HCl to prepare for staining. A primary antibody cocktail of anti-BrdU, anti-fos, and anti-NeuN and a secondary cocktail of three corresponding fluorescing secondary antibodies were used in staining. Dabco was used during cover slipping to prevent any bleaching of the sample during the imaging process. A cryostat was used to cut the hippocampal sections from the brain. Syringes, microplate wells, slides, cover slips, and microscopes were also necessary. The Carl Zeiss Axioimager fluorescent microscope was used for imaging the slides.

Behavioral Procedures

We mated 25 nulliparous female rats. Following mating, the rats were housed alone in standard cages, previously described, with limited contact until they had delivered their pups.

BrdU administration. Within 24 hours following the cessation of delivery, we injected the mothers with a single dose of bromodeoxyuridine (BrdU, 200mg/kg ip). This dosage was comparable to that used in Shors (2004), Shors et al. (2001), and Van Praag et al. (2002). Bromodeoxyuridine (BrdU) is used as a marker of proliferating cells and their progeny (Holmes & Galea, 2002).

Maternal behavior test. The rats were housed with their pups until weaning, approximately 21 ± 2 days post delivery. One week following weaning, the primiparous females were exposed to three newborn pups, and allowed to interact with the pups for one hour. The newborn foster pups (2-7 days old) were randomly selected from the litter

of a healthy lactating donor mother. One pup was placed into each of three corners of the clear cage, and the adult female was placed in the remaining corner. During this interaction time, the animal was continuously observed for the entire hour by video to allow careful observation and analysis of maternal behaviors including the latencies to retrieve the pups, grouping, and crouching (Scanlan, Byrnes, & Bridges, 2006). The first fifteen minutes of the observed video footage was coded continuously. The animals were spot checked every fifteen minutes at 30 minutes, 45 minutes, and 60 minutes. Their location and behavior in relation to the pups was recorded, and the pups were removed and returned to their mother at the end of the 60 minute observation point. The animal was returned to its individual cage for 15 minutes following pup exposure.

EPM test. After the fifteen minute break, the animals were continually observed on the elevated plus maze for five minutes following the basic protocol used in Scanlan, Byrnes, and Bridges (2006). Before beginning the test, three new born foster pups taken from a healthy donor mother were placed on the end of one of the open arms to transfer their odor to the maze. The pups were then placed in the elevated box lid 15 cm from the end of the open arm allowing the female to be exposed to the pup stimuli but not to physically reach the pups. After the pups were in place, the primiparous female was placed on the center intersection of the platform facing the open arm toward the platform holding the pups.

Behavioral analysis. Video observations of the behavioral information were coded by two independent observers. Results were checked with a series of correlations for each variable to ensure that the interrater reliability was high.

In order to move towards the pups the animal will had to overcome any fear, anxiety, or stress of moving out onto the open elevated arm. The number of times the animal moved onto the open arm, the amount of time spent on the open arm, and the distance travelled on the open arm was measured. The animals that scored higher on these measures were categorized as the maternally responsive animals for this test.

During the maternal behaviors tests, we recorded the mothers' latencies to retrieve pups, group pups, and crouch in the first maternal response test to determine which mothers were attentive and which were neglectful. The amount of time the mothers spent grooming pups, retrieving pups, and crouching over pups were recorded as well. We also recorded the total number of pups the mother grouped during the fifteen minute observation. The animals' scores on the these items during the fifteen minutes behavioral observation were combined with the observations of maternal behaviors made at 30, 45, and 60 minutes of the behavioral test into a composite score of maternal behavior. Individual *t* tests were conducted for each animal to determine if their level of maternal behavior differed significantly from the mean score. The animals that scored higher than average were coded as responsive mothers, and the mothers with scores lower than the mean score were coded as non-attentive mothers.

Tissue Processing and Analysis

Collection. Immediately following the elevated plus maze test the animal was injected with an overdose of sodium pentobarbital, and transcardially perfused. This procedure involved pumping the vascular system first with PBS, followed by 4%

Paraformaldehyde (PF) to begin the preservation process. After removing the brain, the tissue was postfixed in PF for overnight, followed by immersion in a 20% sucrose/PBS solution at 4°C. The brain was blocked into three sections and then the block of interest was cut into 50μ sections through to the dentate gyrus with the cryostat at -16°C. Sections were evaluated for anatomical comparison and verification of neuroanatomy. Ten slices from each animal were retained and stored free floating in microplate wells filled with tris buffer (TBS) overnight.

Neural staining procedures. The 50-micron sections through the dentate gyrus were processed for BrdU-immunoreactivity and c-fos expression. They were labeled for NeuN in the same sections to identify mature neurons. Basic fluorescent immunohistochemical techniques were used to stain the brain tissue (Kee, Teixeira, Wang, & Frankland, 2007). Primary and secondary antibodies with fluorescent tags to locate cells triple labeled for BrdU, NeuN, and c-fos receptors were used within the brain tissue following perfusion and fixation.

Staining began with three five minutes washes in tris buffer (TBS) at room temperature. Sections were then incubated at 65 °C in 50% Formamide for two hours. Following incubation the tissue was rinsed in 2xSSC for fifteen minutes at room temperature. In preparation for BrdU labeling the sections were incubated in 2 N HCL for thirty minutes at 37 °C, and rinsed for ten minutes in 0.1 M Borate Buffer at room temperature. The sections were then washed in TBS at room temperature (6 washes at 15 minutes each). Before applying the antibody cocktail the tissue was blocked with TBS++ for one hour. Finally, the primary antibody cocktail of BrdU (sheep anti BrdU polyclonal

antibody; 1:500, GeneTex), c-Fos (rabbit anti c-fos polyclonal antibody; 1:1000, GeneTex) and NeuN (mouse anti NeuN; 1:1000, Chemicon) was applied at 4°C for twenty-four hours.

On the second day of staining, the tissue sections will underwent two fifteen minute washes in TBS at room temperature. Before applying the secondary antibody cocktail, the tissue was rinsed in TBS++ for fifteen minutes. The sections were then incubated with a complementary fluorescent tagged secondary antibody cocktail (donkey anti sheep conjugated with Fluorescein, FITC; 1:500, Jackson Immuno; donkey anti mouse conjugated with Aminomethylcoumarin, AMCA; 1:500, Jackson Immuno; donkey anti rabbit conjugated with rhodamine, Red-X; 1:500, Jackson Immuno) for two hours. Because of the nature of fluorescent staining, each step from this point on was conducted in limited light. To complete the staining process, the tissue was rinsed seven times for fifteen minutes each in TBS at room temperature.

After staining, sections were mounted onto double subbed slides with TBS and allowed to dry overnight. The sections were cover slipped with a special protective solution, Dabco, which prevents the fluorescent stain from bleaching. After drying overnight, the coverslips were sealed in preparation for the microscopic analysis.

