Title

A Novel Model for the Exploration of Social Status in the Male Laboratory Rat: Isolation of Psychosocial Factors and Effects of Early Environment

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By

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Abstract

A Novel Model for the Exploration of Social Status in the Male Laboratory Rat: Isolation of Psychosocial Factors and Effects of Early Environment

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In humans virtually any measure of socioeconomic status (SES), i.e. income, education level, has an inverse association with both physical and mental health outcomes. Common theories to account for this observation involve scarcity of resources in lower SES populations and/or the impact of more challenging social environments, especially in early life, possibly resulting from resource inequality. It is precisely these variables (environment and resources) that confound our current understanding of the neural underpinnings associated with social status. These variables are impossible to control in human beings and, therefore, make us poor subjects from which to begin the study of social disparity.

Animal models of social hierarchy exist, but are not concerned with status as it relates to humans, as such, much of the information we have regarding the neural correlates of social status is derived from nonhuman adult organisms put in transient social environments where rank is determined by the outcomes of aggressive confrontation. Such research is primarily concerned with the effect of rank on the organism, not the underlying factors that may contribute to differences in rank generally and because of this environmental differences prior to these group formations are largely ignored.

We, therefore, developed a novel animal model of social status in which environmental experience was kept equal before group formation, and resource availability equal after. This approach allowed us to identify behavioral characteristics, brain areas, and physiological processes associated exclusively with the psychosocial experience of rank. We accomplished this by forming, to the best of our ability, social groups comprised of rats with identical characteristics (age, weight, sex, early experience) and provided ad libitum access to resources throughout our experiments. Intermittent bouts of competition for resources were used to characterize social rank and we performed oxytocin and vasopressin

receptor autoradiography on brain areas implicated in processing characteristics of social, emotional and stressful stimuli.

Our goal, in addition to controlling environmental experience and resource inequality, was to recreate the graded pattern of effects evident in human SES research to validate the animal model's use as an instrument with which we could explore status relationships and generalize the results to human populations.

Based on the success of this new model we decided to explore the impact that naturally occurring variations in rat maternal care would have on social rank formation. The first experiment in this dissertation was conducted to ensure that differences in maternal care would impact social behavior in the rat in a measurable manner. As this was the case, we conducted the final study included in this dissertation and determined that maternal care does predict eventual social rank, but only in a particular social context. Several findings from the initial study employing our novel animal model were replicated in this experiment, adding to its value as a useful tool with which to explore social status.

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Chapter 1

1. General Introduction

The aim of my research is to ultimately determine whether early environmental differences in maternal care contribute to adult social status and if so, which areas of the brain may reflect this relationship. My aim in this introduction, however, is to provide a background of the major tenets of this research. As such, I first provide a general account of the origin of maternal deprivation research and how in rodents this research has complimented stress research to gain an understanding of particular maternal behaviors responsible for "programming" offspring development. Second, I briefly summarize one line of research that quite elegantly shows how such a process may occur, specifically, how an experience can actually impact physiology in such a way that permanent biological and behavioral change results. Third, I introduce the concept of social status, a phenomenon that has an association with mortality, health and well-being across all westernized countries, and propose a method in which this phenomenon can be studied using techniques and ideas that stem from the previous two topics. Social status, or perhaps more commonly socioeconomic status (SES) in humans negatively correlates with poor health and mortality, but the underlying reasons for this association are difficult to untangle. Many researchers and theorists believe a lack of resources and/or more challenging environments, including early life environment, among lower SES populations are the cause. This supposition does not address that psychosocial factors arising from lack of resources or challenging environments contribute to SES/health disparities. Using information from research on early environment and biological "programming" as a framework for exploration and a means of overcoming confounds that currently hinder SES research I hope to contribute to the understanding of the psychosocial aspect of SES health effects. Lastly, I provide a brief overview of the known actions of the neurotransmitters oxytocin (OT) and arginine vasopressin (AVP) as they are implicated in several forms of social behavior across many different species and likely reflect neurobiological systems involved with the perception of social status.

1.1 Early Life Environments

It is widely recognized that environmental factors and experience early in life play a role in shaping development of both humans and non-humans. As early as the turn of the 20th century, developmental deficits had been documented in cases of institutionally raised infants who as adults displayed pathological behaviors (Bakwin 1942; Bowlby 1951; Spitz 1945). As it turns out, the dominant practice in hospitals of the time (1930s and 40s) was to minimize contact between patients and staff in order to reduce the possibility of spreading bacterial infections. Pediatrician, Harry Bakwin, noticed that pediatric patients failed to gain weight in his hospital despite a sufficiently caloric diet. Only after they were discharged and sent home did they begin to gain weight. Bakwin assumed the reason these children did not gain weight was due to "psychologic neglect" suffered as a result of the minimal-contact policy of the time. He therefore encouraged staff at the Bellevue hospital in New

York to begin "mothering" and playing with the pediatric patients. After this practice was instated the mortality rate of infants under 1 year of age dropped from 35 to 10 percent (van der Horst & van der Veer 2008). Similarly, John Bowlby observed that a lack of parental care experienced by English children whose parents felt it necessary to send them away during the bombings of WWII correlated with the children's delinquent and affectionless behavior as adults (Bowlby 1954). He hypothesized that the lack of parental influence during this time was the cause of their abnormal behavior.

These observations, and many others like them, convinced researchers that the relationship between infant and caregiver not only influences development, but more importantly, can and should be studied empirically. Some talented researchers have since developed creative methods with which to investigate the parent/offspring relationship (as well as other social relationships) and its effects on the development of offspring across many different species.

Harry Harlow, in an effort to produce extremely healthy rhesus monkeys for research purposes, removed them from their mother's care soon after they were born. This protocol was based on the idea of minimal contact that was the prominent practice in hospitals for human offspring at the time. Not surprisingly, he unintentionally created the same type of non-stimulating environment Harry Bakwin combated in his hospital. Harlow noticed abnormal behavior in his developing monkeys, attributed that abnormality to the perturbed maternal relationship he created and decided it was a valuable model for studying the antecedents of love and attachment and the paradigm known as maternal separation (MS) was born. MS simply requires an organism be denied access to its primary caretaker for an extended amount of time during the first few days or months (depending on the species) of life. This paradigm is now used as a tool to explore the effects differential early environmental experiences have on a number of different species of animal. These extended separations result in consistent behavioral phenotypes among mammals, a potential reason for which, and description of, I will address in more detail below.

Years before Harlow created the MS paradigm, a young man named Hans Selye was beginning his career as an endocrinologist searching for hormonal compounds which had not yet been discovered (Perdrizet 1997). In doing so, he noticed many of the substances he researched produced almost identical effects in his laboratory rats. After more observation he realized that often even his control animals, which received injections of saline or some other benign substance, showed effects similar to his experimental animals. Realizing that saline could not effect the physiology of his animals in the same way an experimental substance could and that it was extremely unlikely that the many different endocrine extracts he used in his experiments would produce the same effects, he reasoned that what he was seeing was the result of the process of giving the injections and/or the injections themselves. His idea led to the hypothesis that the body undergoes a generalized process when confronted with any nocuous substance or experience.

This generalized process is now referred to as the stress response. In fact, Selye coined the now familiar word "stress" the way it is commonly used today. According to his theory, simply handling and injecting his rats activated their stress response (he referred to it as the "general adaptation syndrome" at the time) and after prolonged activation, produced the plethora of physiological symptoms he found common to the animals in his experiments. A few of these symptoms such as ulcers, decreased size of immune organs and loss of muscle tone (Selye 1936) are now tell tale signs of being "stressed out". His theory and findings have been supported by evidence and advanced ever since. In fact, Selye's work was the foundation of stress research.

So how was Selye's work important for the study of the parent/offspring relationship? In short, it gave researchers an explanation with which to address a phenomenon that occurs frequently in animal research; that the brief physical manipulation of infant rats and mice somehow allows these animals to differentially cope with stressful stimuli as adults. Without Selye's work, the eventual explanation that the development of the hypothalamic pituitary adrenal (HPA) axis, which is responsible for stress reactivity, was being altered in these animals. Brief manipulations, such as are common in animal research, when they occur in early life sometimes result in a less fearful behavioral phenotype than typical members of the species. Without Selye's insight that the mere manipulation of animals affected HPA activity what has happened in this field since may never have come to pass. This phenomenon, now a common experimental paradigm, has been termed "handling" and results in a behavioral repertoire that stands in stark contrast to the effects of MS. For instance, briefly manipulating rodents in infancy (handling) results in a more exploratory and less fearful behavioral phenotype compared to animals that are not handled in infancy (Ader 1968; DeNelsky & Denenberg 1967b; Pfeifer et al 1976). As such, handling is another example of an experimental paradigm useful for exploring the effects of differential early environmental experiences across species. Moreover, in rodents, the procedure is almost identical to MS; the most salient difference being the amount of time the organism is deprived of its primary caretaker. The simplest form of "handling" is simply the removal of an organism from its mother/caretaker, placement in a separate location (usually a container in an adjacent room) and its return to the mother a few moments later (typically no more than 15 minutes). Like MS, the behavioral effects of handling offspring also last throughout the lifetime (DeNelsky & Denenberg 1967a; b; Meaney et al 1991; Todeschin et al 2009).

1.2 Biological Embedding of Early Experience

As described above, experiences in early life can shape future adult behavior and the physiological mechanisms responsible for that behavior. In humans, correlational evidence abounds which links early experience to adult behavior, but precisely how early experience leads to specific behavioral patterns is difficult to explain. With regard to the current topic of social status, early experience could act at several levels to influence ultimate behavioral patterns. Obviously, early experience could have a direct effect on developing systems responsible for

particular capabilities. For instance, human language learning must occur within a specific developmental time window or language will not be learned in a sufficient manner. This critical/sensitive period represents a mechanism through which early experience can directly affect language skills and if exposure to an environment that provides adequate verbal stimulation does not occur during this developmental time window the result is a language deficiency that persists throughout the lifetime. In a well known longitudinal study following Romanian children who spent more than eight months in an orphanage during the first few years of their life it was found that cognitive and behavioral deficiencies were present in these individuals even after several years of having lived in caring adoptive homes (Rutter 1998). The orphanages these children experienced were renowned for high levels of neglect and minimal stimulation. As these individuals experienced normative levels of care and stimulation after adoption their continued deficits suggest that sensitive periods exist for more complex behavioral and cognitive processes that may be reliant on adequate care and stimulation early in life; a lack thereof constitutes another example of how the early environment can directly affect future behavior. The early environment can affect behavior indirectly too by setting individuals on a developmental trajectory that will potentially limit (or perhaps expand) opportunities for further development. For example, a child that receives adequate verbal stimulation during their critical period for language development will most likely communicate successfully with others. Successful communication in turn may provide the opportunity to engage in other activities that further enrich the development of that child and ultimately places that individual in an environment it would not have had access to without proper language development. The feed forward nature of indirect affects such as these make it difficult to determine the extent to which any particular early experience itself is responsible for any particular behavior in adulthood.

However, evidence that the external world and the experiences we have impact the functionality of our physiology is widespread, but best exemplified by stress and immunology research. Without changing the molecular make up of DNA, epigenetic (non-genetic/environmental) factors can alter the rate at which certain genes are expressed and therefore, the proteins that comprise our bodies. For example, substances responsible for signaling the synthesis (or transcription) of proteins from DNA, known as transcription factors, are differentially expressed in individuals who come from different early life (SES) backgrounds. NF-κB, a transcription factor that regulates the synthesis of pro-inflammatory cytokines (immune cells that tell the body a foreign substance needs to be combated), is elevated in members of low early life SES, while glucocorticoid receptors (GR), a protein responsible for conveying the anti-inflammatory actions of cortisol, is down regulated (Miller et al 2009). Therefore, individuals from low early life SES environments have a hyperactive inflammatory response and a hypoactive anti-inflammatory response, which after several decades of life can exact a negative toll on overall health.

Similar results are seen with respect to other early life environments. One of the best-known epigenetic mechanisms is a process known as methylation, which is seen under conditions of varying maternal care in rats. For as yet unknown reasons, offspring raised by mothers who lick and groom (L/G) their pups very little have the promoter region of their GR gene hypermethylated, which functionally silences the expression of the GR. These animals show heightened reactivity to stress, are more fearful in novel contexts and display higher levels of stress hormones for a longer period of time than animals that are licked and groomed by their mothers more frequently. These differences in behavior and physiology last throughout the animals' lifetimes. A more in depth explanation of how this process occurs and the reasoning for using these techniques in the current research is given in the next section.

1.3 Handling, Maternal Separation and Maternal Behavior

Handling and MS allow researchers to study the impact that a caregivers absence has on an organism. Handling provides the opportunity to explore the effect brief separations have on offspring while MS provides information about much longer separations. These two paradigms each produce consistent neuroendocrine and behavioral phenotypes that persist throughout the lifetime. For instance, MS in monkeys (Seay & Harlow 1965; Young et al 1973) and rats produces animals that as adults display increased levels of fearful behavior and decreased levels of exploratory behavior in novel environments. Additionally, rats that undergo MS show heightened hypothalamic-pituitary-adrenal (HPA) reactivity as they consistently display elevated levels of the glucocorticoid, corticosterone (the primary rodent stress hormone), during stress and reduced glucocorticoid receptor (GR) binding levels in the hippocampus (Ader 1968; DeNelsky & Denenberg 1967a; Francis et al 2002a; Plotsky & Meaney 1993; van Heerden et al 2010). GR in the hippocampus plays an important role in regulating the stress response via negative feedback. By detecting circulating levels of corticosterone, GR signals the cessation of the stress response (Bradbury & Dallman 1989; Sapolsky et al 1991). Conversely, handled animals show virtually the opposite behavioral and neuroendocrine profiles. In novel environments handled animals are less fearful and show heightened levels of exploratory behavior. Rats that undergo handling also show attenuated HPA reactivity. This reduced reactivity is manifested as decreased release of corticosterone during stress and more GR binding in the hippocampus (Francis et al 2002a; Meaney & Aitken 1985; Meaney et al 1988). More GR in the hippocampus allows circulating corticosterone to be detected more effectively, thus shutting down the stress machinery quicker, making the entire system more efficient. Greater efficiency results in less exposure to stress hormones over the course of a lifetime, while chronic overexposure to these hormones is widely theorized to be a major contributor to the deleterious health effects of several physical and psychological disorders. There is also convincing evidence that handling in non-human primates results in a similar resiliency to challenges later in life (Parker et al 2006; Parker et al 2007). Taken together, these data suggest that while prolonged periods of separation produce offspring that have hyperactive stress responses and are behaviorally more fearful, shorter periods of separation

lead to offspring that have attenuated HPA responses and are behaviorally more exploratory.

So what is it about the absence of a caretaker in these paradigms that causes such pervasive and consistent results? Recent research with rats suggests these findings might best be explained by the behavior of the mother after a brief versus prolonged period of separation. Rodent maternal behavior consists of providing warmth through physical contact, nursing, nest building, pup retrieval and the licking and grooming (L/G) of pups. Maternal contact occurs primarily in "nest-bouts" (Caldji et al 1998; Liu et al 1997) that consist of the dam approaching the litter, gathering the pups underneath her and nursing the offspring in an arched-back posture. While nursing in this position, the dam will intermittently engage in L/G of the offspring. It is this L/G behavior that researchers noticed was different between mothers of handled and MS litters.

Following reintroduction of the litter in the handling paradigm dams were noted to engage in more L/G of their offspring (Lee & Williams 1974; 1975; Liu et al 1997). Replacement of the pups in the MS paradigm had no such effect. After careful observation it was found that in both conditions the dams spent an equal amount of time in contact with their pups, but the frequency of nest bouts was increased in handled litters and decreased in MS litters (though they were longer in duration). This led to more instances of L/G in handled offspring, as the arched-back posture during nursing is more prevalent at the beginning of a nest-bout. The ultimate reason nest-bouts and L/G increase after handling is unknown, but these behavioral differences in maternal care consistently delineate dams of handled litters from those of MS litters. Based on this knowledge researchers began to explore maternal care differences in the absence of either the handling or MS paradigms more rigorously.

Naturalistic observations of maternal behavior reveal that L/G behavior is normally distributed among rodent litters (Champagne et al 2003), meaning that some dams engage in very high levels of L/G, some in very low levels and the remainder fall in between, with the greatest number of dams falling in the center of the distribution. Thus, very high and very low L/G dams represent less common behavioral phenotypes among the species, but comparisons between these polarly opposed groups of offspring would lend more insight into the possibility that maternal care is driving the effects seen in the handling and MS paradigms. Thus, researchers began studying the behavioral and neuroendocrine effects of differential maternal care. The results are surprisingly cohesive. Offspring of high L/G mothers display phenotypes that closely resemble those of handled offspring, while offspring of low L/G mothers resemble MS animals. Specifically, low L/G offspring, like MS animals, display more fearful and less exploratory behavior in novel environments (Champagne et al 2008), have fewer GR in the hippocampus (Caldji et al 1998) and when stressed produce higher levels of corticosterone (Francis et al 1999b). A similar cohesiveness is seen with high L/G offspring and handled animals. In novel environments both groups show less fearful and more

exploratory behavior, produce less corticosterone under stress and have higher levels of GR in the hippocampus (Caldji et al 1998). Given the evidence, it seems likely that maternal L/G is indeed driving the effects garnered with handling and MS in rats. A theory, known as the maternal mediation hypothesis, has been proposed which states that differential maternal behavior is responsible for the variation in behavioral and physiological phenotypes of offspring.

Additional evidence supporting maternal care (specifically L/G in rats) as the driving force behind the effects of handling and MS comes from research that uses said paradigms along with various interventions to test whether alternative explanations may be more plausible. For example, stroking pups with a wet camel hair paintbrush (presumably mimicking L/G) during their separation from the dam prevents the effects of MS, whereas other tactile interventions including pinching, light stroking, limb movement and vestibular stimulation administered at the same intervals and durations fail to prevent these effects (Pauk et al 1986). This suggests that many forms of tactile stimulation, which do not resemble maternal L/G have no preventative power on the effects of MS. In a related study, pups reared in the complete absence of a dam (fed via gastric canula) were similarly stroked with a paintbrush either in the anogenital area only (to promote waste release) twice a day (two bouts of brushing every day not two individual brush strokes; likewise for the 8/day group) or over the entire body including the anogenital area 8 times per day. Results showed that rats brushed twice per day were more fearful as adults than those brushed 8 times/day and upon giving birth displayed low levels of licking and grooming towards their own litter, whereas rats brushed 8 times/day displayed species-typical L/G levels (Lovic & Fleming 2004). In order for the preventive effects to be realized, L/G must occur sufficiently often enough (at least 8 times/day) and/or in a naturalistic manner (entire body). This suggests that naturalistic L/G-like stimulation prevents the effects of MS despite a complete absence of the dam. In order to test whether pharmacologically induced perturbations of maternal behavior had an effect on handled offspring researchers administered chlordiazepoxide (an anxiolytic) or saline solution (to the control group) to dams while their litter was absent. Chlordiazepoxide treatment reduced dam nursing and grooming behavior, but left other maternal behaviors intact and did not reduce overall activity as compared to control dams. Offspring of control dams (in this case normal handled offspring) displayed less fearful behavior than treated dams, suggesting that the lack of L/G received by offspring of chlordiazepoxide treated dams prevented the usual decrease in fearful behavior associated with handling (D'Amato et al 1998). As mentioned earlier, elevated levels of GR in the hippocampus are seen in both handled and high L/G animals. A study looking at GR in the Hippocampus of 7 day old pups found that pups which had been stroked with a paintbrush every day prior to day 7 already displayed more GR than non-stroked animals (Jutapakdeegul et al 2003). Additionally, compared to rats, mice lick and groom their offspring very little. Therefore, if L/G is responsible for the anxiolytic effects seen in high L/G rat offspring then mice reared by primiparous lactating rat dams should display less fearful behavior than mice reared by non-maternal nulliparous rats or mice reared normally. In fact, researchers found exactly these

results even after controlling for differences between the milk of the two species. These rat-reared mice even displayed attenuated stress reactivity compared to their mice reared counterparts (Rosenberg et al 1970). It therefore appears to be extremely likely that L/G during infancy, rather than length of separation per se, has a substantial impact on the future behavior of a rat.

In addition to GR in the hippocampus, maternal L/G has been implicated in other neurological differences. Variations in the amount and location of many neurotransmitters, their receptors and levels of genetic transcription factors have been discovered in the central nervous system of adult rats that appear to be driven by differences in the amount of L/G received in the first few days after birth (Caldji et al 2003; Francis et al 1999a; Weaver et al 2004). These findings lend additional support to the notion that differential early environmental experience in the form of maternal care has an impact on other, as of yet, unexplored behaviors (including social behavior that may contribute to effects of social status) and their neurological antecedents.

1.4 Human Social Status

Social status is a potent determinant of health in humans. Those lowest in social class/status exhibit the highest incidences of mortality and the poorest levels of mental and physical health in a given population (Marmot & Rose 1978; Marmot & Shipley 1996; Marmot & Smith 1991). These findings might not be surprising given that those lowest in social status are often the most impoverished and vulnerable members of society. What may be surprising to some is the graded relationship between health and social status. Using socioeconomic status (SES) as a measure of social rank a graded, continuous association has been identified between rank and morbidity (Adler & Boyce 1994). Individuals at any given social rank will have better health outcomes relative to those lower in social rank and worse health outcomes when compared to those higher in social rank (Singh-Manoux et al 2004). This gradient with respect to social status and health suggests that one's relative place in society matters greatly.

So what is social status? Depending on the perspective from which you are asking the question it could refer to many things. Are you an executive or a manager; first string or second string; male or female; religious or secular? There is no limit to the number of factors that can be used to define and group people, nor the number of societies a person can belong to, or the characteristics that invoke status within them. The reality is that social status, however you define it, is an emergent process of life within a society.

With regard to the status/health phenomenon described above, social status is usually defined by the amount of money one makes in relation to others. The same health gradient, however, can be found with other (non-monetary) measures that stratify society. Education level of an individual or of an entire region, neighborhood crime rate, immigration status, type of employment, to name but a

few; all these means of measuring status reveal the same status/health gradient. Traditionally, income and education are the most widely utilized measures of social status. Theoretically though, social status is a concept that reflects an individual organism's ability to secure resources in relation to other members within that society. These resources do not necessarily have to be tangible. Psychological support, for example, is a resource of great importance, but determining who is more capable of attaining it is a problem unto itself. It is partly the complexity behind the meaning of the term 'social status' that makes it such a difficult and enjoyable thing to study. For instance, the perfect measure would necessarily generalize to all contexts (interpersonal, communal, national, global), include all types of resources (physical, psychological, etc.) and address the means with which to attain them. The impossibility of this task results in two things: First, the continued quest for more complete measures and second, the necessity to use common factors to study the status/health gradient (e.g. income/education). It is true this gradient exists with respect to all-cause-mortality when looking at most any factor that stratifies society in a hierarchical manner, but when addressing specific illnesses, disorders, or diseases the contribution of social status may be more or less of an impact than in another specific malady. Therefore, the manner in which social status is measured is informative and subtle variations in its operationalization can lead researchers to the impact specific status factors have on particular health outcomes and whether (or to what degree) those status factors actually contribute to a specific malady or the status/health gradient in general.

For instance, when using income as the lens through which we explore the status/health gradient two things become apparent, namely, that the absolute wealth of a population and the extent of income inequality within a population have different effects on health patterns. A country whose per capita income is extremely low will show health improvements over the entire population as per capita income increases. This improvement in population health tapers off though once a great enough level of wealth has been attained. At this point the distribution of wealth within the population determines the relative improvement or decrement in health associated with a change in social status (Kaplan et al 1996; Wilkinson 1997). In countries where the income of the richest and poorest individuals are very similar the difference in health between those two individuals is very small (but still present) while the greatest health disparities are found in countries where income inequality is greatest. Therefore, relatively speaking, it is better to be the poorest person in a poor country that has little income inequality than it is to be the poorest person in an affluent country with great income inequality. According to these findings, clearly the more money you have the better your health, but what accounts for the larger health disparity in populations that have a wider economic gap?

One explanation for such a phenomenon is that better health care can be afforded with greater income; however, countries such as Canada, England, Denmark and Sweden, which have forms of socialized health care and are relatively affluent still exhibit this health/status gradient (Thomsen et al 2005; Winett 1997). In fact, several studies exploring the relationship between status and health in

countries without socialized medicine have found that after controlling for availability and quality of health care, a health gradient still persists with regard to social status. Therefore, it seems the inequality of income rather than inequality of medical care due directly to unequal income presents a more intriguing challenge in explaining the status/health disparity. If differential health care isn't responsible for social status health effects due to income inequality then what is going on?

Perhaps individuals with the good fortune of having better health attain a higher status because of their superior physiology? Is it correct to assume that low SES hinders health or could poor health simply limit ones ability to ascend the strata of society? Given the correlational nature of the evidence behind the status/health gradient and the complexity of the factors that ultimately contribute to it, it is difficult to conclude with absolute certainty that this isn't the case. In fact, it seems likely that status would be affected by health to some extent. In the most extreme sense, having a serious disability would very likely require more resources to live a lifestyle commensurate with healthy members of society and limit the opportunities for upward mobility and increase the probability of downward mobility. If those with better health are constantly moving upward in society and those with poor health are moving down then it seems logical that a status/health gradient would emerge in society and even amplify the association between income inequality and poor health. This theory, known as the health-selection hypothesis, has existed in one form or another since the 19th century and while instances of poor health causing downward social mobility are a reality the extent of this effect is negligible.

The opposing theory is referred to as the social causation hypothesis. Social causation refers to social status as the driving force behind health disparities and support for this theory over the health-selection hypothesis abounds. For example, the movement of individuals from higher to lower status does not confer a mortality rate of the lower status achieved, nor does upward mobility decrease rates of mortality to a similar level. In fact, those individuals do not differ markedly from members of their original status in terms of mortality rate (Bartley & Plewis 1997; Boyle et al 2009; Chandola et al 2003). In addition, it has been found that when rates of health related movement within the work force are compared to mortality rates of those same individuals after retirement that mortality rates applicable to the most lengthy position held are realized (Chandola et al 2003; Fox et al 1985).

Several unhealthy behavioral habits contribute to the status/health gradient as well. For instance, substance (tobacco, alcohol, etc.) abuse, a high-fat diet and inactive lifestyle are correlated with social status, as are several other behaviors that directly impact long-term health, however, after statistically controlling for these known covariates a strong negative status/health relationship still exists (Krieger et al 1997).

1.5 Subjective social status and psychosocial impact

What this tells us is in addition to behaviors directly linked to our physical socioeconomic condition there is undoubtedly another consequence of status that

has an impact on health and longevity. It is common knowledge that our perception of the world around us influences our physiological responses to myriad stimuli. As a highly social species, we must inexorably determine for ourselves how to best navigate the complex milieu that is society. We begin acquiring this information as soon as we are able to interact with our social environment. In doing so, we discover in countless situations and under ever changing circumstances how we measure up to others. As a result, we have at any given time in our lives a perception, whether accurate or not, of our relative value within society. This perception influences how we react to social situations throughout our lives and produces as a trait characteristic the way in which we feel about ourselves in relation to the rest of society generally, as well as a state characteristic that is manifest in specific acute social circumstances. At the trait level, for example, despair or low self worth may emerge amongst members of low social classes. This trait could easily be caused by the constant necessity to compare their social reality against that of the majority of society, which in terms of simple material possessions seem to be better off than they. Low self worth is seen in individuals suffering from major depressive disorder. Suffering from depression has been shown to correlate with increased risk of mortality. Therefore, the perception of being a less prosperous member of society can potentially put an individual at risk of developing psychological abnormalities that shorten the lifespan (Matthews et al 2000; Modin et al 2010). Trait characteristics, once learned, are difficult to amend. State characteristics determine the manner in which an individual responds to acute social challenges and can vary greatly depending on the social environment. These may play a protective role in buffering against the negative effects of trait characteristics. A common, but salient example would be the difference between how one feels in the company of good friends versus work colleagues.

I'd like to introduce the concept of psychosocial factors as the state and trait characteristics that exist in social species. The point being that psychosocial factors associated with social status most likely contribute to the decrement in health seen with lower status. Evidence for this possibility can be inferred from recent studies showing that subjective social status (SSS) is as potent a predictor of health related outcomes as SES (Adler et al 2008; Gianaros et al 2007; Ostrove et al 2000). SSS is simply measured by placing a mark on a symbolic ladder signifying where you feel you lie in status compared to the rest of society (Adler et al 2000). This measure arguably reflects psychosocial factors that objective measures of SES cannot account for. This insight was the impetus for chapter 3 of this dissertation. Psychosocial factors attributable to social status have not been, and probably cannot be, studied in isolation from resource inequality or early-life environmental differences in humans. Using an animal model of social hierarchy in which resources are equal and early life environments are the same may expose behavioral, physiological and neurological correlates that can inform human research.

Finally, mounting evidence suggests that early life social status may have as much, or more, of an effect on later health as current SES (Campbell et al 2008; Chen & Matthews 2001; Cohen et al 2004; Cohen et al 2010). Measures of childhood SES

include the participant's subjective retrospective estimate (on a hypothetical ladder) or their reporting of certain objective variables during childhood. Some common objective measures include mother's level of education, father's occupation or simply whether a participant's parents owned their own home. Most studies report an association between health and childhood SES that is as strong or stronger than the association between adult SES and health (Galobardes et al 2008). Given the importance of the impact of early life environment on future behavior we decided to take advantage of the naturally occurring differences in L/G of rat offspring to determine if maternal care would be predictive of adult social rank. This experiment is presented in chapter 4 of my dissertation.

1.6 Oxytocin and Vasopressin

Arginine vasopressin (AVP) and oxytocin (OT) are two closely related and highly conserved neuropeptides involved in the social behavior of several species of animals and their activity, both direct and indirect, is widespread throughout the central nervous system (CNS) REF (McEwen 2004) for review. Both AVP and OT are synthesized in neurons of the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus as well as the amygdala. OT and AVPergic projections from the PVN innervate the hypophyseal portal system where OT and AVP are directly released into peripheral circulation as hormones or where their action causes the release of neurotrophins from the anterior pituitary, which cause the release of yet more hormones from endocrine structures throughout the body. The PVN is primarily responsible for activating the stress response. Alongside corticotrophin releasing hormone (CRH), OT and AVPergic cells of the PVN are responsible for the release of adrenocorticotropic hormone (ACTH), a neurotrophin, from the anterior pituitary that signals the release and manufacture of glucocorticoids (the major "stress" hormones) from the adrenal cortex. These glucocorticoids allow the mobilization and use of stored glucose for the purpose of physiologically responding to a perceived stressor. Similarly, additional OT and AVPergic neurons of the PVN innervate brainstem and spinal cord nuclei to activate the sympathetic branch of the autonomic nervous system via the release of catecholamines from the adrenal medulla and postganglionic cells that innervate the viscera. OT and AVP neurons from the PVN also innervate several areas of the limbic system (McEwen 2004), a system integral for processing and detecting emotional stimuli. Several, if not all, brain areas involved in determining whether an encountered stimulus is stressful and/or emotionally valenced innervate the same OT and AVPergic PVN neurons responsible for the actions described above. Basically, information from virtually every sensory system is combined and processed by several brain areas in order to determine an appropriate response to any and all external stimuli.

Within the CNS AVP is additionally synthesized in the suprachiasmatic nucleus of the hypothalamus, locus coeruleus (the primary source of serotonergic neurons in the brain), bed nucleus of the stria terminalis (BNST), the septal area, and the diagonal band of Broca (DBB). These neuropeptides act within the CNS differently from classical neurotransmitter systems. Synaptic activity of OT and AVP

is thought to be minimal with most communication occurring in a paracrine fashion over relatively vast distances (compared to neurotransmitters) via extracellular fluid. While specific actions of centrally released OT and AVP (and many other NTs) are largely unknown in most brain areas the use of inverse microdyalisis studies have become the gold standard in addressing this problem and new information is becoming available rapidly.

1.6.1 Social Effects of AVP and OT

As the first hormone ever synthesized, in 1953, the peripheral actions of OT are well known. It is most well known for its role in uterine contractions during parturition and milk ejection during lactation in mammals, including humans. Other well-known actions of OT include the regulation of several types of social behavior in laboratory animals (discussed in the following chapters) as well as more complex social behaviors in humans. For example, human males administered intranasal OT are more effective at inferring the mental state of another individual based on photographs of the face (Domes et al 2007b). This effect coincides with an increase in the amount of time subjects spend looking at the eye region of facial stimuli (Guastella et al 2008). Intranasal OT also increases trust (Kosfeld et al 2005), causes faces paired with shock to be interpreted as 'sympathetic' rather than aversive (Petrovic et al 2008), and during an economic decision making task fails to prevent participants from investing further in an individual who has previously returned less than the invested amount, even though the investor knows the investee was capable of doing so (Baumgartner et al 2008). These findings support the role of OT as an important mediator/moderator of social behavior in humans; the effect of which is not necessarily adaptive as is the case in the latter two findings. That OT activity in the CNS has a prosocial influence, though, is the more relevant finding in terms of linking the data from human and non-human research.

Intranasal administration of AVP has similarly yielded some very interesting sexually dimorphic effects on human social behavior. In males, AVP administration results in more agonistic facial expressions toward images of unfamiliar male faces and decreases their subjective rating of "friendliness" of those images. Interestingly, when similarly administered AVP, women respond to unfamiliar female faces with more affiliative facial expressions and rate those faces as more friendly (Thompson et al 2006).

More specific social behaviors, such as recognition and attachment, have been linked to the actions of AVP and OT in many rodent and non-human primate species. For example, prairie voles, a monogamous specie of vole, form pair bonds after mating with a conspecific, but do not form pair bonds after mating when treated with either an AVP (males) or OT (females) antagonist. However, in the absence of mating they will form pair bonds when treated with OT or AVP (Williams et al 1994; Winslow et al 1993). OT is thought to act on the nucleus accumbens in female prairie voles upon mating to drive pair bond formation through enhanced reward value, while AVP is proposed to act on the lateral septum of male prairie voles (Fergusson et al., 2002), perhaps through increased emotional salience of the

partner. Also, OT knock out (OTKO) mice, which lack the gene responsible for the production of OT are socially amnesic. However, after administration of OT these mice recover social memory as evidenced by a decrease in exploratory behavior towards a previously encountered conspecific. OT has been suggested to act in the medial amygdala to drive this behavior. Additionally, AVP receptor knock-out (AVP1αRKO) mice that lack the gene required to produce the AVP receptor subtype 1α also lack social recognition memory. Again, expression of this receptor, specifically in the lateral septum (LS), via viral vector overcomes the lack of social recognition. Although AVP has been shown to be involved in some aspects of conditioning, such as habituation and extinction, the AVP1αRKO mice show no deficit in non-social memory such as object recognition.