Microscopic Analysis and Quantification

The sections were imaged on our Carl Zeiss Axioimager fluorescent microscope. The number of triple labeled BrdU/c-fos/NeuN cells in the dentate gyri in both the left and right hemispheres of the pup exposed mothers were quantified in each section and

averaged to give one unilateral score for each animal. Four sections per rat were counted. Only brain tissue that was adequately stained as compared to negative control samples was quantified and analyzed. Tissue samples were coded and analyzed by researchers blind to the behavioral data.

Using the AxioVision software program, each image was evaluated by selecting the areas that displayed triple staining as indicated by the level of fluorescence. The threshold level of areas to be included in quantification was set at 15%, 25%, or 35% of the overall gray-level displayed. The value used in quantification was dependent on the overall exposure of the individual image. Using these set values allowed the method of quantification to remain objective across all of the images. The total area showing triple-staining in the dentate gyrus was summed for each hemisphere, and then averaged to give one overall score for each animal. The total area in micrometers squared was divided by the average area of an individual neuron in the dentate gyrus of a rat (Roy, Seidler, & Slotkin, 2002). This transformation has no effect on the statistical significance of the results, but allows for easier understanding of the neurobiological relationship being studied.

Statistical Analysis

Variables were tested for normality using a one-sample Kolmogorov-Smirnov test. A two-tailed t-test was used to evaluate the relationship between the number of triple labeled cells in the dentate gyrus and the maternal behavior group from the first

observational test, responsive mothers and non-attentive/neglectful mothers. Because the individual variables were continuous, they were analyzed using bivariate correlations.

General Linear Model was used to evaluate the addition of the number of pups as an additional variable in the elevated plus maze task. The number of pups was included as a covariate, and was ruled out as a possible confounding variable as the model was very significant ($F(2,21) = 12.27$, $N = 25$, $p < 0.001$).

Behavioral data were analyzed multidimensional scaling (MDS). MDS is a data reduction technique used to uncover a "hidden structure" to a set of data (Kruskal & Wish, 1978). MDS refers to graphical models that provide a spatial representation of the similarity structure of variables, which is not possible with standard parametric statistical techniques such as Factor Analysis. Using correlations, the relationships (i.e. proximities) among variables can be displayed graphically. The variables are represented by a set of points in two or higher dimensional space (a map). Thus, the closer two or more variables are on the map, the more highly correlated they are, while the farther apart they are, the less correlated they are. In order to "map" all of the variables into a desired space (two dimensional or greater), a certain lack of fit has to be accepted. This lack of fit is referred to as the s-stress. The values of s-stress range from 0 (perfect fit) to 1 (worst possible fit). The aim of MDS is to find a map of the variables that minimizes the s-stress for a given number of dimensions (Kemmler et al., 2002). The number of dimensions can be likened to the number of latent underlying factors in the dataset. Thus, when choosing the number of dimensions to represent the data, one must consider 1) the number of variables in the model (Kruskal & Wish, 1978), 2) the lack of fit (s-

stress value), given the number of dimensions, 3) an index of fit of the model (r squared value or RSQ), and 4) interpretability of the dimensions. The first point addresses the fact that for each dimension of the data, there should be approximately 4 variables entered into the model. Thus, for a 2-dimensional map, approximately 8 variables should be used. The second point addresses how well the MDS map actually "fits" the data. Stress values below .15 are typically deemed acceptable (Diekhoff, 1992). The third point addresses the variance accounted for within the model. As is the case with any regression analysis, one must consider the amount of variance being accounted for. Typically, RSQ values of .8 or higher are desirable (Schiffman, Reynolds, & Young, 1981). Finally, one must pick a solution based on interpretability of the dimensions. Parsimony is crucial to interpreting the "map" of any given dataset.

Nonmetric unfolding MDS can be used to produce both a map of stimulus variables (behaviors, in this case), based on the averaged inter-variable proximities from the subjects, and a subject map. This approach is useful when trying to map individual proximities to particular variables or group of variables. When subjects can be naturally grouped in 2 or more classes (such as good / bad mothers), nonmetric unfolding MDS is useful in mapping the association of subject groups with specific variables. In this study, nonmetric unfolding MDS was used to examine differences in 1) the patterns of behavior among the good and bad mothers, 2) the patterns of neural activity among the good and bad mothers, 3) and the patterns of association between behavioral and neural variables. Individual scores across each variable were transformed into z-scores prior to analysis.

Results

Maternal Behavior

Because subject were not randomly assigned to experimental groups, but rather placed into groups based on the maternal behavior they displayed the twenty five subjects were not evenly divided between the two levels of the independent variable of maternal behavior. The responsive mother group contained eleven females which scored above the mean maternal behavior score of 10.36, *S.D.* = 4.42. The neglectful group consisted of fourteen females that scored lower than the mean.

Significant differences in the amount of mature neurons which developed immediately following parturition showing c-fos activation in mothers that responded maternally versus those that did not were detected, $t_{23} = 5.3$, $N = 25$, $p < 0.001$ (see Figure 1). The attentive mothers had significantly more triple labeled neurons in the area of interest ($M = 108.01$, *S.D.* = 57.5) than the non-responsive mothers ($M = 26.09$, *S.D.* = 9.90).

Several of the individual factors showed significant correlations with the number of c-fos activated mature neurons showing BrdU inclusion (see Table 1). The latency to retrieve the first pup ($M = 734.57$ s, *S.D.* = 268.89), and the latency to begin grouping the pups ($M = 794.88$ s, *S.D.* = 230.42), were both negatively correlated to the number of triple labeled cells ($r = -0.526$, $p = 0.007$ and $r = -0.432$, $p = 0.031$). The amount of time the mother spent retrieving pups ($M = 7.55$, *S.D.* = 16.2) was positively correlated with

the number triple labeled neurons ($r = 0.390$, $p = 0.054$) as was the number of pups grouped ($r = 0.405$, $p = 0.045$).

Contrary to original expectations latency to initially contact the pups ($M = 20.92$, $S.D. = 76.28$) was positively correlated with the number of triple labeled cells ($r = 0.508$, $p = 0.010$) and the amount of time spent investigating the pups ($M = 109.49$, $S.D. = 42.5$) was negatively correlated ($r = -0.263$, $p = 0.20$, n.s.).

EPM

The total time spent on the open arms of the elevated plus maze ($M = 7.55$, $S.D. = 16.2$) was positively correlated with the number of c-fos activated neurons also incorporating BrdU ($r = 0.411$, $p = 0.023$) (see Figure 3). Total time spent on the open arm of the plus maze also correlated positively with the amount of time spent retrieving pups ($r = 0.556$, $p = 0.004$), and the number of pups grouped ($r = 0.651$, $p = 0.000$) (see Table 2). Total time on the open arms was negatively correlated with both grouping latency ($r = -0.582$, $p = 0.002$) and latency to retrieve the pups ($r = -0.680$, $p = 0.000$).

Total time on the open arm was also associated with the number of pups in the mothers' litter, and with the number of males in her litter (see Table 3). Both of these factors were positively correlated with the amount of time she spent on the open arms of the elevated plus maze ($r = 0.437$, $p = 0.029$ and $r = 0.500$, $p = 0.013$).