For these reasons, it was decided that a likely system to explore with regard to social status included the neurotransmitters OT and AVP.

Chapter 2

Effects of Maternal Separation on Rat Social Behavior

2.1 Introduction

Early life experiences play a profound role in the development of mammals. In the rodent, early experiences such as postnatal infant handling result in long lasting and stable behavioral and endocrinological differences of adult offspring. Prolonged maternal separation (MS) in rats produces offspring with a heightened hypothalamic-pituitary-adrenal (HPA) system, as well as increased anxiety- and depressive-like behaviors, which persist throughout the lifespan (Huot et al 2001; Plotsky & Meaney 1993). Daily handling of rat pups (for a brief 15 minute period) over the first two weeks of life produces offspring with attenuated HPA activity in response to stress. These offspring also display less anxiety-like behaviors compared to controls. (Levine 1957; Levine et al 1956; Meaney et al 1988). These relatively simple (and seemingly brief) experiences during the postnatal developmental period result in long-term alterations of phenotype and underscore the critical role of environmental influences on the developing organism.

Much is known about the effects of MS on behavior and the developing HPA axis in mice and rats (Kaffman & Meaney 2007; Liu et al 1997; Meaney et al 1985). In several other species evidence exists that MS effects behavioral measures of fear and anxiety as well (Spencer-Booth & Hinde 1971; Suomi et al 1975). However, significantly less is known about how social behavior may be affected by varying environmental conditions such as MS. In the present study a prolonged MS paradigm (3hr/day; postnatal days 1-14) was used to perturb the early postnatal period in the rat. Again, little is known about how this postnatal manipulation may alter the regulation and expression of social behaviors in the developing rat and while debate exists regarding the mechanism of action surrounding the MS model, in rats it has been employed successfully for decades as an effective tool to explore the potent effects early developmental conditions exert on the growing organism.

Most studies to date employing the MS paradigm assess the outcome of adult animals. Little attention has been focused on exploring how the physiology and behavior of the developing adolescent animal may be affected. Adolescence is a period in humans characterized by increases in social interactions (Iervolino et al 2002), especially towards peers (Larson et al 1996). Rats display a similar behavioral phenotype as social interactions and play behavior peak during adolescence and are directed disproportionately toward same age conspecifics (Pellis & Pellis 1990; Primus & Kellogg 1989). It is theorized that increased peer relations serve as a tool by which organisms acquire the skills needed to become independent and survive in a social group (Harris 1995; Smith 1982). Behavioral

similarities between our two species suggest we share common biological mechanisms that underlie social behavioral development and support the use of rats as a means to explore this possibility.

Oxytocin (OT) and arginine vasopressin (AVP) are neuropeptides highly implicated in the regulation and expression of a variety of social behaviors including social memory (Dantzer et al 1987) (Benelli et al 1995), parental care (Bamshad et al 1993; Fahrbach et al 1985; Keverne & Kendrick 1992), territoriality (Ebner et al 2000; Ferris et al 1984), pair-bonding (Williams et al 1994; Winslow et al 1993) and trust (Baumgartner et al 2008; Kosfeld et al 2005) to name a few. In the rat, qualitative differences in early life experiences (as measured by maternal care) are associated with differences in oxytocin receptor (OTR) and vasopressin V1a receptor levels in brain regions of adult offspring (Francis et al 2000; Francis et al 2002b). Developmental regulation or programming of these receptors might be expected to emerge earlier during the life course, in particular, during the adolescent period when social interactions are heightened.

We, therefore, designed the present study to investigate the effects of MS on adolescent social behavior, and putative neurobiological mediators of social behavior in the laboratory rat.

A social interaction task was used to measure social behaviors between unfamiliar adolescent offspring of the same condition (either MS or animal facility reared (AFR)). Offspring were tested again during adulthood to assess whether variations during adolescence persisted into adulthood. While much research involving social interaction tasks like the one used here focus on aggressive or play behavior, others have assessed approach and withdrawal behaviors that are considered either social or non social (Winslow & Camacho 1995; Winslow et al 1993). The present study uses the latter categorization to measure relative social investigation (affiliative) and non-social exploratory (avoidant) behavior.

This study was planned to be a first pass at the effects of early environment on social behavior and, as such, female offspring were excluded in order to bypass the cyclical effects of hormone variation on the data. OT and AVP receptors were quantified at both adolescence and adulthood.

2.2 Methods

Animals

Long-Evans rats purchased from Charles River Laboratories (St. Constant, Québec, Canada) were mated and the resulting male offspring randomly assigned to one of two conditions: MS or AFR. Parent animals were housed individually for one week after delivery to habituate them to the new environment. Animals were bred housing a single male and female together until the male was observed to ejaculate twice. At this point males were returned to their home cage and females housed

individually. Breeding occurred over the span of 4 consecutive days at which time all females had undergone estrous and successfully mated. All animals had unlimited access to food and water while housed in clear plastic cages lined with bedding material under a 12 hour light-dark cycle (lights on at 0700 h) at a constant temperature of 21 ± 2°C and humidity of 55 ± 5%. Clean cages were provided twice weekly during gestation then cage changes varied by condition after birth. After weaning all animals received cage changes twice per week. Male offspring (N=63) were weaned and weighed at postnatal day (PND) 21 and non-littermates were housed in groups of three for the remainder of the experiment. The Office of Laboratory Animal Care at the University of California, Berkeley, approved this study.

Maternal Separation and Animal Facility Reared Conditions

MS

On PND 1-14 dams were removed from the home cage while pups were removed and placed together in a separate, but identical, cage. Once pups were out of the home cage mothers were replaced. Pups remained separated from the mother for 3 hours in a separate, adjacent room then returned to the home cage with the mother. Separations were conducted during the middle of the light phase. Clean cages were provided only at PND 10 in accordance with animal facility guidelines. Beyond PND 14 and until weaning all litters received typical AFR care.

AFR

These litters underwent standard facility maintenance (cages changed twice weekly) from birth until weaning. Mothers and pups were not separated for any appreciable amount of time during these changes.

Social Interactions

At PND 35, representative of rat adolescence (Witt 1994) , and again during adulthood (PND 105-110) a social interaction task was conducted to assess social behavioral phenotypes of offspring. All animals were weighed one day prior to testing. All testing was recorded for later scoring and conducted during the light portion of the day in a dimly lit room. Equipment was cleaned after each trial to eliminate potential olfactory influences on subsequent trials.

Procedure

Two weight-matched, non-littermate animals from the same condition were simultaneously placed on opposite sides of a large transparent square plastic cage (90cm x 90cm) for 10 minutes. A two-minute delay allowed the animals to habituate to the novel-testing arena; thus, behaviors were scored for each animal individually at the beginning of the third minute, leaving 8 minutes (480 seconds) of test data for analysis per animal. Three sets of behaviors were scored. First, 'social interaction', consisted of approaching, following, sniffing, licking or touching the other animal. Second was 'non-social exploration' which consisted of withdrawal of the animal being scored from the other animal as well as moving throughout the cage without

noticeably attending to or contacting the other animal. And finally, 'self-grooming', which consisted of periods of either inactivity or stationary self-grooming was recorded. These behaviors were hand scored by an individual blind to the condition of the animals under observation.

Receptor Autoradiography

Adult Animals were sacrificed at PND 105-110 upon completion of adult behavioral testing. Adolescent animals (n = 10; 5 MS, 5 AFR) were sacrificed upon completion of behavioral testing. Following decapitation, brains were quickly removed, placed on powdered dry ice, then stored at −80°C until needed. Brains were then sliced in 20µm coronal sections containing the nucleus accumbens, lateral septum, and amygdala. Sections were mounted onto Permafrost slides and stored at −80°C until the time of assay. For OTR binding, [^{125}I]-ornithine vasotocin analogue [(^{125}I)OVTA] was employed [vasotocin, d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Orn^8, (^{125}I)Tyr^9-NH_2]; 2200 Ci/mmol]; (NEN Nuclear, Boston, MA, USA). For V1a receptor binding, ^{125}I-lin-vasopressin [^{125}I-phenylacetyl-D-Tyr(ME)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH_2]; (NEN Nuclear) was used. Sections were allowed to thaw to room temperature (RT) and then immersed in 0.1% paraformaldehyde for 2 min to optimize tissue integrity. Sections were then rinsed two times in 50 mM Tris-HCl (pH 7.4) at RT for 10 min and incubated for 60 min at RT in a solution of 50 mM Tris-HCl (pH 7.4) with 10 mM $MgCl_2$, 0.1% bovine serum albumin, 0.05% bacitracin, and 50 pM ^{125}I-lin-vasopressin or 50 pM ^{125}I-OVTA. Non-specific binding was determined by incubating adjacent sections with the radioactive specific ligand as well as 50 µM of unlabelled Thr4, Gly7 oxytocin, a selective oxytocin ligand (Peninsula Laboratories, Belmont, CA, USA) or 50 µM of unlabelled [1-(β-mercapto-β,β-cyclo-pentamethylene propionic acid),2-(O-methyl)-tyrosine]-arg^8-vasopressin, selective for the V1a receptor. Following incubation, sections were washed 3 × 5 min in 50 mM Tris-HCl (pH 7.4) with 10 mM $MgCl_2$ at 4°C, followed by a final rinse in this same buffer for 30 min on ice. Slides were then quickly dipped in cold dH_2O and rapidly dried with a stream of cold air.

Sections were apposed to Kodak BioMaxMR film (Kodak, Rochester, NY, USA) with ^{125}I microscale standards for 72 h. Autoradiographic ^{125}I-receptor binding was quantified from film using MCID image analysis software (Linton, Cambridge, England). An average of nine sections per animal per area were scored using the Paxinos and Watson (2004) rat atlas as a reference.

Statistical Analyses

Behavior and Autoradiography

All between group comparisons were conducted using one-way analysis of variance (ANOVA). Within group comparisons (age effects) were conducted using paired samples t-tests. All data was analyzed using version 17 of SPSS (Armonk, NY, USA).

2.3 Results

Social Interaction

MS rats engage in more social interaction ($F(1,61) = 19.299$, $p < .001$) and less non-social exploratory behavior ($F(1,61) = 18.769$, $p < .001$) as adolescents than do facility raised animals (Fig. 1). However, as adults, MS animals are indistinguishable from the AFR group in both social behavior ($F(1,43) = .014$, p = ns), and non-social exploratory behavior ($F(1,43) = .153$, p = ns, Fig. 1). While the amount of time spent engaged in social behavior declines significantly with age in both groups, this decline in MS rats ($t(22) = 5.11$, $p < .001$) is more pronounced than the AFR group ($t(21) = 2.63$, p =.016). Additionally, the duration of non social exploration increases significantly with age in both groups, again with more extreme increases shown in MS animals ($t(22) = -6.58$, $p < .001$) compared to AFR animals ($t(21) = -3.12$, $p = .005$).

Receptor Autoradiography

No differences were found between MS and AFR conditions in the amount of OTR binding in any brain area measured during adolescence or adulthood. However, comparisons between age groups revealed significant increases in OTR binding with age in the amygdala under both conditions (MS: $F(1,8) = 9.76$, $p = .014$, AFR: $F(1,8) = 10.88$, $p = .011$) and in the lateral septum for the AFR group ($F(1,8) = 15.21$, $p = .005$). Significant decreases were seen with age in OTR binding for both groups in the nucleus accumbens (MS: $F(1,7) = 32.24$, $p = .001$, AFR: $F(1,7) = 32.36$, $p = .001$).

Differences in V1aR binding were found between MS and AFR animals during adolescence in the lateral septum ($F(1,8) = 9.75$, $p = .014$) and amygdala ($F(1,8) = 9.05$, $p = .017$). In each area the MS group displays significantly higher levels of binding than AFR animals (Fig. 3). In adulthood no differences between groups were found (Fig. 2). A common significant decrease in V1aR binding was seen with age in each group across all brain areas except the lateral septum of AFR animals. (MS: Nacc $F(1,8) = 18.17$, $p = .003$; LS $F(1,8) = 8.35$, $p = .020$; Amyg $F(1,8) = 37.90$, $p < .001$; AFR: Nacc $F(1,8) = 70.90$, $p < .001$; Amyg $F(1,8) = 88.03$, $p < .001$).

2.4 Discussion

During adolescence MS offspring are significantly more social and less non-social than AFR offspring. This behavioral difference disappears by adulthood suggesting that increased social interaction during adolescence, possibly driven by the actions of V1a receptors in the amygdala and lateral septum, in the MS group has a compensatory effect that eventually renders them indistinguishable from AFR animals.

Elevated levels of V1a receptor binding are evident in the lateral septum of adolescent MS animals compared to AFR animals. The concomitant increased level of social investigation in MS rats is in line with much evidence supporting the notion that V1a receptors in the lateral septum of rats are necessary for species typical recognition of conspecifics. A species of rat deficient in AVP, the Brattleboro, shows

distinct deficits in social recognition (Engelmann & Landgraf 1994), while normal adult male Wistar rats given an AVP antagonist after exposure to a novel juvenile spend more time investigating the same juvenile after 30 minutes than control rats given saline injections (Dantzer et al 1987). The lack of a decrease in investigation time indicates impaired recognition of the previously encountered animal. Similarly, transgenic mice lacking V1a receptors are extremely impaired at social recognition, however, inserting V1a receptors into the lateral septum of these animals, but not other brain areas, via viral vector restores social recognition (Bielsky et al 2005). Given these findings, V1a receptors in the lateral septum appear to be necessary for social recognition among various rodent species.

So why do adolescent rats with higher levels of V1a receptors in the lateral septum spend more time engaged in social investigation? The lack of maternal care received by these animals apparently alters neurological systems necessary for the development of species typical social behavior. As sociality is phylogenetically vital to the continuation of any social species, perhaps a sensitive period for the acquisition of appropriate social behaviors extends into adolescence or beyond. That differences in social investigation, non social exploration and V1a receptor binding are absent by the time MS and AFR rats reach adulthood supports the notion that MS animals are capable of acquiring species typical social behavior through the exaggerated investigative social behavior they display during and possibly before adolescence via the action of V1a receptors in the lateral septum. Lukas et al. (2009) report increasing levels of V1a receptor binding within the lateral septum in normal rats as a function of age. As MS animals possess more V1a receptors in the lateral septum as adolescents it would seem that these animals, presumably because their early environment denied them normative neurological development, expressed elevated levels of V1a in order to compensate for the deficit in species typical behavior. That said, a mechanism that allows for, recognizes, or determines normal social behavior is unknown. Though, the combined evidence described above, as well as the current findings point to an AVP dependent system that is still malleable enough at adolescence to compensate for deficits in social behavior due to differential maternal care in early life.

How might this system operate? Many AVPergic afferents of the lateral septum originate in the medial amygdala (MeA) and BNST (another limbic structure)(Caffé et al 1987). Too little AVP activity in the lateral septum may be the consequence of abnormal amygdala activity, which then may lead to upregulation of V1a receptors in the LS. This abnormal amygdala activity may be the catalyst for increased social interactions among MS rats. Raggenbass et al. (2008) have hypothesized that lateral septal neurons, which are mostly GABAergic and therefore inhibitory, are under GABAergic inhibitory influence by interneurons innervated by the AVPergic MeA and BNST. These interneurons synapse with most, if not all, lateral septal neurons (Risold & Swanson 1997) and activation of the AVPergic system causes inhibition of lateral septum neurons, thereby causing disinhibition (activation) of lateral septum target sites, one of which is the PVN (the command center for stress responses). If AVPergic signaling is absent in this circuit, LS

inhibition of target sites is achieved. Hence, PVN stress circuitry would not be engaged and the animal would be free to engage in social interaction without normal recognition of, or the emotional valence attributed to, conspecific cues. Some evidence for this possibility already exists. In comparison to Wistar rats that display little aggression, highly aggressive (arguably, engaged in more social behavior) Wistar rats release low levels of AVP from the LS. Interestingly, when AVP is administered to the LS of low-aggression animals they display more anxiety-like behavior while high-aggression rats given an AVP antagonist to the LS display less social investigation behavior (Beiderbeck et al 2007).

Additionally, the MeA lies where main and accessory olfactory information first converge in the brain and has been demonstrated to be important in interpreting chemosensory signals in rodents (Blanchard et al 2005; Kang et al 2009; Lehman et al 1980; Petrulis & Johnston 1999). For instance, exposure to either reproductive or predator odor results in distinct patterns of immediate early gene expression (a measure of neural activity) within the MeA (Meredith & Westberry 2004). I would argue that social behavior is dependent on accurate interpretations by the amygdala of species typical social cues that in MS animals are not learned before adolescence and therefore deprives the LS of AVPergic input (activity of AVPergic neurons in the amygdala) leading to upregulation of V1a receptors. Interestingly, researchers working on olfactory mediated amygdala activity recently showed that decreased activity in the amygdala prevents male mice from avoiding or approaching, respectively, predator odor or female mouse urine (Samuelsen & Meredith 2011). As rodents rely on olfaction more so than any other sensory modality it is logical to assume that MS animals could misinterpret chemosensory cues relevant for social interactions. Elevated levels of social interaction then eventually allow for the proper interpretation of conspecific social behavior by the amygdala (through repeated reinforcement) and subsequently "teach" lateral septum neurons through AVPergic activity. This activity ultimately normalizes V1a receptor expression in the LS and allows for disinhibition of the PVN in appropriate social contexts thereby reducing social investigation to a species typical level. Whether this testable hypothesis is accurate will rely on further research.

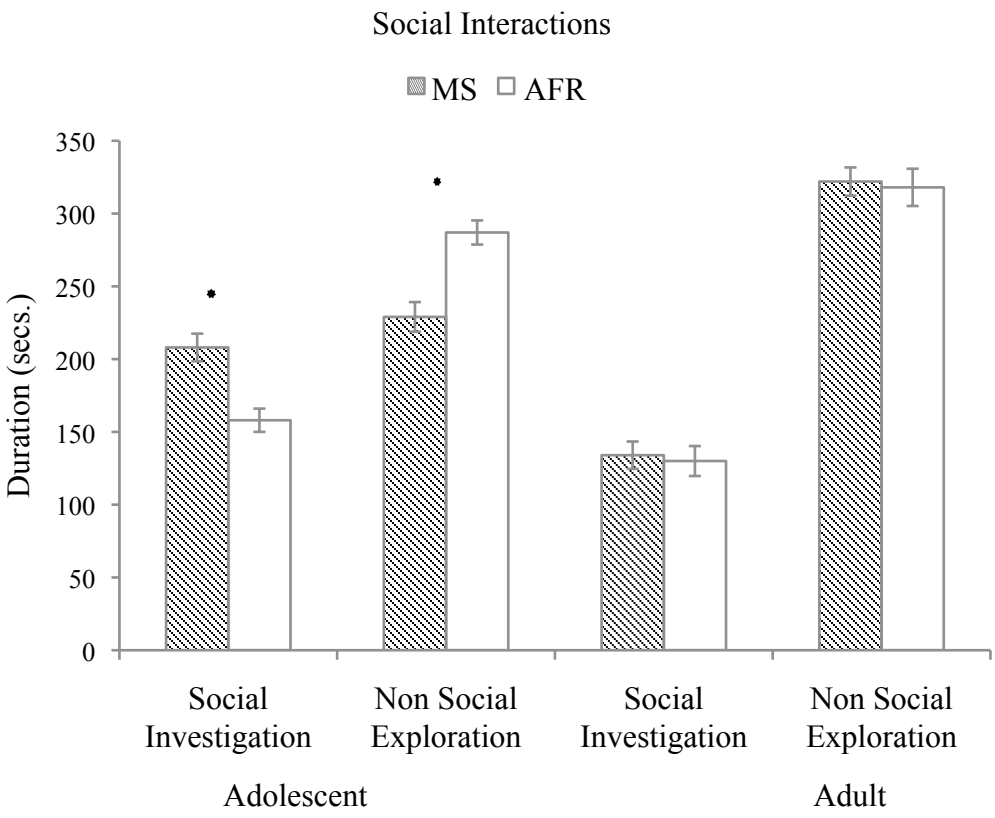


Figure 1.
Mean social investigation and non-social exploration times as adolescents and adults. *Indicates significant difference ($p<.05$) between MS and AFR.

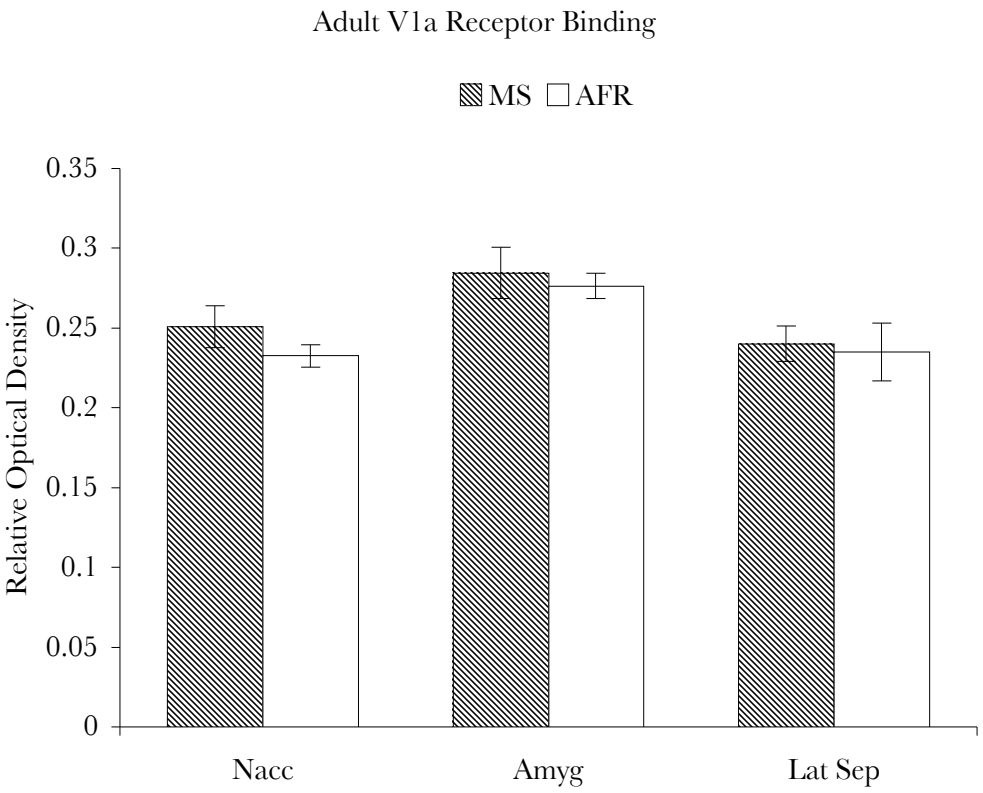


Figure 2.
V1a receptor binding during adulthood in the nucleus accumbens, amygdala, and lateral septum. No Sig. Diffs.

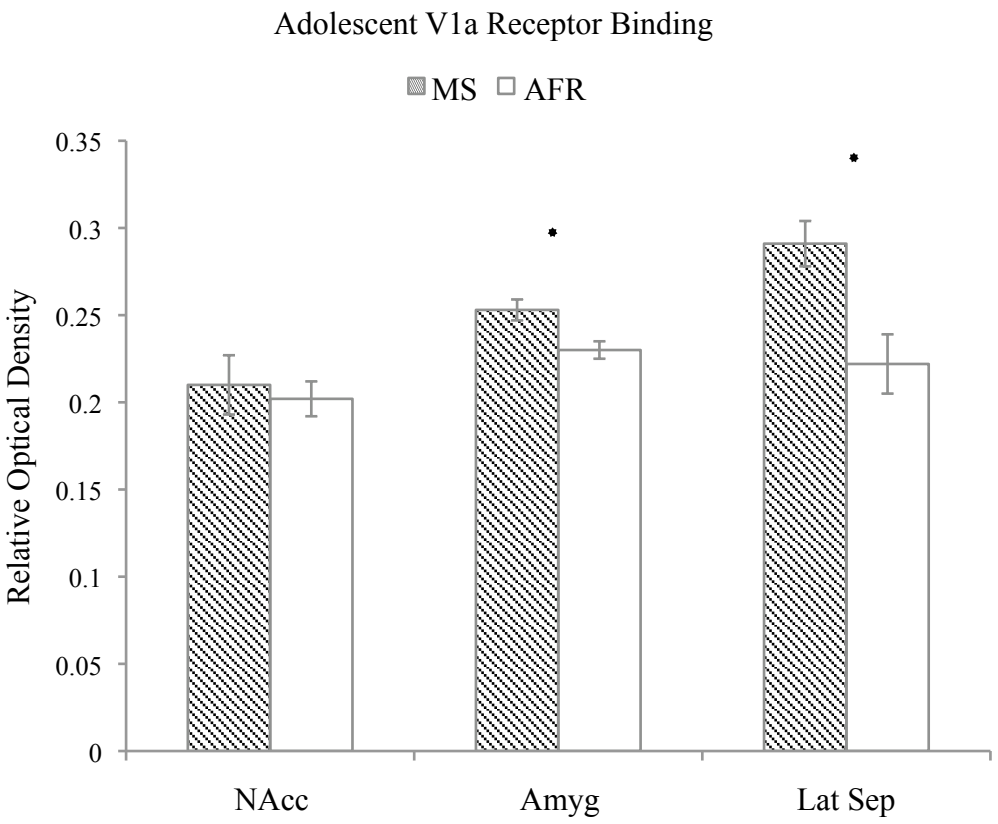


Figure 3.
V1a receptor binding during adolescence in the nucleus accumbens, amygdala, and lateral septum. *Indicates significant difference ($p<.05$) between MS and AFR.

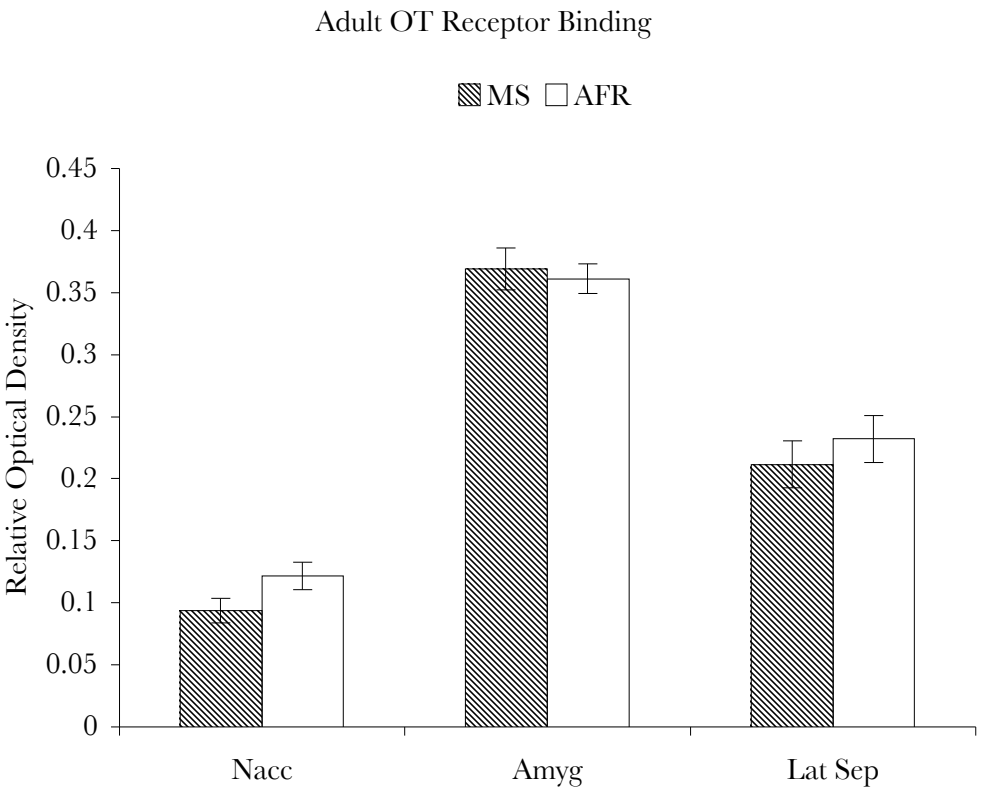


Figure 4.
OT receptor binding during adulthood in the nucleus accumbens, amygdala and lateral septum. No Sig. Diffs.

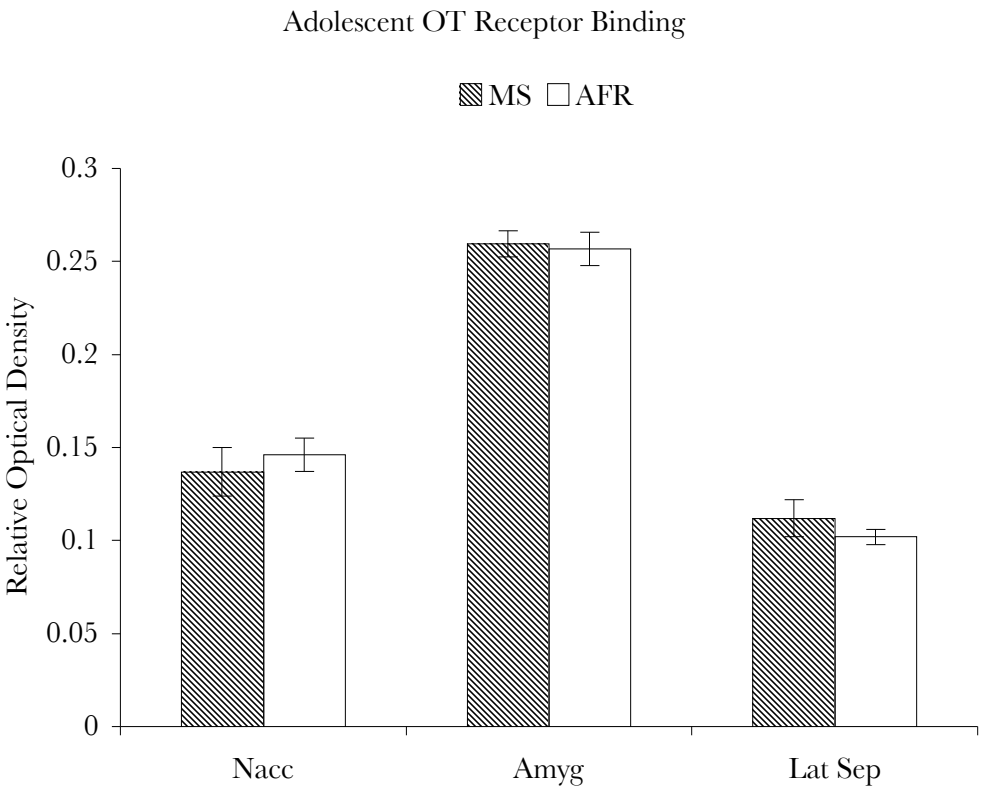


Figure 5.
OT receptor binding during adolescence in the nucleus accumbens, amygdala and lateral septum. No Sig. Diffs.

Chapter 3

Behavioral, Endocrine and Neural Effects of Social Status in a Novel Paradigm Capable of Isolating Psychosocial Factors

3.1 Introduction

Social status is a potent determinant of health in humans. Those lowest in social status exhibit the highest incidences of mortality and the poorest levels of mental and physical health in a given population (Marmot & Rose 1978; Marmot & Shipley 1996; Marmot & Smith 1991). These findings might not be surprising given that those lowest in social status are often the most impoverished and vulnerable members of society. What may be surprising to some is the graded relationship between health and social status. Using socioeconomic status (SES) as a measure of social rank a graded, continuous association has been identified between rank and morbidity (Adler & Boyce 1994). Individuals at any given social rank will have better health outcomes relative to those lower in social rank and worse health outcomes when compared to those higher in social rank (Singh-Manoux et al 2004). Graded SES disparities are not only found when looking at physical health, but are present in rates of most psychiatric disorders and in measures of normal cognitive function in adults and children. Obviously, this gradient with respect to social status and health suggests that one's relative place in society matters greatly.

Many researchers have proposed that more or less challenging social environments (Matthews & Gallo 2011), inequality of resources (Lynch & Kaplan 1997) and psychosocial factors (Gianaros et al 2008) associated with social status are responsible for this disparity. Unfortunately, it is not possible to address these issues separately or experimentally in humans since causality remains such an elusive component of human research. Causality is difficult to address for several reasons, the most notable of which is simply an overwhelming lack of control over participants' prior experiences and exposure to confounding factors such as abuse or illness. Environmental experiences early in life are known to have profound effects on development in both human and non-human animals, some of which can persist throughout the lifetime. For instance, in humans, extreme neglect in the form of social isolation during infancy results in affectionless behavior in adulthood (Bowlby 1954; Rusby & Tasker 2008), while in rats it results in a more fearful behavioral phenotype throughout life (MacrÌ et al 2004). These examples merely highlight the sensitivity of the developing organism to environmental experiences and the necessity for control over confounding factors in status research. While controlling human environments for research purposes is possible for brief periods of time, experiential differences between subjects before the start of a study may influence the results of any research in unverifiable ways (hence random and representative sampling). The use of animals, however, provides a means through which resources and other environmental factors may be controlled (or at least measured) from before conception and throughout the lifetime. In the current study we use this strategy to infer psychosocial parameters exclusively attributable to

social status and to then determine what and how specific environmental conditions impact those status/rank parameters.

Much animal research dealing with social hierarchy has already been conducted in rodents and non-human primates. Most primate studies, however, suffer from the same limitations as human research. Laboratory based primate work often utilizes animals that have been subjects in prior research. Often the prior research is not uniform across all animals in a given experiment and is tantamount to human research in terms of differential environmental experiences. Field based research contains the same lack of control over the previously mentioned confounds as well. Therefore, to adequately study status/health disparities, a controlled animal model of social status representative of human sociality is needed. Unfortunately, a large majority of controlled laboratory rodent studies that make use of social hierarchy simply use it as a means of administering chronic social stress. These studies are not concerned with why or how a particular animal comes to occupy a particular rank, but rather the effect of rank (usually subordination) on specific health outcomes such as heart disease (Sgoifo et al 1997) or metabolic syndrome (Tamashiro et al 2007). These studies also traditionally assess rank based on the outcomes of aggressive encounters between animals, offensive and defensive wound patterns, or simply weight gain or loss. Classically, the dominant animal in a rodent social group has been identified as the one that either weighs the most or has lost the least amount of weight since group formation (Raab et al 1986). These measures of dominance are of little use in human translational research. Additionally, social groups in these cases are almost exclusively composed of adult animals whose prior experiences may not be similar (bred in house or purchased from a breeder, flown or trucked for delivery, housed singly or in pairs) since mention of experience before group formation is seldom included in publications (Gentsch et al 1988). Lastly, most of these social groups are transient with animals remaining in contact with each other no more than two weeks and often for only portions of days (Blanchard et al 2001). None of these regularly employed paradigms of social status are representative of typical human sociality (nor do they mean to be) and are therefore less valid as tools to study the antecedents of social disparity.