The number of times the animals moved onto the open arm nearest the pups i.e. the front arm ($M = 2.42$, $S.D. = 1.04$) was positively correlated with the total number of mature neurons labeled for BrdU and c-fos activity ($r = 0.367$, $p = 0.039$) (see Figure 4).

The number of times on the front arm also showed a positive correlation with the number of pups grouped in the maternal behavior observation ($r = 0.440, p = 0.028$), and showed a negative correlation to latency to retrieve the pups ($r = -0.374, p = 0.066$) and latency to group the pups ($r = -0.500, p = 0.011$).

The number of moves onto the front arm is also positively correlated with the number of male pups in the litter ($r = 0.543, p = 0.006$) (see Table 3).

The total time spent on the arm closest to the pups was positively correlated with the measures of maternal behavior number of pups grouped ($r = 0.586, p = 0.002$) and amount of time spent retrieving pups ($r = 0.511, p = 0.009$). The total time on the front arm was negatively correlated with both grouping latency ($r = -0.484, p = 0.014$) and retrieving latency ($r = -0.595, p = 0.002$) (see Table 2). Total time spent on the front arm is also correlated positively with the number of pups in the mothers' litter ($r = 0.437, p = 0.029$) and the total number of males in the litter ($r = 0.013, p = 0.500$) (see Table 3).

The amount of time spent on the first half of the open arm closest to the center of the maze was negatively correlated with the maternal observation measures retrieving latency ($r = -0.751, p = 0.000$) and grouping latency ($r = -0.694, p = 0.000$). The amount time spent on this portion of the arm was positively correlated with amount of time the mother spent retrieving pups during the maternal behavior observation ($r = 0.685, p = 0.000$) and to the number of pups grouped ($r = 0.683, p = 0.000$) (see Table 2). The amount of time spent on the first half of the open arm also has a positive correlation to the number of male pups in the mothers litter ($r = 0.432, p = 0.035$) (see Table 3).

The amount of time spent on the second half of the open arm which is closest to the pups was positively correlated to the number of male pups in the mothers' litter (r = 0.428, p = 0.037).

No relationship was found between the directions in conjunction with distance the animal travelled on the open arm and the number of triple labeled neurons. We also did not find any relationship between the number of pups in a mothers' litter or the gender ratio of the litter and the number of mature neurons with c-fos activation and BrdU incorporation.

On the basis of previous results, the following measures were considered for the multidimensional scaling analysis (MDS) of good and bad mothers: time spent in maternal behavior (crouching, retrieving, and grooming the pups); time spent in exploration; time spent in different locations of the elevated maze (total time spent in the open arms, time spent in the close section and time spent in the far away section); and number of neurons activated in the dentate gyrus.

The 2 dimensional (2-D) solutions for good and bad mothers can be seen in Figure 5. This solution yielded a stress score of .03 and an RSQ value of .97. These stress scores and RSQ values indicate that these models are explaining a large percentage of the variance and that the data are not being distorted to fit the model.

Dimension 1 was positively correlated with exploration and negatively with maternal behavior and number of cells activated, thus representing a lack of interest / motivation in mothers, whereas dimension 2 was positively correlated with neural activation and time spent in the far away portion of the elevated maze (an indicator of

maternal anxiety), thus representing intense activity in the brain following high motivation in maternal behavior and anxiety due to the elevated maze. A negative, linear combination of the two dimensions (shown in Figures 5 by a diagonal line drawn to separate the graph in two sectors), provided 2 primary clusters of behaviors: 1) neural activation is very high when females fight their anxiety of being in the open arm since they need to retrieve their pups; 2) other forms of maternal behavior (e.g., grooming) do not require so much neural activation, but still they are involving the same neural pathways (same side of Dimension 1 in the map). This map indicated that exploring the pups, can be regarded just a form of generalized exploration tapping into other neural mechanisms not measured by the triple labeled counts of neurons in the dentate gyrus. Overall, taking into account the relative, independent effects of each variable, this map shows that good mothers are not less anxious *per se*, they are able to win over their anxiety because they prioritize the needs of their pups on their fear/anxiety response, via a neural mechanisms localized in the dentate gyrus.

Discussion

The results confirmed our expectations that mothers that behave maternally show more selective neural activity in response to the presence of pups in mature neurons that developed after birth when the mother was first interacting with the pups. Figures 6, 7, and 8 confirm that the staining techniques used measured the desired targets, BrdU, c-fos, and NeuN. These figures show triple labeled cells in the dentate gyrus of a maternally responsice mother at 40x, 63x, and 100x respectively. The blue stained areas are the

mature neurons (stained for NeuN). The smaller red (c-fos) and green (BrdU) areas within the cells, which appear yellow where they overlay each other are showing that these neurons were activated and born during the time period of interest.

It is important to note that one of the responsive mothers actually acted out violently towards the pups. That particular subject began by showing great interest in the pups, but within the fifteen minutes of behavioral observation became infanticidal and killed two of the foster pups. The remaining twenty four mothers were not aggressive towards the pups, but rather simply showed little to no interest to the pups at all. Although this animal was included in the responsive mother group, she had the lowest number of triple labeled cells (Figure 2). This suggests that there is a continuum of maternal behavior and neuronal activity ranging from very low levels of activation being associated with infanticide and very high levels of activation being associated with full maternal responsiveness.

Figure 2 shows the correlation between triple labeled cells and the composite maternal behavior score. Unfortunately the graph does show significant heteroscedasticity. At higher ranges, three subjects go in opposite directions. However, this graph does display that all the responsive mothers do have higher values of triple labeled cells, with the exception of two one of which was the infanticidal mother.

All of the animals in this study were primiparous. This differs from previous research in which the amount of c-fos expression and neurogenesis were compared between primiparous and nulliparous animals (Scanlan, Brynes & Bridges, 2006). Since we are making the fine comparison of mothers with other types of mothers it is not surprising

that all groups showed some c-fos activity in mature neurons in the presence of pups that were born within 24 hours of the parturition. But even though all groups showed some selective activity, not all groups displayed maternal behavior. Perhaps there is a threshold level of new cells that must be activated in the presence of pups to illicit maternal behaviors.

The negative correlation between the number of triple labeled cells and the amount of time spent investigating and exploring pups was initially a surprising results. However, the results are in line with the other results, because just showing an interest in the presence of pups shows a general curiosity, but not a maternal interest in the pups. This also applies to the positive correlation seen between the latency of initial contact with pups. Although some animals initially responded faster to the pups, they were not displaying maternal behavior once reaching the pups. And in fact these animals displayed a response that seemed almost frantic or anxious.

The elevated plus maze test continued to confirm the hypothesis that mothers who respond more maternally i.e. less anxiously on the plus maze had more triple labeled cells. The more time the animal spent on the open arm, and the more times the animal moved onto the open arm nearest the pups the more c-fos activated mature neurons were found in the dentate gyrus. Several of the measure of maternal behavior on the elevated plus maze also correlated with measures of the traditional maternal behaviors in the observational test confirming that both tests were measuring levels of maternal behavior as seen in Table 2.

Another interesting relationship was seen between elevated plus maze behavior and the number of male pups in the litter. Results showed that animals with more male pups were less anxious on the elevated plus maze. They spent more time on the open arms of the maze, and moved onto the open arms more often. They also spent more time on the open arm closest to the pups (see Table 3). It is interesting that these factors influenced the animals' curiosity and feelings of anxiety, but showed no relationship with the display of maternal behaviors during the observational test.

The results of this study demonstrate the simply evaluating the quantity of cell proliferation as it relates to maternal behavior might not be enough to explain many of the individual variation in the displays of maternal behavior. Leuner, Mirescu, Noiman, and Gould (2007), Daunaudery et. al (2007), and Ruscio (2008) et. al highlight the importance of pregnancy and the presence of young on cell proliferation. The results of the current study add another component to these previous results. It is important to evaluate the quality of the new neurons developing as the female brain adapts for motherhood rather than strictly the quantity.

These results show that mothers, whose brains incorporate cells that are specifically activated in the presences of pups, are more responsive to young and display more maternal behaviors than those who do not. This is not to say these females were not adequate mothers. All of the females in this study lactated, groomed, and weaned their young successfully. However, there seems to be some breakdown of maternal responsiveness ranging from very good to good to average to bad to very bad mothers. It

is possible that some mothers are simply more motivated to care for their young and others simply are not as invested in motherhood.

Another theory is that the mother's inability to respond maternally to her pups is related to disruptions in the brains ability to regulate stress and anxiety in ways other successful mothers can. The hippocampus which is shown to be related to maternal behaviors has also been connected with mediators of depressive behaviors in female rats (Frye & Walf, 2004). If this is the case it is possible that the disruption of maternal behavior could relate to the baby blues and episodes of post partum depression. Future research will be needed to address this theory.

This study demonstrates that there are differences in the processes that are necessary for the formation of the maternal brain which results in changes in neurological phenomenon of mothers that fail to display typical maternal behaviors. Alternate studies are focusing on the differences in selective activity between mothers exposed to pups and those not exposed to pups. Future research could also include comparisons of primiparous animals with nulliparous controls both exposed to pups or novel objects. A third alternative would be to continue the secondary exposure to pups for an extended period of time. This option might allow for a more detailed looked into the different levels of maternal behavior.

Maternal behavior, arguably the most significant behavior for the propagation of the mammal, should not go unnoticed by health researchers. Depression is one of the most common perinatal complications (Gaynes et. al, 2005). Baby blues affect fifty percent of the women who give birth, and roughly ten percent of mothers experience post

partum depression (Brunton & Russell, 2008) however the neurobiological causality is still unclear (Zonana & Gorman, 2005).

Mothers suffering from depression are less responsive to their infants, experience more parenting stress, and actually view their infant more negatively than non depressed mothers (Forman et. al 2007). These damaging effects on the parent child relationship were found to be lasting even after the post-partum depression period passed. Traditional treatments for depression such as the use of tricyclic antidepressants and selective serotonin reuptake inhibitors might not be an option for breastfeeding mothers (Whitby & Smith, 2005; van Broekhoven & Verkes, 2002). A greater understand of maternal motivation and responsiveness would benefit health researches as they seek to alleviate these negative maternal experience for the both the mothers and infants.

Previous studies suggest that motherhood influences aspects of cognition, emotionality, neural plasticity, and neural health across the female lifespan. Finding that even one of these effects generalizes from rats to humans opens the door to the investigation of variables that may provide therapeutic benefits for existing neurobiological threats to the female brains. These threats could include but are not limited to depression, anxiety disorders, maternal-offspring interactions and neural degeneration. Although generalizations from rats to humans should be made with appropriate caution, the natural maternal rat serves as a valuable model for investigation. It is essential to generate competent biomedical models of the complex reproductive-related neuroendocrine and behavioral modifications in the female that may contribute to previously mentioned states and conditions.

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Table 1

Cells Triple Labeled with NUEN/BRDU/CFOS in the Dentate Gyrus Correlated with Factors of the Maternal Behavior Observational Test

	Triple Labeled Cells	
	r	*p – value*
Grouping Latency	-0.482	0.009
Retrieving Latency	-0.568	0.002
Exploring Latency	0.525	0.004
# of Pups Grouped	0.498	0.007
Seconds Spent Retrieving	0.487	0.008
Composite Maternal Behavior Score	0.646	0.001

Table 2

Elevated Plus Maze Test Factors Correlated with Maternal Behavior Observation Test Factors

	Correlations - *r*				
	OpenArmFront	OpenArmTotal	OpenArmFar	OpenArmClose	TimesonFront
RetrievingLat	-.595**	-.680**	-.261	-.751**	-.374*
RetrievingSec	.511**	.556**	.184	.685**	.340*
Grouped#	.586**	.651**	.315	.683**	.440*
GroupingLat	-.484**	-.582**	-.130	-.694**	-.500**

**. Correlation is significant at the 0.01 level (1-tailed).

*. Correlation is significant at the 0.05 level (1-tailed).

Table 3

Elevated Plus Maze Test Factors Correlated with Litter Size and Gender Ratio

		Litter Size (M=12.84)		# of Male Pups (M=6)	
	M	r	*p – value*	r	*p – value*
Total Time Open Arm	58.02	0.351	0.085	0.441	0.031
# Times on Front Open Arm	2.42	0.467	0.019	0.543	0.006
Total Time on Front Open Arm	35.09	0.437	0.029	0.500	0.013
First Half Front Open Arm	23.45	0.368	0.071	0.432	0.035
Second Half Front Open Arm	11.64	0.373	0.066	0.428	0.037