Our method of group formation differs markedly and necessarily from prior methods simply because we are interested in answering different questions. As mentioned above, three reasons such a status/health disparity is believed to exist deals with resource inequality, more or less challenging environments and psychosocial factors arising from occupation of a particular status. It is the first two variables that confound our current understanding of the psychosocial aspect of social status. Therefore, in order to isolate the psychosocial effects of differential social status we created groups of animals that have equal access to resources and very similar, if not identical, environmental experiences before group formation. Our groups were, therefore, composed of same sex, same age, non-littermate, weanling rats matched on weight and amount of maternal care (observed levels of licking and grooming) received. All animals had equal access to resources (bedding,

food and water) throughout the experiment while intermittent bouts of competition for water and chocolate were used to assess rank. Additionally, we assessed anxiety like behavior in all animals prior to group formation in order to determine whether this pre-existing characteristic was predictive of adult social rank. Such a paradigm has never been utilized to study social status in rodents, so formation of a hierarchy under these conditions was not assured, however, the results of this study provide evidence that this novel paradigm results in similar patterns of graded behavioral and physiological outcomes known to exist in humans and has much utility in untangling the complex problem of human social status health disparities.

Arginine vasopressin (AVP) and oxytocin (OT) are two closely related and highly conserved neuropeptides involved in the social behavior of several species of animals and their activity, both direct and indirect, is widespread throughout the central nervous system (CNS). Recently, these neuropeptides have garnered much attention in their role as mediators/moderators of social behavior in both humans and non-humans alike. Since we are interested in the psychosocial effects of status we decided to investigate OT and AVP receptor distributions within brain areas implicated in processing social, emotional and stressful stimuli. Our results shed light on brain areas potentially involved in the psychosocial perception of relative social status and provide a stepping-stone from which to devise potential interventions that combat the pathological effects of differential social status.

3.2 Methods

Animals

Male Long-Evans rats born in our colony from animals originally purchased from Charles River were used in the study. Parent animals were housed individually for one week after delivery to habituate them to the new environment. On day 8 a single male and female were housed together for another 6 days. A total of 40 pairs of animals were mated. After 6 days of cohabitation each pair was again individually housed and females were under animal facility care until parturition (PND 0). All animals had ad libitum access to food (Purina Rat Chow, Purina Mills, St. Louis, MO.) and tap water. Temperature was maintained at 20 ± 2°C, humidity was maintained at 50 ± 5% and a light/dark cycle of 12-h (lights on at 0700 h) was maintained throughout the study. The Office of Laboratory Animal Care at the University of California, Berkeley, approved this study.

Maternal Observations

Beginning on PND 1, litters were observed at three different time intervals (6-8am, 12-1pm and 6-8pm) for a total of five hours per day over the next 7 days. Over these 7 days cages were maintained by the researchers to minimize any differences in care the litters may have received. The 6-8 am and pm intervals flanked lights on and lights off (7am and 7pm, respectively) time points in order to acquire a more representative and complete measure of maternal care. Red lights were used to illuminate litters during the lights-off portions of the observations and

turned off after observations were completed for that time interval. For each litter, maternal behavior was recorded once every two minutes during each of the observational periods for a total of 1050 data points per litter. Maternal behaviors recorded were "on", mother covering the nest of pups while stationary and usually sleeping/resting, "L/G", mother actively licking and grooming any pup in the litter while on the nest and "off", mother not on the nest of pups. "Off" encompassed all other maternal behaviors, such as eating, drinking, self-grooming or sleeping off the nest. An index of maternal care was calculated for each litter by simply dividing the number of L/G observations by the total number of observations.

Group Formation

Male offspring from litters that fell within one standard deviation of the maternal care index were used to form social groups. These animals were weighed the day prior to group formation (PND 23). On PND 24, four non-littermate males as closely matched on weight and maternal care index as possible were housed in clear guinea pig cages measuring 20in x 16in x 8.5in. A total of 10 cages were housed in this manner (N=40) while another 10 males were housed in pairs in traditional rat cages (19in x 10.5in x 8.5in) also matched as described above. Pair housed animals served as controls for the larger four-animal social groups.

Social Competitions

Water

Competition for water began after depriving all animals of water for 6-8 hours. The introduction of a single water bottle signaled the beginning of a competitive bout. All animals within a cage had equal access to the single bottle and more than one animal could access the bottle at any given time. The coat of each animal in a cage was colored red, black, or green or left white (animals left white were handled as though they were being colored) before each bout in order to clearly distinguish their identity. Each bout was digitally recorded and a researcher blind to the experimental condition of the animals measured the amount of time each animal spent drinking from the water bottle.

Chocolate

Before group formation miniature chocolate chips were intermittently placed in all cages of litters in order to acclimate the animals to its palatable nature. During competitions a small portion of chocolate was melted to a dish, allowed to harden, then the dish was attached to the inside wall of the animals' home cage with double-sided tape. The dish was small enough that only one animal could access the chocolate at any given time. Once again, each bout was recorded and the amount of time each animal spent eating the chocolate was measured by an individual blind to the condition of the animals.

Rank Assessment

Animals with larger eating/drinking times were deemed dominant over those with smaller times and assigned a rank of 1 (subordinates) to 4 (dominants) accordingly. Final ranks were assigned by averaging the rank scores of all animals

from the last water and food challenges (PND 163 and 165 respectively). In the event of a tie the food competition (PND 165) ranking was used to assign rank.

Light/Dark Box

A box (100cm x 50cm x 20cm) made of half transparent (light) and half opaque (dark) Plexiglas was used for this task. The opaque portion was separated from the translucent by an opaque wall with a hole in it that rats could move through to enter the light half of the box. To begin each trial, rats were placed in the opaque portion headfirst through the hole that separated the two halves of the box. The researcher then left the room while the trial was digitally recorded. Each trial lasted 5 minutes (300 seconds) and was later scored by an observer blind to the condition the rats belonged to. Behaviors scored were "latency", time taken to first enter (all four limbs of the animal through the doorway) the light portion of the box, "pokes", number of times the nose (or more) of the animal broke the plane of the doorway, but then reentered the dark portion of the box and "light time", amount of time the animal spent in the light portion of the box. This task was performed intermittently throughout the course of the study (at PND 23, 35, 48 and 73).

Forced Swim Task

To measure depression-like behavior a forced swim task was used. "Pools" made of transparent, cylindrical Plexiglas measuring 30cm in diameter and 70cm in height were filled halfway with water (25°-27°C). On day 1, rats were individually placed in this tank for 15 minutes to learn the futility of struggling to escape from the container. The next day, rats were placed in the tank again for 5 minutes while the trial was recorded. Fecal matter was removed from the water after each animal on day 1 and the water was changed after 4 or 5 animals depending on the cleanliness of it. On day 2, water was changed after every trial. An observer blind to the condition of the animals scored the amount of time each animal spent climbing (vigorously pawing at the side of the tank), actively swimming, the amount of time spent immobile (a sign of depressive-like behavior) and the latency to first become immobile. Immobility is considered a biomarker of depression since anti-depressant medication will increase the amount of time an animal persists in trying to escape.

Cognitive Performance

A syringe puzzle task was used to measure cognitive performance. Rats were first given a training trial in which they were individually exposed to a clear plastic syringe with chocolate melted to the middle of the plunger and taped to the floor of either their home cage or a novel plastic box (counterbalanced over all animals). For this 90 second training trial the plunger was pulled out making the chocolate immediately accessible and rats were free to explore and eat the chocolate during this time. A second trial (puzzle trial) was conducted in the same cage two hours later with the plunger pushed into the syringe making the chocolate inaccessible unless the animal pulled the plunger out of the syringe far enough to expose the chocolate (~2cm). The animal successfully "solved" the puzzle if they gained access to the chocolate. Latency to solve the puzzle was the dependent measure of this task

and the trial was ended after 120 seconds if unsuccessful. The next day all animals repeated the task described above, but in the opposite setting (novel box or home cage).

Restraint stress

A single acute restraint-stress task was performed on adult animals. Rats were removed from their home cage between 7a.m. (the beginning of the "light" portion of their day) and 10a.m. then immediately had the tip of their tail clipped and approximately .5 ml of blood was collected from the wound in 1.5ml plastic tubes with snap on lids. This process was not performed after 10am to prevent the natural circadian release of corticosterone from positively skewing the amount measured. This initial blood sample was collected no later than one minute from the retrieval of the animal from its home cage (basal) to ensure that the presence of corticosterone in the blood, which is released in response to the task, was avoided. Animals were then placed in conical plastic sleeves (Decapicones) of which the larger end was taped shut. This rendered the animal incapable of moving. An opening in the small end of the sleeve allowed the animal to breath normally while restrained. After 15 minutes of restraint a second blood sample was collected (peak) and the animal was released back into its home cage. Blood was subsequently collected from the animal once every 30 minutes after release for the next 90 minutes (30, 60, & 90) for a total of 5 samples (basal, peak, 30, 60, 90) per animal. All blood samples were collected in 1.5ml plastic tubes and placed in an ice water bath to prevent coagulation. Once all samples were collected they were centrifuged at 5000 rpm for 10 minutes. The plasma supernatant was then aliquoted from the samples and stored at -20°C until the time of the assay. If the tail wound started to scab over and prevented sample collection the tail was placed in warm water and massaged to remove the coagulated blood and allow bleeding. This process was accomplished in a matter of seconds. Once bleeding resumed the tail was dried with a paper towel and blood was collected as described above. Animals were held in the left arm cradled against the standing experimenter's torso while the experimenter's fingers held the base of the tail to prevent the animal from getting loose. With the right hand, the experimenter ejected blood from the tip of the tail by stroking the tail from base to tip in a "milking" motion. The resulting blood dripped into collection tubes sitting in a tube holder on a table in front of the experimenter.

Corticosterone EIA

Measurement of all plasma corticosterone was performed according to the instructions of the corticosterone enzyme-linked immunosorbent assay (EIA) kit (Assay Designs) with the following exception. The dilution of plasma to assay buffer that yielded optimal concentrations of corticosterone as compared to the standard curve of the assay was 5µl of plasma to 95µl of buffer, or 1:20 dilution. Plates were analyzed at 405nm using a BioRad cell plate reader. All samples were run in duplicate.

Receptor Autoradiography

Adult Animals were sacrificed at PND 170 upon completion of adult behavioral testing. Following decapitation, brains were quickly removed, placed on powdered dry ice, and stored at −80 °C. Brains were then sliced in 20μm coronal sections containing the medial frontal cortex, nucleus accumbens, lateral septum, and amygdala. Sections were mounted onto Permafrost slides and stored at −80 °C until the time of assay. For OTR binding, [^{125}I]-ornithine vasotocin analogue [(^{125}I)OVTA] was employed [vasotocin, d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Orn^8, (^{125}I)Tyr^9-NH_2]; 2200 Ci/mmol]; (NEN Nuclear, Boston, MA, USA). For V1a receptor binding, ^{125}I-lin-vasopressin [^{125}I-phenylacetyl-D-Tyr(ME)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH_2]; (NEN Nuclear) was used. Sections were allowed to thaw to room temperature (RT) and then immersed in 0.1% paraformaldehyde for 2 min to optimize tissue integrity. Sections were then rinsed two times in 50 mM Tris-HCl (pH 7.4) at RT for 10 min and incubated for 60 min at RT in a solution of 50 mM Tris-HCl (pH 7.4) with 10 mM $MgCl_2$, 0.1% bovine serum albumin, 0.05% bacitracin, and 50 pM ^{125}I-lin-vasopressin or 50 pM ^{125}I-OVTA. Non-specific binding was determined by incubating adjacent sections with the radioactive specific ligand as well as 50 μM of unlabelled Thr4, Gly7 oxytocin, a selective oxytocin ligand (Peninsula Laboratories, Belmont, CA, USA) or 50 μM of unlabelled [1-(β-mercapto-β,β-cyclo-pentamethylene propionic acid),2-(O-methyl)-tyrosine]-arg^8-vasopressin, selective for the V1a receptor. Following incubation, sections were washed 3 × 5 min in 50 mM Tris-HCl (pH 7.4) with 10 mM $MgCl_2$ at 4 °C, followed by a final rinse in this same buffer for 30 min on ice. Slides were then quickly dipped in cold dH_2O and rapidly dried with a stream of cold air.

Sections were apposed to Kodak BioMaxMR film (Kodak, Rochester, NY, USA) with ^{125}I microscale standards for 72 h. Autoradiographic ^{125}I-receptor binding was quantified from film using the NIH Image program (http://rsb.info.nih.gov/nih-image/). An average of nine sections per animal per area were scored using the Paxinos and Watson rat atlas as a reference.

3.3 Results

Behavioral Measures

Hierarchy Formation

Ranking of animals on the final water and food competitions, on PND 163 and 166, respectively, were significantly correlated with rankings from competitive bouts in young adulthood (PND 66, 75 and 78; r's = .37-.75).

Data collected before group formation (maternal care, L/D behavior and weight) did not predict eventual adult social rank, nor did weight at any given time predict or significantly correlate with eventual rank. The widely used method of determining hierarchical social status based exclusively on weight is clearly not beneficial using our novel paradigm. The use of our paradigm allowed a stable hierarchy to emerge in which violent confrontation was minimal (according to our

experiences while interacting with the animals throughout the study and a lack of physical injury sustained by nearly every animal). Hierarchies in which stability is not sustained are characteristically more violent and exact a higher level of stress on the animals that bear the brunt of these confrontations (Sapolsky 2004). In such a social environment weight gain and maintenance would be more reflective of hierarchical rank.

Light/Dark Box

At PND 73 a significant main effect of rank was revealed in the amount of time animals spent in the illuminated portion (a measure of exploratory behavior or lessened anxiety) of the Light/Dark box, $F(3,47) = 2.898$, $p = .046$ (Fig. 4). The results reveal a graded relationship between rank and anxiety-like behavior similar to that seen in human beings (Evans & Kim 2007). Dominant animals (M = 159.8, SD = 39.6) spent more time in this portion of the box than most other groups (Mid1 M = 160.1, SD = 69.0; Mid2 M = 128.4, SD = 52.8; Sub M = 100.5, SD = 77.2). Post hoc analyses did not reveal any simple effects, but a trend toward significance was seen between the dominant and subordinate groups, Tukey post hoc, p=.052. Final rank was not associated with earlier measures (PND 23, 35 or 48) of L/D box behavior, which suggests that pre-hierarchy (PND 23) measures of exploratory/anxiety-like behavior do not drive eventual adult social rank. Rather, the data suggests that hierarchical rank affects levels of anxiety/exploratory behavior beginning between late adolescence (PND 48) and young adulthood (PND 73).

Porsolt Forced Swim Task

A significant main effect of rank was found in the amount of time (in seconds) spent actively trying to escape (termed "climbing") from the forced swim apparatus (Fig. 5), $F(3,34)= 4.005$, $p = .015$ (Fig. 5). Dominant animals (M = 99.3, SD = 12.2) displayed the most climbing behavior while subordinate animals (M = 75.2, SD = 22.0) displayed the least, while mid animals (Mid1 M = 97.2, SD = 21.4; Mid2 M = 76.3, SD = 23.5) fell between the dominant and subordinate groups. Post hoc tests revealed no simple effects between ranks.

Puzzle Task

A significant main effect of rank was reflected in the latency (in seconds) to approach the exposed chocolate on a syringe during training trials in the familiar home cage, $F(3,44) = 4.415$, $p=.008$ (Fig. 3). A Tukey Post hoc analysis, p=.009 revealed a simple effect between the dominant (M = 8.8, SD = 5.5) and subordinate (M = 37.4, SD = 35.1) groups, while a trend toward significance, p=.07, was seen between subordinate and mid 1 animals (M = 11.4, SD = 9.2). A trend towards significance was seen in the amount of time differentially ranked animals took to solve the syringe puzzle in the familiar home cage (p=.09). This trend showed a graded pattern with respect to rank with higher-ranking animals solving the task more quickly than lower ranks. While a significant difference in cognitive processes were not seen in this task the graded pattern of results is worth mentioning, as it is reminiscent of graded cognitive patterns seen in humans.

Physiological Measures

Stress Reactivity

A significant main effect of rank was found in the total amount of corticosterone (Cort) (pg/ml) detected during a restraint stress task and the 90 minutes of recovery immediately following, $F(3,34)= 4.374$, $p=.01$ (Fig. 6). Dominant animals had the most robust response (M = 1485, SD = 848) followed by mid1 animals (M = 1366, SD = 519), mid2 animals (M = 1276, SD = 736) and subordinate animals (M = 797, SD = 468). Subordinate animals displayed a blunted Cort response in comparison to all other groups. Other studies have reported blunted stress responses in humans suffering from post traumatic stress disorder (Yehuda et al 1991) and in subsets of chronically socially stressed rats (Albeck et al 1997). Blunted stress responses in our subordinate animals may result from the chronic social stress inherent in occupying the least dominant position in the hierarchy.

Receptor Autoradiography

All receptor-binding values are expressed as units of relative optical density. A significant main effect of rank was reflected in oxytocin receptor binding levels in the nucleus accumbens (NAcc), $F(3,29)=4.622$, $p=.009$ (Fig. 7), frontal association cortex (FrA), $F(3,41)=4.413$, $p=.009$ (Fig. 9), orbital frontal cortex (OFC), $F=(3,40)=2.836$, $p=.05$, and central amygdala (CeA), $F(3,22)=3.988$, $p=.021$ (Fig.8). A trend towards significance was seen in the amount of OTR binding of the entire medial frontal cortex taken from measurements in the FrA, OFC, cingulate gyrus, pre-limbic cortex and infralimbic cortex, $F(3,37)=2.73$, $p=.058$. Throughout the entire medial frontal cortex binding was greater in subordinate animals than in all other groups. In the specific regions of the FrA and Orb subordinates differed significantly from most other ranks (FrA, Sub M = .044, SD = .015, Mid2 M = .024, SD = .016, Mid1 M = .027, SD = .012, Dom M = .027, SD = .018; Orb, Sub M = .063, SD = .024, Mid2 M = .041, SD = .02, Mid1 M = .05, SD = .009, Dom M = .048, SD = .018). Dominant animals displayed higher levels of OTR binding in the NAcc than all other groups (Dom M = .307, SD = .081, Mid1 M = .257, SD = .059, Mid2 M = .201, SD = .048, Sub M = .249, SD = .041). In the CeA higher levels of OTR binding were seen in mid animals with the greatest values appearing in the Mid2 group (Mid2 M = .331, SD = .04, Mid1 M = .314, SD = .023, Dom M = .286, SD = .031, Sub M = .261, SD = .058).

The binding of vasopressin receptor subtype 1a (V1a) did not differ significantly by rank in any brain area measured. Differences in V1a distribution have been found in rats due to variations in maternal care received in the first few days of life. We may not have detected any V1a receptor differences because our study consisted of groups of animals that were matched on levels of maternal care before group formation.

3.4 Discussion

Groups initially comprised of weaning aged (PND 23), non-littermate animals matched on sex, weight and maternal care with equal access to resources formed stable social hierarchies, as measured by competition for chocolate and water, without intervention (Fig. 1). None of the measures collected before group formation (L/D behavior, weight, maternal care) predicted eventual adult social rank, nor did weight correlate with rank at any point in the study. In fact, growth curves for all animals were almost identical (Fig. 2).

Several behavioral and physiological measures, however, were significantly different between adult social ranks and varied in a graded manner commonly seen in human data. These findings lend support to the validity of our animal model as a means of exploring rank/health disparity relationships within the human population. For example, like humans, animals in our study showed a graded difference in anxiety related behavior in which subordinate animals took significantly more time to approach a novel stimulus (syringe task) than more dominant animals (Fig. 3). A similar result occurred at PND 73 with respect to the amount of time animals spent in the illuminated portion of a L/D box. Again, subordinate animals spent significantly less time in the illuminated area (indicative of higher levels of anxiety) than more dominant animals (Fig. 4). Several studies have revealed a similar relationship between social status and anxiety in humans (Kessler 1979; Modin et al 2010). Likewise, as adults, a significant difference in the amount of time spent actively struggling to escape from a tank in the forced swim task was seen between ranks. Again, this difference occurred in a graded manner in which dominant animals struggled more than relatively less dominant animals (Fig. 5). Increases in the latency to become immobile in the forced swim task are widely accepted as being indicative of depressive-like behavior in rodents. As reported here, subordinate animals display more depressive-like behavior, a finding that mirrors human results of status/depression prevalence rates (Modin et al 2010; Yu & Williams 1999).

Directly related to anxiety and depression are stress and stress hormones. Not surprisingly, we found a significant effect of rank on the amount and duration of corticosterone (Cort), a stress hormone, released during an acute stressor (restraint stress) and consequent recovery (Fig. 6). Subordinate animals displayed a blunted response while relatively more dominant animals released larger amounts. A blunted stress response is frequently seen in humans that have experienced traumatic events (as in PTSD) (Yehuda et al 1991) or chronic exposure to stressful environments (Miller et al 2009). In humans, a lower social status is accompanied by more frequent and qualitatively more intense challenging life events (Carroll & Smith 1997). Repeated chronic activation of the HPA axis by challenging environmental circumstances is theorized to wear down this system resulting in atypical physiological responses to stressful stimuli (Goymann & Wingfield 2004). Albeck et al. (1997) reported that a subset of subordinate rats in his visible burrow system (VBS) model of chronic social stress displayed a blunted stress response. He termed these animals “non-responders” and found a portion of their HPA axis was deficient in corticotropin releasing hormone (CRH) receptors. Fewer CRH receptors

could be responsible for the blunted Cort response seen in his non-responders and while his VBS model results in more violent encounters between animals than ours it is possible that the psychosocial perception of occupying a subordinate status is more traumatic/stressful for some animals than it is for others. A similar phenomenon may be occurring in our subordinate animals to a lesser degree and undetected by our analyses, which could account for the low levels of Cort seen in our subordinate animals. Whether differences in CRH receptor density is the cause or effect of Albeck's results is unknown and measures of CRH were not performed in the current study, but the possibility that subordination is differentially perceived psychosocially by particular animals still remains.

Receptor Autoradiography
Nucleus Accumbens

Significantly greater levels of oxytocin receptor (OTR) binding were seen in the nucleus accumbens (NAcc) of dominant animals (Fig. 7) and may be intuitive given the nature of this reward related structure. As an integral component of the mesolimbocortical reward/reinforcement pathway, the NAcc is active whenever an appetitive stimulus is experienced, such as the consumption of food or the act of sexual intercourse. Though speculative, it is possible that the dominant animal in a hierarchy would experience competitive social interactions as more appetitive than their subordinate counterparts and engage in social behaviors that potentially confer dominance. Recent research has shown that OTergic afferents of the NAcc arise from collaterals of magnocellular neurons in the hypothalamic PVN and SON (Ross et al 2009), the same neurons that comprise the neurohypophyseal portal system. Therefore, arguably, NAcc OTR is affected directly by the same circuitry responsible for the release of OT into peripheral circulation. Peripheral OT levels rise in response to several types of physiological stimulation, such as parturition and suckling, for review see (Richard et al 1991). OT release in the PVN in particular has been demonstrated in rats via suckling, parturition, aggression, defeat and mating. Endogenous OT levels in the NAcc, however, has not been explored in rats under any of these behavioral conditions, but peripheral and intracerebroventricular (i.c.v.) OT administration has repeatedly produced alterations in neurotransmitter profiles within the NAcc. These studies are primarily concerned with drug abuse intervention strategies, but underscore the importance of OT activity within the NAcc under appetitive, motivation/reward-based conditions. The only study to date that has manipulated exogenous OT in the NAcc of rats found OT increased hindpaw withdrawal latency to thermal and mechanical nociception and altered opiate activity within the NAcc (Gu & Yu 2007). While not particularly relevant, this work also supports the modulatory action of OT within the NAcc. Interestingly, in rats, activation of the NAcc may be associated with a general increase in appetitive behavior (Hull et al 2002) rather than an increase in any one specific behavior. Increased expression of OTR in the NAcc could signal that dominant animals perceive competitive social interactions as more appetitive than other members of the hierarchy. Further studies will have to determine whether OT activity in the nucleus accumbens increases appetitive social behavior in rats.

Are dominant animals predisposed to higher levels of OTR expression in the NAcc or do these animals learn that competitive social interactions are appetitive which in turn increases the expression of OTR? Unfortunately, my research design is unable to address this question, but other research would suggest that the heightened expression of OTR in the NAcc is environmentally/experientially driven. For instance, differences in the expression of OTR do not vary greatly between closely related species of polygamous rodents including meadow voles, mice and rats (Insel & Young 2001). Monogamous prairie voles, though, express high levels of OTR in the NAcc compared to polygamous meadow voles. Amongst several rodent species whose reproductive strategy is polygamous there is far less OTR expression in the NAcc. The variation that exists between species practicing differential reproductive strategies most likely arose from evolutionary forces preferentially selecting one strategy over the other. That the morphology of OT immunoreactive fibers are highly conserved across rodent species (Ross et al 2009) regardless of reproductive strategy is another indicator that environmental experiences drive differences in receptor expression between members of the same species. Therefore, the variation in OTR expression that exists between dominant and non-dominant animals in the NAcc is probably not a function of evolutionary selectivity or genetic predisposition, but driven by differences in psychosocial perception between members of differing ranks. Additionally, environmental differences between prairie and meadow voles, which practice different reproductive strategies, are minimal, as they inhabit neighboring ecologies that are similar in virtually every way. Differential OTR expression between these two species is not a product of living in distinct environments, but a reflection of evolutionary forces that drove separate reproductive strategies.

Definitive evidence that dominant animals achieve their status because of the way they perceive competitive social interactions is lacking. The possibility that dominant animals become dominant because they are predisposed to higher levels of OTR within the NAcc is still valid. Further research will need to be conducted to determine the directionality of this effect, but it is clear that OTR expression within the NAcc is involved in the formation of social hierarchies in rats.

Amygdala

The amygdala has long been known to be involved in the detection, processing and expression of fear/anxiety in humans and non-humans alike, recently however, OT has been implicated in the functionality of the amygdala across mammalian species as well. For instance, i.c.v. administration of an OT antagonist eliminates all immediate early gene expression (a measure of neuronal activity) in the medial amygdala of mice to relevant (predator) and irrelevant (bovine urine) odors, and renders them unable to recognize conspecifics (Samuelson 2011).

Humans given OT intranasally show decreased amygdala activity to images of faces (Domes et al 2007a), fear inducing stimuli (Kirsch et al 2005) and affectively negatively conditioned stimuli (Petrovic et al 2008). Additionally, humans with a

particular variant of the OT gene have been shown to have larger amygdala volumes than those without the variant (Furman et al).

More recently, research has delineated the potential contributions of sub regions of the amygdala to various aspects of fear/anxiety and related processes. Particularly relevant for the current data is the contribution of the CeA and its sub regions. Many efferents of the CeA project to the hypothalamus and brainstem where they are important in the expression of autonomic fear responses to acute, highly predictable stimuli (as opposed to more contextually lengthy, ambiguous stimuli) such as would be the case when interacting with conspecifics. In the present study mid animals displayed significantly higher OTR binding in the CeA than subordinate and dominant animals (Fig. 8). This finding may be interpreted in light of recent research showing that OT activity within sub regions of the CeA produce either inhibition in medial central amygdala (CeM) neurons or excitation of lateral central amygdala (CeL) neurons (Huber et al 2005). Combined, these findings suggest that mid animals may deem socially relevant rank related stimuli more neutral than either dominant or subordinate animals because they have a larger distribution of OTR within both the CeM and CeL divisions of the CeA. This distribution may manifest perceptually and/or behaviorally in such a way that mids find rank related social stimuli neither as fearful nor non-fearful as the other two ranks. For instance, it is possible that in dominants a majority of OTR lie in the CeM where rank related social stimuli triggers OT release and results in inhibition of the fear pathway, while in subordinates excitation of the fear pathway is achieved by the same rank related stimuli because OTR is more localized in the CeL. Because the spatial resolution of the current OTR binding data does not lend itself to delineations of this precision (between CeM and CeL) further study will be required to explore this possibility.

Frontal Cortex

Frontal Association Area and Orbitofrontal Cortex

Subordinate animals displayed greater levels of OTR binding in both the FrA and the OFC compared to all other ranks of animals. The frontal association area (FrA), as delineated in Paxinos and Watson (2004), consists of the most rostral portion of the frontal cortex and extends caudally an enormously small amount (~500μm). Though considered a portion of the neocortex it does not have a well-defined internal granule layer (Preuss 1995) and has historically been combined with larger sections of rat cortex so that many past researchers who report data from the FrA are referencing completely different areas, for examples see (Hambrecht et al 2009; Holson & Walker 1986; Kolb 1974; Preuss 1995). Therefore, a representative comparison of previous research and the FrA data I have reported is not included. Instead, a combination of amygdalar and frontal cortex activity as it resembles human data will be discussed.

The human homologue of the rat FrA would arguably be the prefrontal cortex (PFC), which consists of several subdivisions, but in our species it is exponentially

larger, subserves countless actions, does not fully develop until early adulthood and is widely accepted as an inhibitory mechanism that keeps more "primal" neural functions from being carried out. In short, it has been posited as the area of the brain most responsible for the unique quality of being human. Though almost nothing is known about the rat FrA and it obviously isn't utilized in the same manner as the human PFC, it is interesting to note that there are several findings linking the amygdala and human frontal cortices to both social status and OT action reminiscent of the findings in rats reported here.

In humans, fMRI studies have shown the ventromedial prefrontal cortex is functionally connected to the amygdala when viewing emotionally valenced images of faces (Heinz et al 2005), while perceived parental social status during early childhood correlates with greater amygdala activity while viewing images of angry faces (Gianaros et al 2008). Furthermore, self reported rankings of social status correlate with gray matter volume in the medial frontal cortex (Gianaros et al 2007). In a study that created artificial social ranks through the use of a video game in which participants were led to believe they were competing with other individuals the dorsolateral prefrontal cortex was activated when viewing a more dominant player while the amygdala became more active when hierarchies were unstable (Zink et al 2008). Given this information, it is not unlikely that a pathway sensitive to social rank related information could exist between the amygdala and frontal cortex of both humans and rats.

Kolb (1974) found that lesioning the OFC of rats resulted in more aggressive encounters towards male conspecifics but not toward predator-like stimuli or territorial threat. This finding was later replicated (De Bruin et al 1983) and a role in inhibiting aggression was attached to the OFC of the rat. It is not known whether OT activity in the rat OFC is excitatory or inhibitory, but if it is indeed excitatory it stands to reason that subordinate animals may be deemed as such in this study because of a tendency to be less aggressive toward same sex conspecifics and do not compete for chocolate or water as vigorously as other animals. Very little else is known about the OFC of the rat but given its proximity to the FrA and the findings mentioned above it could very easily be a component part of the pathway from the frontal cortex to the amygdala of the rat.

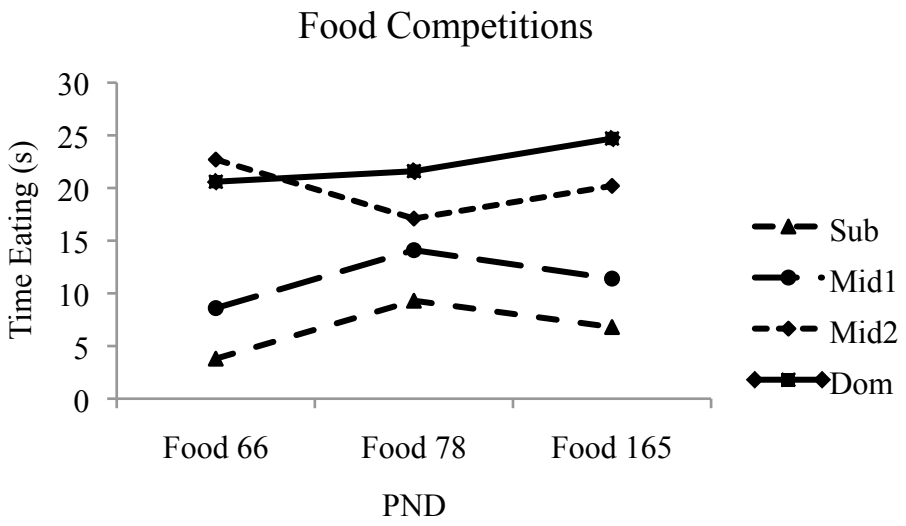
3.5 Conclusion

If simple images of human faces elicit responses from the amygdala and frontal cortex that correlate with social status and OT activity, it stands to reason that this sort of immediate processing is occurring in rats when they encounter one another in their environment as well. Just as we are sensitive to social cues like the expression of anger and sadness, rats identify socially relevant characteristics in their species. These perceptions of and responses to socially relevant stimuli presumably affect the behavior of the animal they are occurring in just as our neural responses to stimuli affect our behavior. If these assumptions are valid, then it is possible that similar neurotransmitters and neural substrates are involved in the

psychosocial perception of relevant social cues that influence relative social positions within a society in both rats and humans and likely most other mammals. In terms of the neural structures that result in or are the result of the unique psychosocial perceptions that accompany social status we have shown that in three limbic structures OTR distribution is an integral component of this process and does not reflect differences in available resources or environmental experiences prior to group formation present in other paradigms. These findings highlight the NAcc, CeA and frontal cortex as promising areas in which interventions can be targeted to attenuate the negative effects associated with differential social status. Our behavioral findings may help to further the knowledge of the contribution of psychosocial states attributable to social status differences on a variety of emotional disorders. Lastly, this novel paradigm results in rank differences that mimic the graded behavioral outcomes prevalent in human research of social status and health making it a valuable animal model with which to examine other aspects of health/status disparities that may be relevant to humans.

Figures 3.6

a.



b.