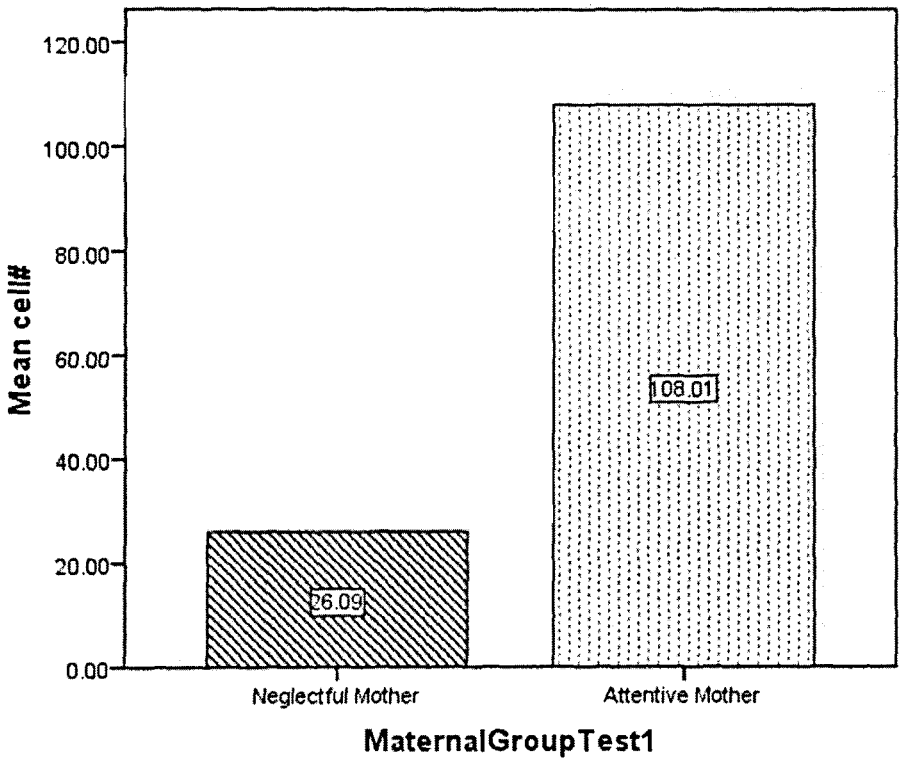

Figure 1. Mean number of mature neurons showing c-fos activation and BRDU incorporation in the dentate gyrus factored by Maternal Behavior Group. Difference between the groups is significant ($t_{23} = 5.3$, $N = 25$, $p < 0.001$).

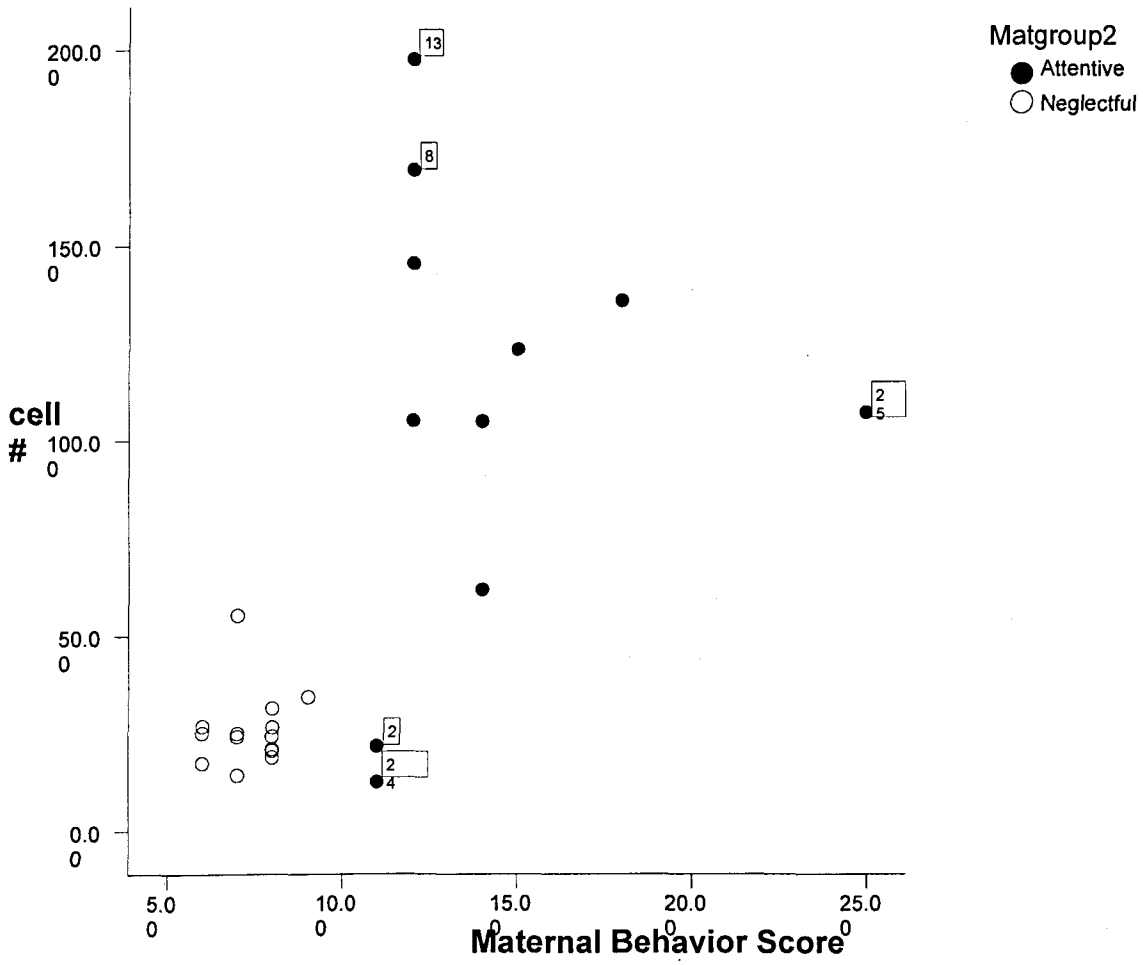

Figure 2. Correlation between triple labeled cells and the composite maternal behavior score. Although number 24 is listed as a responsive mother, this animal was infanticidal.