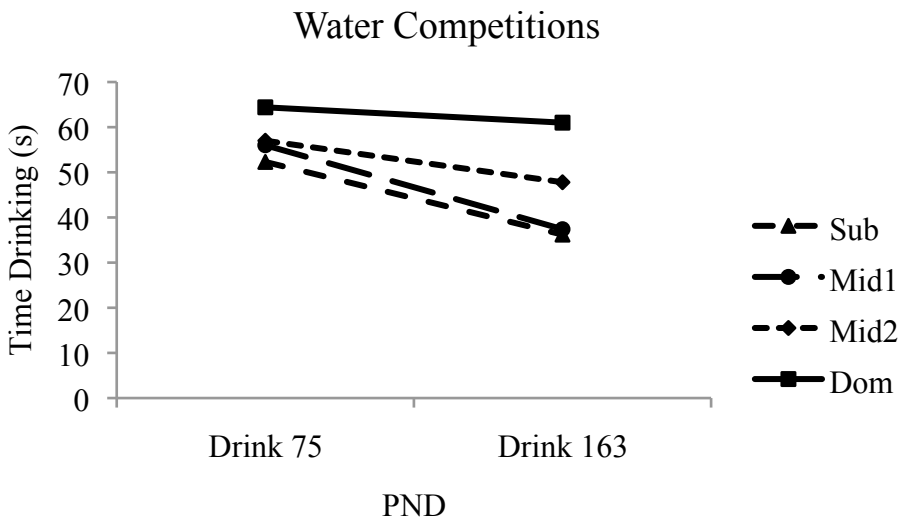


Figure 1.
Results of competitive bouts for (a) food and (b) water show a high degree of stability from early adulthood onward.

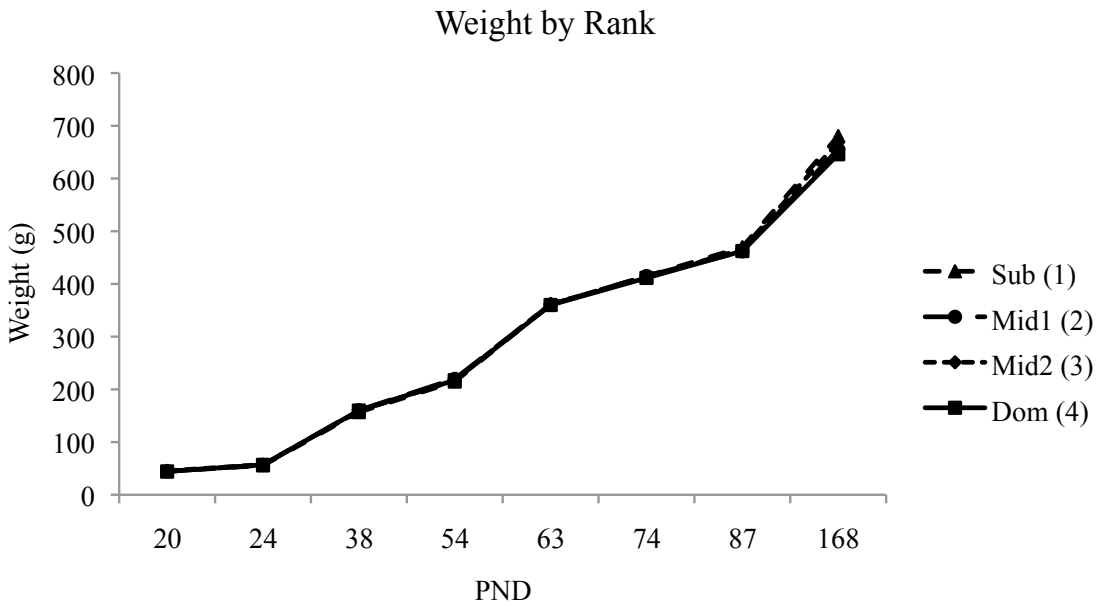


Figure 2.
Weight did not differ by rank throughout the entire study. The growth curves for all ranks are virtually identical until a miniscule amount of variation appears in middle adulthood (PND 168).

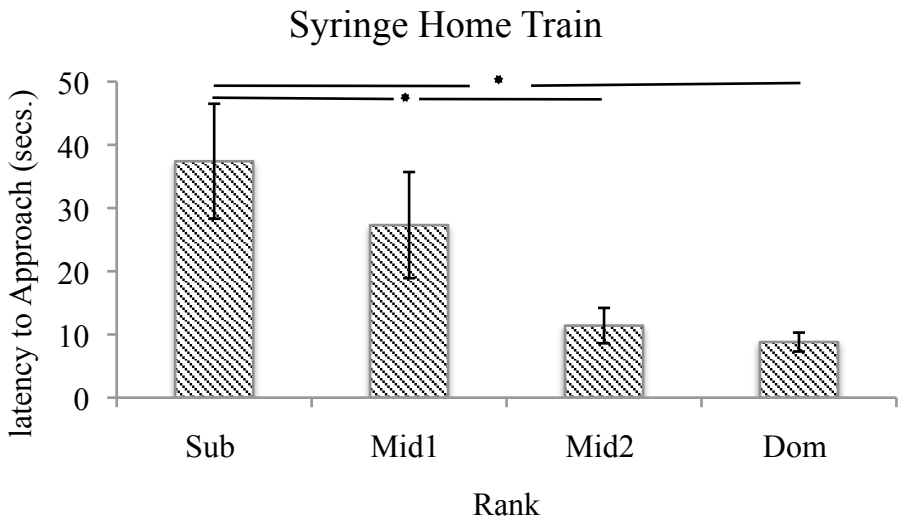


Figure 3.
A significant main effect of rank (p=.008) was revealed in the latency to first approach a syringe with chocolate on the plunger during the training portion of a cognitive task. Post hoc tests revealed a simple effect between subordinate and dominant animals (p=.oo8) and a trend towards significance between subordinate and Mid2 groups (p=.07).

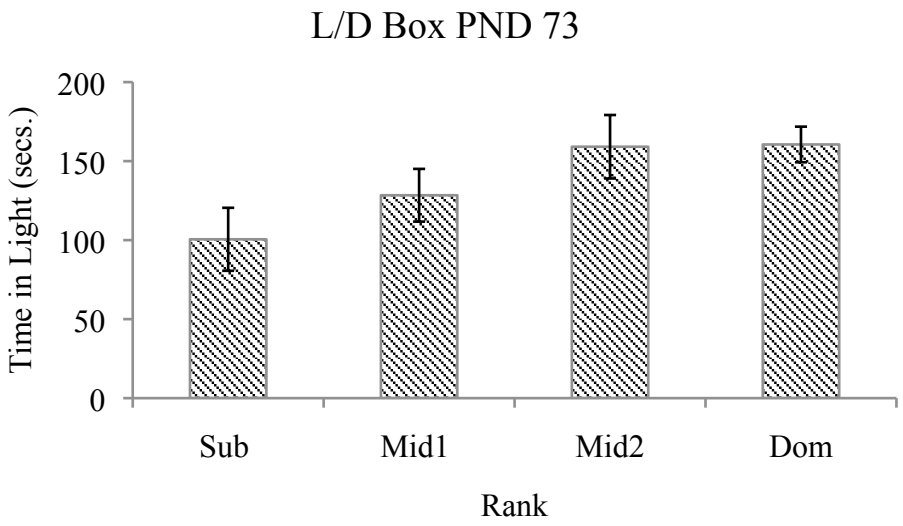


Figure 4.
A significant main effect of rank (p=.046) was present in the amount of time spent in the illuminated portion of the L/D box at PND 73. Again, this pattern of rank difference occurred in a graded manner.

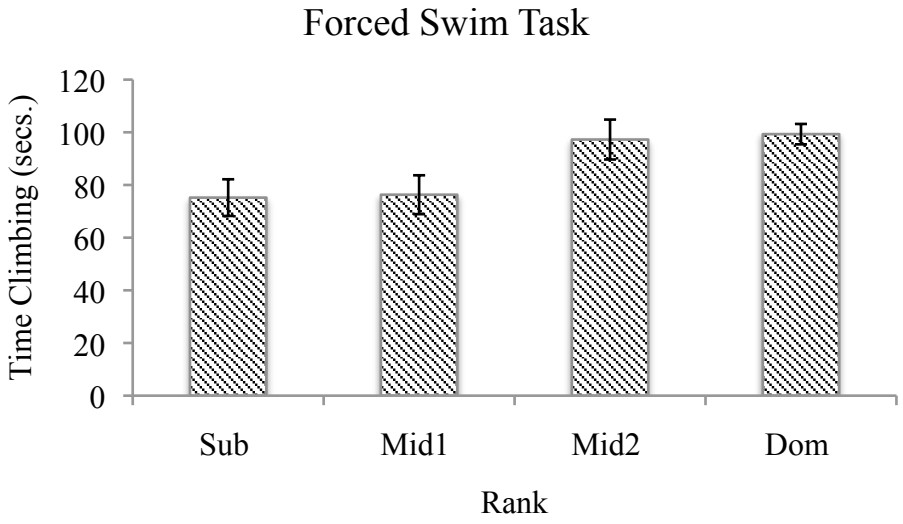
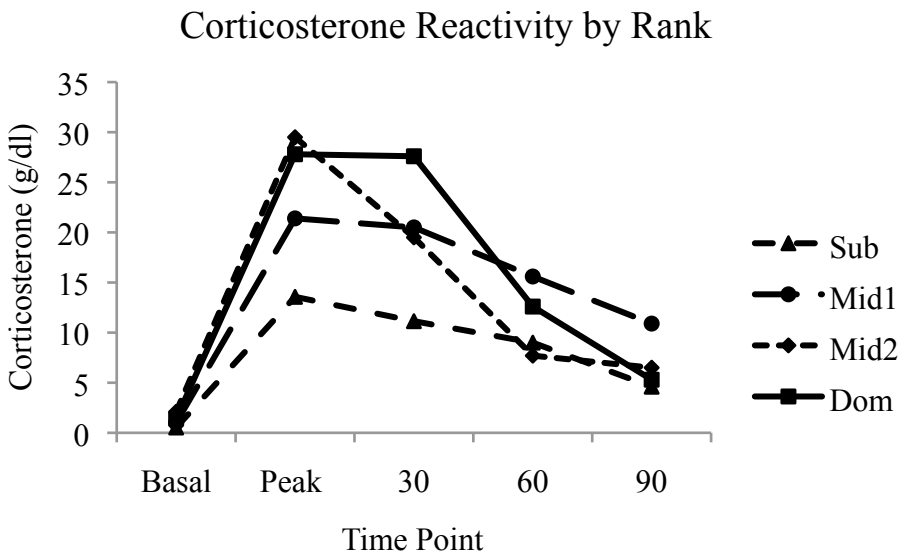


Figure 5.
A significant main effect of rank (p=.015) was revealed in the duration of time spent actively trying to escape from a water filled tank in the forced swim task. This is an ethologically relevant measure of depressive like behavior in rats and these results are quite similar to the graded relationship seen among humans with respect to prevalence of depression across social strata.

a.



b.

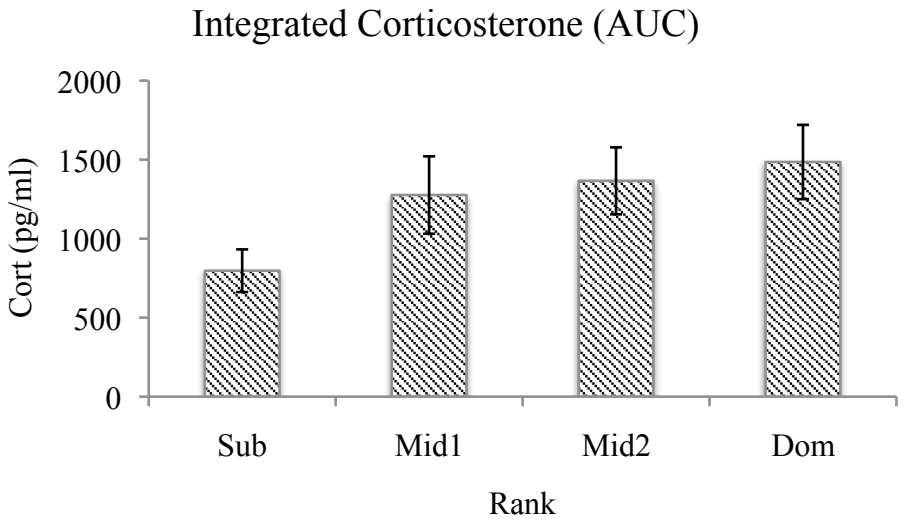


Figure 6.
Endocrine reactivity profiles across ranks in response to an acute restraint stress task at (a) baseline, peak and 30, 60 and 90 minutes after release from restraint and (b) the total amount of corticosterone experienced over the duration of the entire task by rank. Omnibus ANOVA revealed a main effect of rank ($p<.05$) as measured by the area under the curve (AUC) of total cort released. A graded relationship is present in the pattern of total cort released as a function of rank.

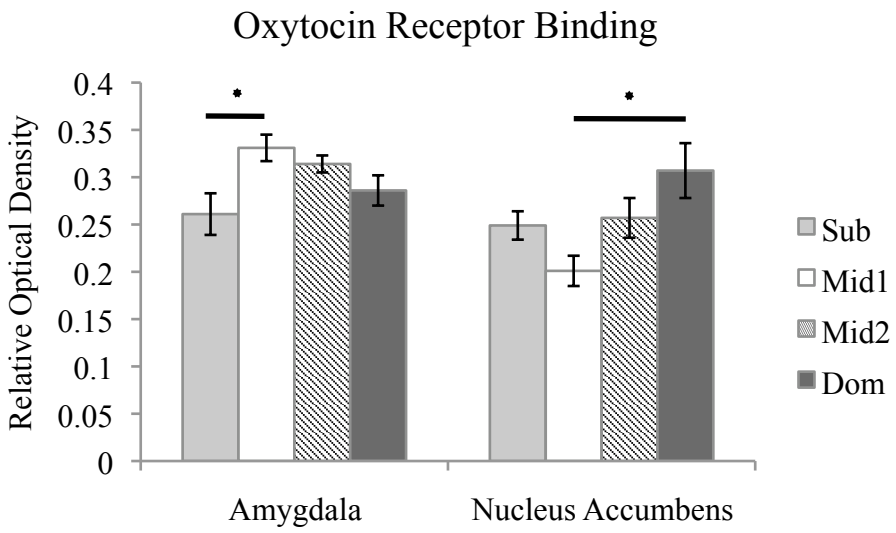


Figure 7.
A significant main effect of rank was seen in the density of OTR binding in the Amyg (p=.021) and NAcc (p=.009) of adult animals. Post hoc analyses revealed a simple effect between subordinate and Mid1 animals in the Amyg (p=.017) and between Mid1 and dominant animals in the NAcc. (p=.005).

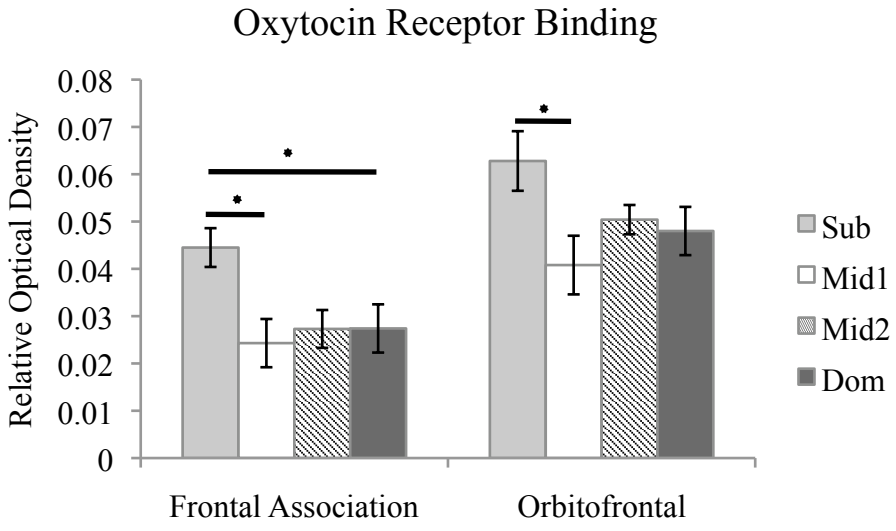


Figure 8.
A significant main effect of rank was seen in the density of OTR binding in the FrA (p=.009) and OFC (p=.05) of adult animals. Post hoc analyses revealed two simple effects in the FrA, one between subordinate and Mid1 animals (p=.016) another between subordinate and dominant animals (p=.04). In the OFC a simple effect was found between subordinate and Mid1 animals (p=.04).

Chapter 4

Influence of Early Environment on Social Position Within and Between Populations

4.1 Introduction

It is common knowledge that early life constitutes an important developmental window in which adverse experiences can have lasting behavioral, physiological and psychological effects. It is also well known in animal research that perturbations of parental care in early life can have developmental consequences. Many researchers have used this knowledge to further our understanding in several areas of research spanning from medicine to cognition to psychology.

As described in the introduction of this thesis, maternal separation is a paradigm that has been used for several decades as a means of perturbing early environments. It was from this paradigm that we came to know that maternal behavior in rats has a programming effect on the development of the HPA axis. This axis has been described elsewhere in this dissertation, but is being mentioned again because it is a crucial component in the expression, processing and appraisal of threatening stimuli within our environment. Abnormalities in this system could easily affect other types of behaviors reliant on the accurate appraisal of potential threats, including conspecific social cues. Obviously, I am referring to social behavior and the way in which variations in maternal care could manifest in a social environment.

Social status and maternal behavior are widely researched, though the contribution of maternal care to the process of social hierarchy formation is unknown. Researchers of socioeconomic status in humans are acutely aware that early life adversity could be a contributing factor to the health disparity present in society. For purely ethical, practical and logistical reasons they are simply incapable of conducting experiments that would shed light on the problem. Using our animal model of hierarchy formation, however, along with the ability to measure maternal behaviors known to be important for the development of relevant neurobiological mechanisms we can explore the impact that differential maternal care has on social hierarchy formation. This model and technique can be used to study many aspects of hierarchy formation and characteristics of social rank amongst populations that differ in a known variable. Here we use it for the first time to determine whether natural variations in maternal care confer a predisposition towards dominant or subordinate social status. We also attempt to assess characteristics of rank across populations that consist of varied levels of maternal care. Additionally, we collect behavioral measures of anxiety and motivation before group formation to assess their causal role in rank formation.