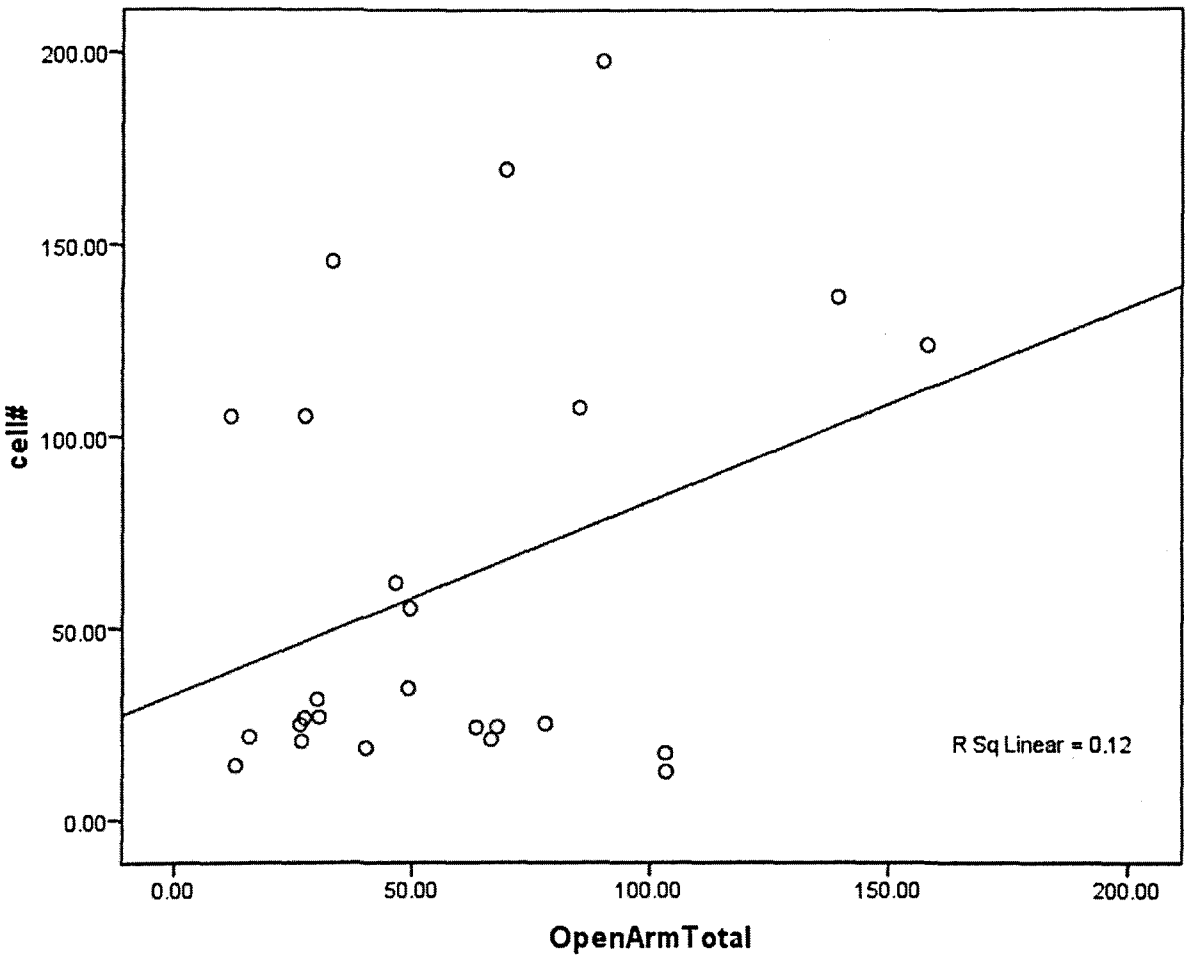

Figure 3. Number of mature neurons showing c-fos activation and BRDU incorporation in the dentate gyrus correlated with number of seconds spent on the open arms of the EPM maze.

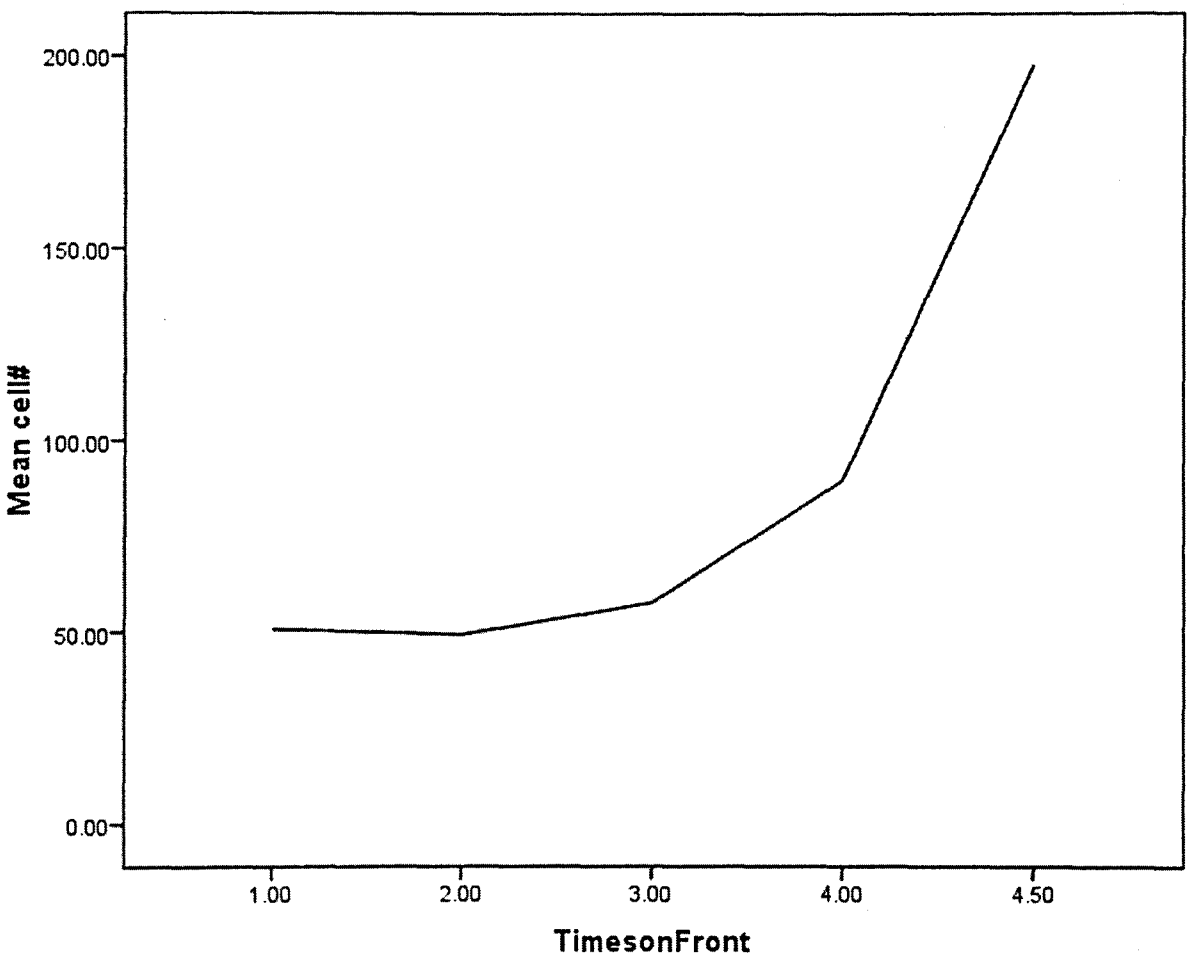

Figure 4. Mean number of mature neurons showing c-fos activation and BRDU incorporation in the dentate gyrus correlated with number of moves onto the front open arm.

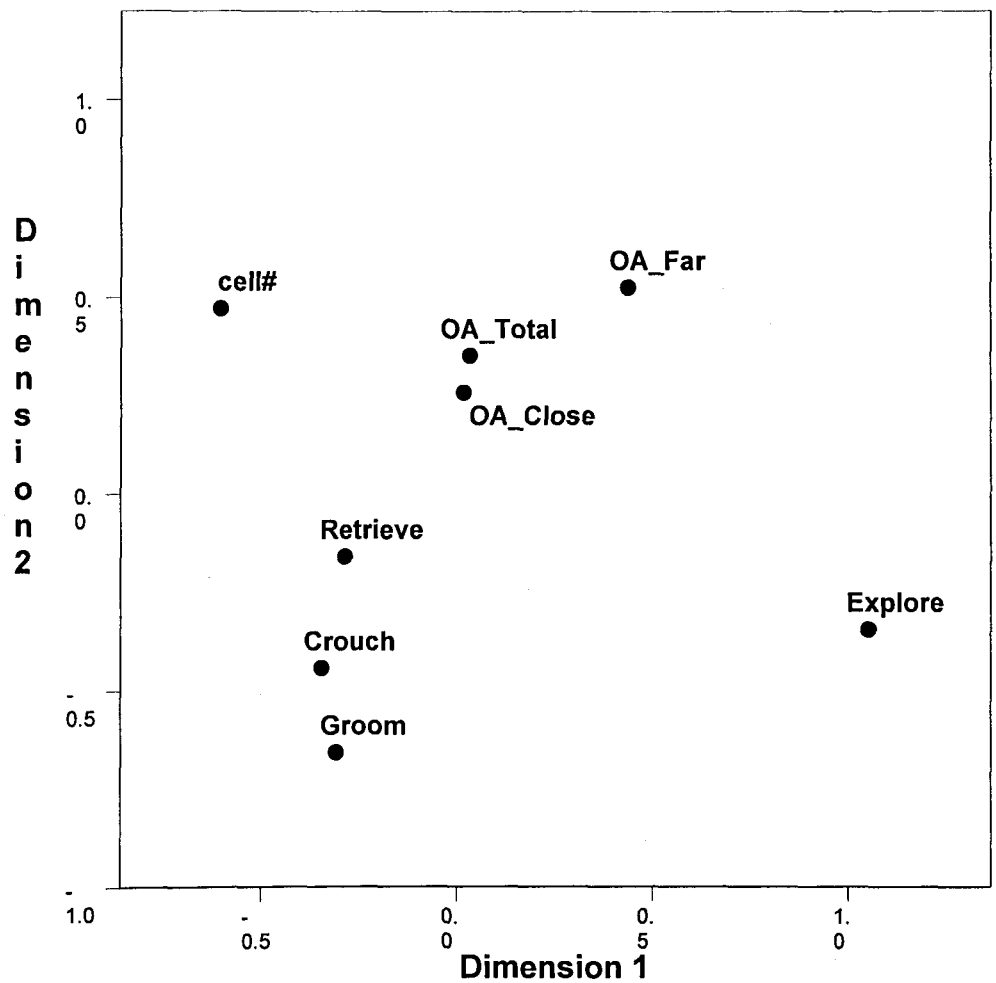

Figure 5. Multidimensional scaling (MDS) map of the relationships among maternal behavior (retrieving, grooming, and crouching), locations (OA_Total = time spent in the open amrs; OA_Far = time spent in the far portion of the maze; OA_Close = time spent in the close portion of the maze), time spent exploring the environment, and number or cells activated.

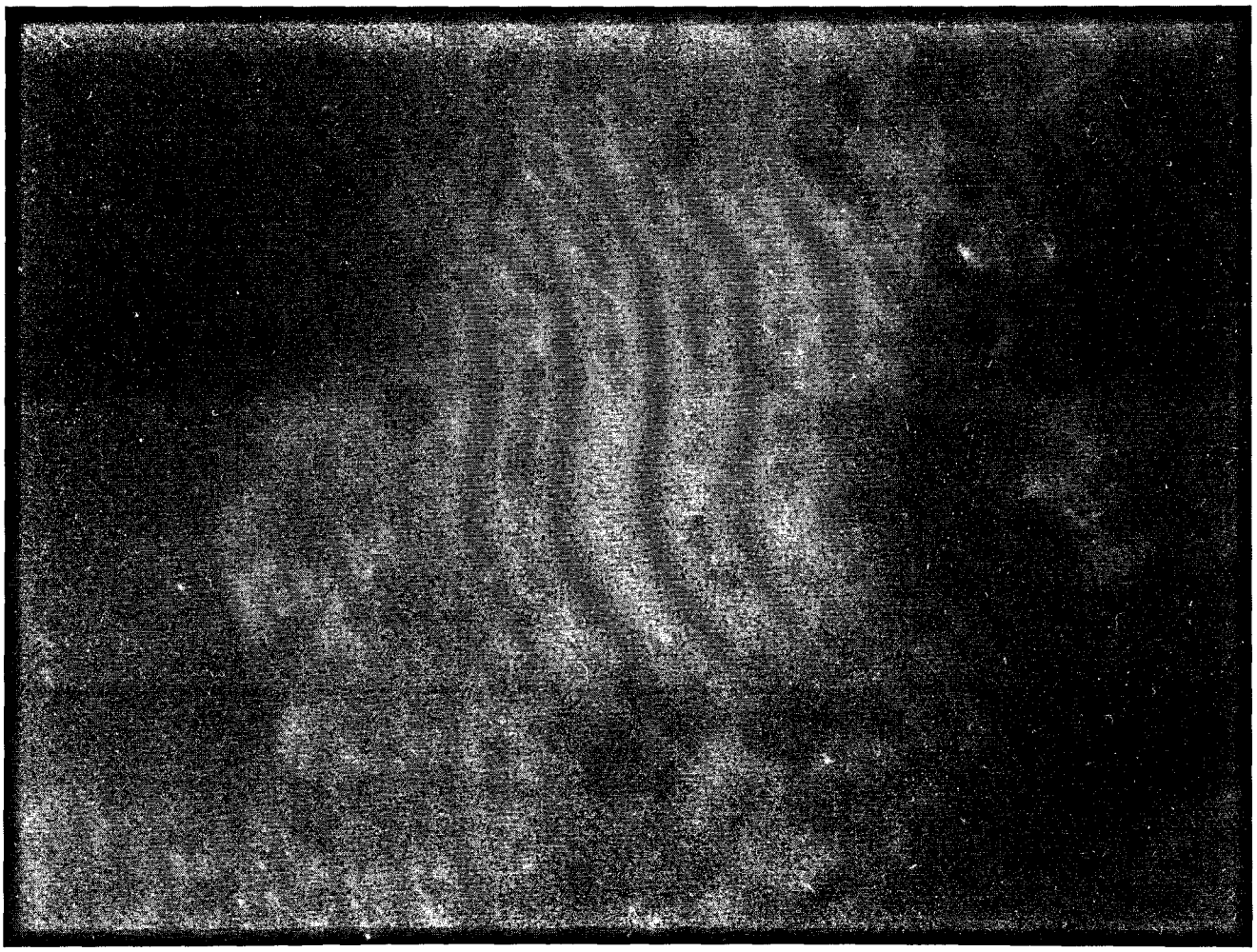

Figure 6. Triple labeled cells for BrdU, c-fos, and NeuN in the dentate gryrus of the attentive maternal rat brain at 40x.

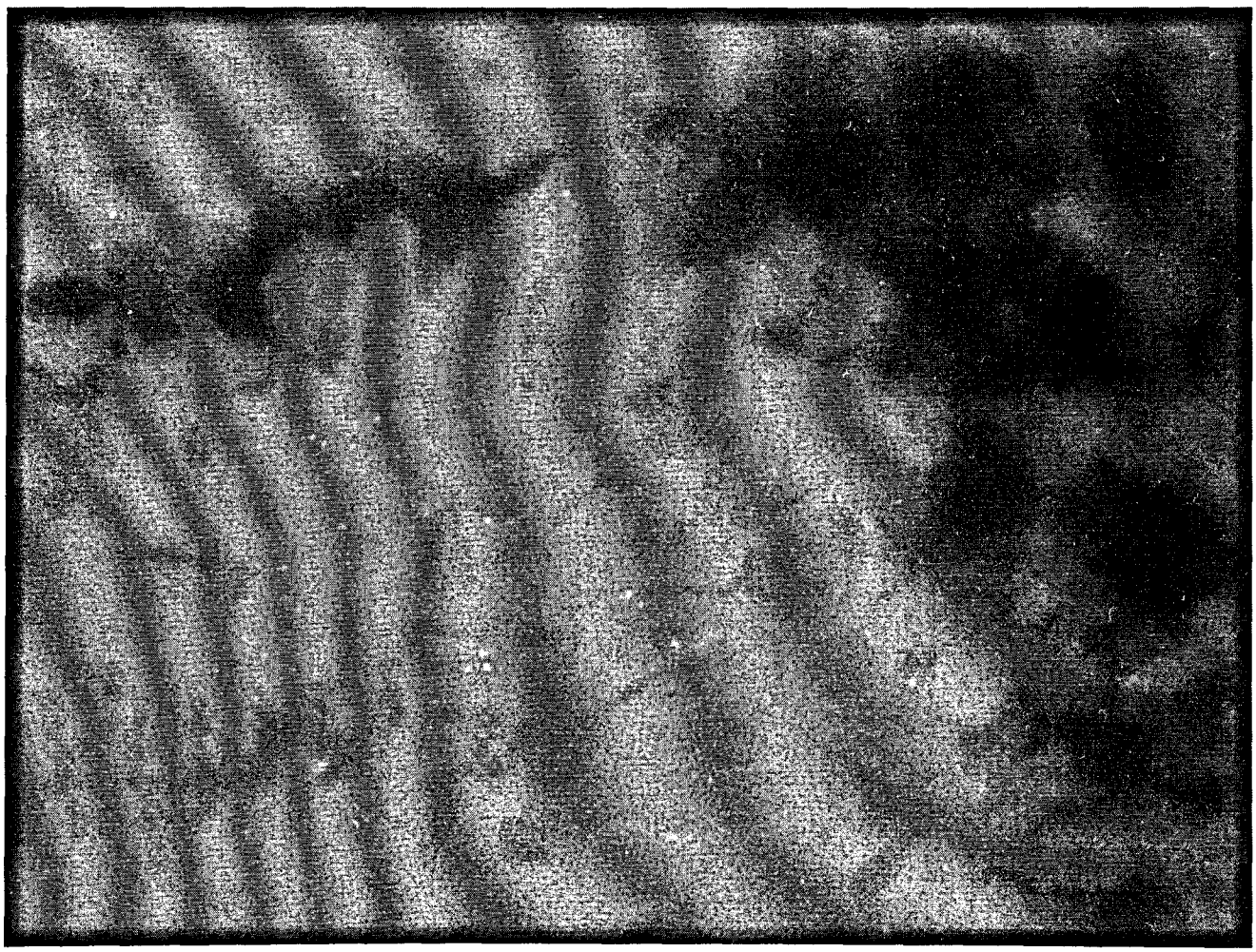

Figure 7. Triple labeled cells for BrdU, c-fos, and NeuN in the dentate gryrus of the attentive maternal rat brain at 63x.

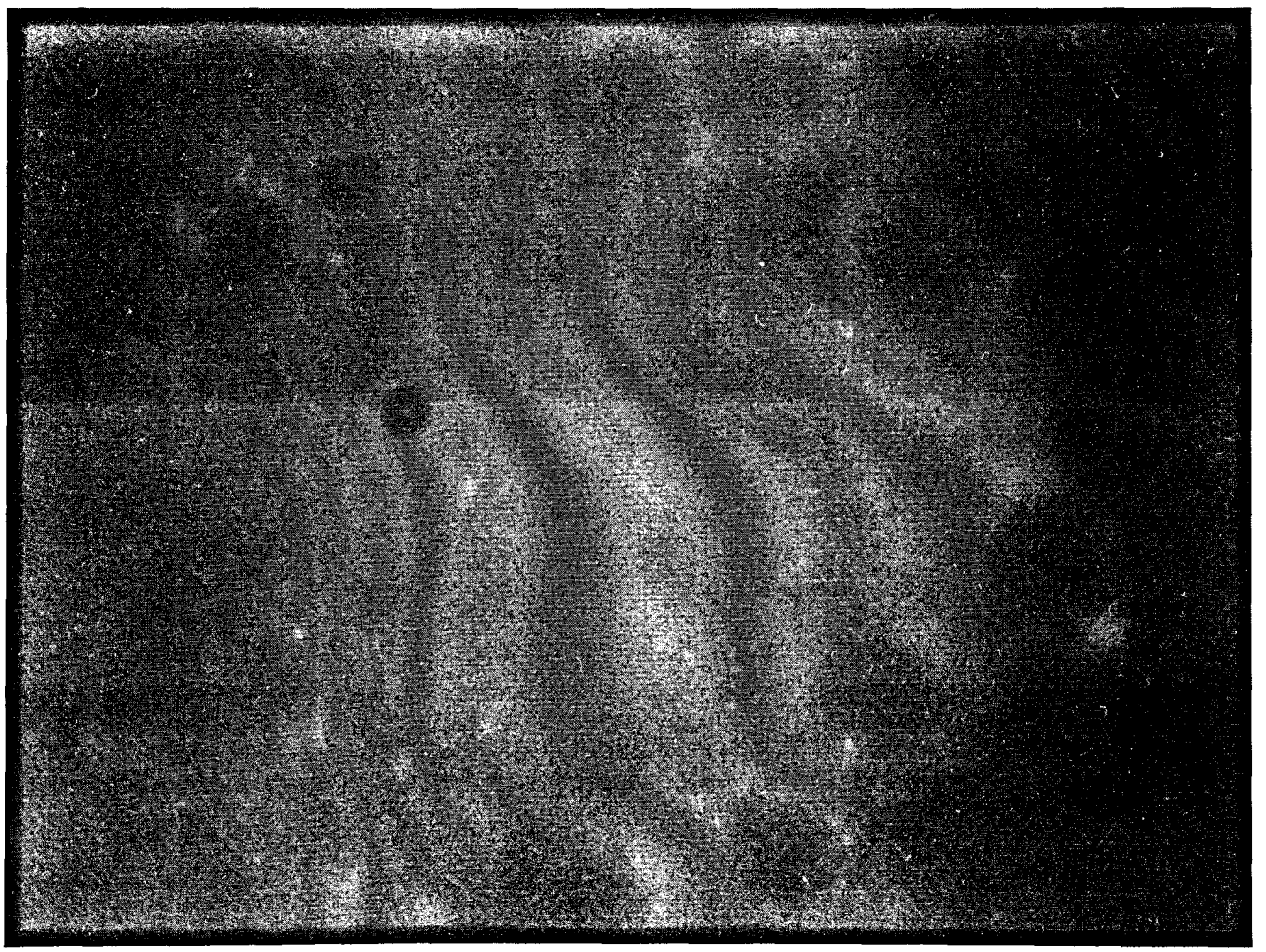

Figure 8. Triple labeled cells for BrdU, c-fos, and NeuN in the dentate gryrus of the attentive maternal rat brain at 100x.

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