To accomplish this we will create four distinct conditions that vary by housing make-up. As before, all animals will be matched on all variables (save one). The first condition consists of pair housed rats from different maternal care backgrounds (highs housed with lows). The next three conditions will consist of rats that are matched on level of maternal care and housed in groups of three. These three conditions, though, differ in amount of maternal care received such that condition two is composed of cages of rats all from the same maternal background (highs housed with highs), condition three consists of mids housed with mids and so on for a total of four conditions. More detailed methods are given below and levels of receptor binding are examined as before.

4.2 Methods

Animals

Male Long-Evans rats born in our colony from animals originally purchased from Charles River were used in the study. Parent animals were housed individually for one week after delivery to habituate them to the new environment. On day 8 a single male and female were housed together for another 6 days. A total of 40 pairs of animals were mated. After 6 days of cohabitation each pair was again individually housed and females were under animal facility care until parturition (PND 0). All animals had ad libitum access to food (Purina Rat Chow, Purina Mills, St. Louis, MO.) and tap water. Temperature was maintained at 20 ± 2°C, humidity was maintained at 50 ± 5% and a light/dark cycle of 12-h (lights on at 0700 h) was maintained throughout the study. The Office of Laboratory Animal Care at the University of California, Berkeley, approved this study.

Maternal Observations

Beginning on PND 1, litters were observed at three different time intervals (6-8am, 12-1pm and 6-8pm) for a total of five hours per day over the next 7 days. Over these 7 days cages were maintained by the researchers to minimize any differences in care the litters may have received. The 6-8 am and pm intervals flanked lights on and lights off (7am and 7pm, respectively) time points in order to acquire a more representative and complete measure of maternal care. Red lights were used to illuminate litters during the lights-off portions of the observations and turned off after observations were completed for that time interval. For each litter, maternal behavior was recorded once every two minutes during each of the observational periods for a total of 1050 data points per litter. Maternal behaviors recorded were “on”, mother covering the nest of pups while stationary and usually sleeping/resting, “L/G”, mother actively licking and grooming any pup in the litter while on the nest and “off”, mother not on the nest of pups. “Off” encompassed all other maternal behaviors, such as eating, drinking, self-grooming or sleeping off the

nest. An index of maternal care was calculated for each litter by simply dividing the number of L/G observations by the total number of observations.

Group Formation

Non-littermate male offspring were housed with weight-matched conspecifics in all conditions. Three populations of 24 animals were group-housed three per cage with conspecifics that matched their maternal L/G background. Offspring from litters that fell one standard deviation above the mean of the measured maternal care index were used to form 8 cages of high L/G rats. Animals that fell one standard deviation below the maternal care mean were similarly used to create a cohort of low L/G rats (also 8 cages). A cohort that consisted of average maternal care backgrounds formed the "mid" group. In these three conditions (N = 72) animals in each cage were matched as closely as possible on maternal care received, not merely with others from the same distribution of maternal care received. A fourth cohort of non-littermates was housed in pairs that were weight matched, but came from opposite maternal backgrounds. Ten cages (N=20) of pair housed animals consisted of one high L/G rat and one low L/G rat to determine if differential early experience would drive social rank formation. All animals from all conditions were weighed the day prior to group formation (PND 23). On PND 24, theses animals were housed as described above in clear guinea pig cages measuring 20in x 16in x 8.5in (group housed) or in traditional rat cages (19in x 10.5in x 8.5in (pair housed)). Groups were composed of three animals rather than four, as in the previous study (Ch. 3), since mid1 and mid2 animals did indeed fall between dominant and subordinate animals in a graded manner.

Social Competitions

Water

Competition for water began after depriving all animals of water for 6-8 hours. The introduction of a single water bottle signaled the beginning of a competitive bout. All animals within a cage had equal access to the single bottle and more than one animal could access the bottle at any given time. The coat of each animal in a cage was colored red, black, or green or left white (animals left white were handled as though they were being colored) before each bout in order to clearly distinguish their identity. Each bout was digitally recorded and a researcher blind to the experimental condition of the animals measured the amount of time each animal spent drinking from the water bottle.

Chocolate

Before group formation miniature chocolate chips were intermittently placed in all cages of litters in order to acclimate the animals to its palatable nature. During competitions a small portion of chocolate was melted to a dish, allowed to harden, then the dish was attached to the inside wall of the animals' home cage with double-sided tape. The dish was small enough that only one animal could access the chocolate at any given time. Once again, each bout was recorded and the amount of time each animal spent eating the chocolate was measured by an individual blind to the condition of the animals.

Rank Assessment

Animals with larger eating/drinking times were deemed dominant over those with smaller times and assigned a rank of 1 (dominants) to 3 (subordinates) accordingly. Final ranks were assigned by averaging the rank scores of all animals from the last water and food challenges (PND 111 and 109 respectively). In the event of a tie the food competition (PND 109) ranking was used to assign rank.

Thatcher Britton/Open Field

Animals were tested at PND 22 (before group formation) and 39 for anxiety and motivation to approach/consume chocolate. This was done to determine whether baseline differences in the motivation to eat chocolate were actually determining what we measured as high or low status. The testing apparatus consisted of a square transparent Plexiglas box measuring 90x90cm. A 40x40cm box was demarcated in the center of the floor of the testing apparatus (the four sides parallel with the walls) with masking tape. The inner portion of this box was deemed the 'center' and an animal was recorded as occupying this center area once its four limbs were inside of it. A trial began when a single animal was placed in the corner (same for all animals) of the box. A mini chocolate chip (Nestle's) was placed in the center of the inner box before any individual trial began. The latency for the subject to enter the center of the testing apparatus, amount of time spent in the center of the apparatus, the latency to make contact with the chocolate chip and the consumption of the chip was recorded for each animal. Each trial was ended after 5 minutes regardless of performance. After each trial the apparatus was thoroughly cleaned and allowed to dry in order to prevent the potential influence of olfactory cues from affecting the subsequent subject.

Light/Dark Box

A box (100cm x 50cm x 20cm) made of half transparent (light) and half opaque (dark) Plexiglas was used for this task. The opaque portion was separated from the translucent by an opaque wall with a hole in it that rats could move through to enter the light half of the box. To begin each trial, rats were placed in the opaque portion headfirst through the hole that separated the two halves of the box. The researcher then left the room while the trial was digitally recorded. Each trial lasted 5 minutes (300 seconds) and was later scored by an observer blind to the condition the rats belonged to. Behaviors scored were "latency", time taken to first enter (all four limbs of the animal through the doorway) the light portion of the box, "pokes", number of times the nose (or more) of the animal broke the plane of the doorway, but then reentered the dark portion of the box and "light time", amount of time the animal spent in the light portion of the box. This task was performed intermittently throughout the course of the study (at PND 21, 38, 46 and 73).

Cognitive Performance

A syringe puzzle task was used to measure cognitive performance in adult rats (PND 117 and 118). Rats were first given a training trial in which they were

individually exposed to a clear plastic syringe with chocolate melted to the middle of the plunger and taped to the floor of either their home cage or a novel plastic box (counterbalanced over all animals). For this 90 second training trial the plunger was pulled out making the chocolate immediately accessible and rats were free to explore and eat the chocolate during this time. A second trial (puzzle trial) was conducted in the same cage two hours later with the plunger pushed into the syringe making the chocolate inaccessible unless the animal pulled the plunger out of the syringe far enough to expose the chocolate (~2cm). The animal successfully "solved" the puzzle if they gained access to the chocolate. Latency to solve the puzzle was the dependent measure of this task and the trial was ended after 120 seconds if unsuccessful. The next day all animals repeated the task described above, but in the opposite setting (novel box or home cage).

Restraint stress

An acute restraint-stress task was performed on animals as young adults (PND 70) and adults (PND 163). Rats were removed from their home cage between 7a.m. (the beginning of the "light" portion of their day) and 10a.m. then immediately had the tip of their tail clipped and approximately .5 ml of blood was collected from the wound in 1.5ml plastic tubes with snap on lids. This process was not performed after 10am to prevent the natural circadian release of corticosterone from positively skewing the amount measured. This initial blood sample was collected no later than one minute from the retrieval of the animal from its home cage (basal) to ensure that the presence of corticosterone in the blood, which is released in response to the task, was avoided. Animals were then placed in conical plastic sleeves (Decapicones) of which the larger end was taped shut. This rendered the animal incapable of moving. An opening in the small end of the sleeve allowed the animal to breath normally while restrained. After 15 minutes of restraint a second blood sample was collected (peak) and the animal was released back into its home cage. Blood was subsequently collected from the animal once every 30 minutes after release for the next 90 minutes (30, 60, & 90) for a total of 5 samples (basal, peak, 30, 60, 90) per animal. All blood samples were collected in 1.5ml plastic tubes and placed in an ice water bath to prevent coagulation. Once all samples were collected they were centrifuged at 5000 rpm for 10 minutes. The plasma supernatant was then aliquoted from the samples and stored at -20°C until the time of the assay. If the tail wound started to scab over and prevented sample collection the tail was placed in warm water and massaged to remove the coagulated blood and allow bleeding. This process was accomplished in a matter of seconds. Once bleeding resumed the tail was dried with a paper towel and blood was collected as described above. Animals were held in the left arm cradled against the standing experimenter's torso while the experimenter's fingers held the base of the tail to prevent the animal from getting loose. With the right hand, the experimenter ejected blood from the tip of the tail by stroking the tail from base to tip in a "milking" motion. The resulting blood dripped into collection tubes sitting in a tube holder on a table in front of the experimenter.

Corticosterone EIA

Measurement of all plasma corticosterone was performed according to the instructions of the corticosterone enzyme-linked immunosorbent assay (EIA) kit (Assay Designs) with the following exception. The dilution of plasma to assay buffer that yielded optimal concentrations of corticosterone as compared to the standard curve of the assay was 5μl of plasma to 95μl of buffer, or 1:20 dilution. Plates were analyzed at 405nm using a BioRad cell plate reader. All samples were run in duplicate.

Receptor Autoradiography

Adult Animals were sacrificed at PND 165 upon completion of adult behavioral testing. Following decapitation, brains were quickly removed, placed on powdered dry ice, and stored at −80 °C. Brains were then sliced in 20μm coronal sections containing the medial frontal cortex, nucleus accumbens, lateral septum, and amygdala. Sections were mounted onto Permafrost slides and stored at −80 °C until the time of assay. For OTR binding, [^{125}I]-ornithine vasotocin analogue [(^{125}I)OVTA] was employed [vasotocin, d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Orn^8, (^{125}I)Tyr^9-NH_2]; 2200 Ci/mmol]; (NEN Nuclear, Boston, MA, USA). For V1a receptor binding, ^{125}I-lin-vasopressin [^{125}I-phenylacetyl-D-Tyr(ME)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH_2]; (NEN Nuclear) was used. Sections were allowed to thaw to room temperature (RT) and then immersed in 0.1% paraformaldehyde for 2 min to optimize tissue integrity. Sections were then rinsed two times in 50 mM Tris-HCl (pH 7.4) at RT for 10 min and incubated for 60 min at RT in a solution of 50 mM Tris-HCl (pH 7.4) with 10 mM $MgCl_2$, 0.1% bovine serum albumin, 0.05% bacitracin, and 50 pM ^{125}I-lin-vasopressin or 50 pM ^{125}I-OVTA. Non-specific binding was determined by incubating adjacent sections with the radioactive specific ligand as well as 50 μM of unlabelled Thr4, Gly7 oxytocin, a selective oxytocin ligand (Peninsula Laboratories, Belmont, CA, USA) or 50 μM of unlabelled [1-(β-mercapto-β,β-cyclo-pentamethylene propionic acid),2-(O-methyl)-tyrosine]-arg^8-vasopressin, selective for the V1a receptor. Following incubation, sections were washed 3 × 5 min in 50 mM Tris-HCl (pH 7.4) with 10 mM $MgCl_2$ at 4 °C, followed by a final rinse in this same buffer for 30 min on ice. Slides were then quickly dipped in cold dH_2O and rapidly dried with a stream of cold air.

Sections were apposed to Kodak BioMaxMR film (Kodak, Rochester, NY, USA) with ^{125}I microscale standards for 72 h. Autoradiographic ^{125}I-receptor binding was quantified from film using the NIH Image program (http://rsb.info.nih.gov/nih-image/). An average of nine sections per animal per area were scored using the Paxinos and Watson rat atlas as a reference.

4.3 Results

Effects of L/G and Interactions Between Rank and Housing Condition

Maternal care did not predict eventual social rank in pair housed animals, which differed only in the amount of L/G received (p=.9), nor was there a significant main

effect of rank across the three different populations of group housed animals in any behavior measured. Within each of these individual populations, though, several interesting results emerged. Among the cohort of high L/G animals the small amount of variation in L/G received was a significant predictor of eventual social rank, $F(2,21)=4.124$, $p=.031$ (Fig. 1). No other condition showed this effect.

Interactions between rank and maternal background emerged in stress reactivity at PND 70, $F(4,49)=3.622$, $p=.012$ (Fig. 2a) and latency to approach a syringe containing chocolate in an unfamiliar setting $F(4,58)=3.202$, $p=.025$ (Fig. 2b). Significant interactions were also seen in OTR binding density in the amygdala, $F(4,57)=2.599$, $p=.046$ (Fig. 3a) and bed nucleus of the stria terminalus (BNST), $F(4,60)=3.162$, $p=.02$ (Fig. 3b).

Rank Effects

In the high L/G condition significant rank effects were seen at PND 46 in latency to enter the illuminated portion of the L/D box, $F(2,21)=4.352$, $p=.026$, time spent in the center of an open field baited with chocolate (PND 22), $F(2,21)=3.883$, $p=.037$ in the Thatcher-Britton (T-B) version of the open field task and latency to make contact with the chocolate during another T-B task at PND 39, $F(2,21)=3.675$, $p=.043$ (Fig. 4). The mid L/G condition similarly showed a significant effect of rank in the amount of time spent in the center in the T-B (PND 22) task, $F(2,20)=7.342$, $p=.004$, (Fig. 5) though the direction of the effect was dissimilar. A single effect of rank was seen in each of the three L/G conditions in OTR binding, each in a different area. The low L/G condition significantly differed in OTR density in the amygdala, $F(2,16)=4.83$, $p=.023$, the mid L/G condition in the BNST, $F(2,20)=7.159$, $p=.005$ and the high L/G group in the NAcc $F(2,21)=4.754$, $p=.02$ (Fig. 6). In the pair housed condition an effect of rank was seen in both the training and testing portions of the syringe puzzle task in the home cage, train $F(1,15)=6.01$, $p=.027$, test $F(1,16)=5.276$, $p=.0351$, the latency to enter the light portion of the L/D box (PND 73), $F(1,16)=5.048$, $p=.039$ and the amount of time spent in the light $F(1,16)=5.125$, $p=.038$ and also in approach, $F(1,16)=6.28$, $p=.023$ and withdrawal, $F(1,16)=6.093$, $p=.025$ behavior in a social interaction task during adulthood (Fig. 7).

4.4 Discussion

L/G Effects and Interactions

The major goal of this dissertation was to determine whether early life differences in the form of maternal care would affect the eventual adult social position a rat would come to occupy. According to my results it is difficult to conclude with certainty and like so many questions in scientific research the answer so far is “it depends”. Based on the results of the pair housed cohort of animals it would seem that maternal care has little to do with eventual rank. These animals were matched on every characteristic that was in our power to control and purposefully differed solely in the amount of maternal care they received as infants. Despite this, no difference in rank formation was associated with this manipulation

(Fig. 1). Ironically, one group's maternal background was predictive of social position. Animals matched on high levels of maternal care showed a significant effect of L/G received on adult social status such that animals that received the highest levels of maternal care became subordinate and those that received the least became dominant (Fig. 1). What this finding suggests is that maternal care does not uniformly affect all populations. While a small amount of variation among high L/G animals confers a social consequence a large amount of variation among polarly opposed groups has no detectable effect at all on social status.

Several interactions between rank and L/G-based housing groups did emerge. Stress reactivity at PND 70 revealed that high L/G animals produced the greatest and almost least amounts of corticosterone depending on rank and displayed a pattern of reactivity in opposition to both low and mid L/G groups. Subordinate highs release just over half as much cort as meso and dominant highs, while subordinates of both low and mid groups release the largest amounts of cort in their groups (Fig. 2a). Data on the dynamics of social hierarchies and stress reactivity would suggest that based on these results there is more competition for dominant status among meso and dominant rats in the high L/G group and more reinforcement of status on subordinates by dominants in the low and mid L/G housing groups (Sapolsky 2004). An interaction in the latency to approach (not solve) the syringe puzzle task in a novel setting reveals that mid dominant and low subordinate rats are less fearful/more exploratory in a novel setting than differentially ranked animals from other groups. While mid dominants are quickest to approach low dominants are the slowest (Fig. 2b)

OTR binding in the Amyg and the BNST both show an interaction between L/G group and rank (Figs. 3a and 3b, respectively). In the amyg, high subordinates show the least density of binding while low subordinates show the greatest binding densities. This pattern reverses itself in dominant animals with low dominants showing the least amount of binding and high dominants showing the greatest. In the BNST, high and low L/G groups seem not to vary much by rank in OTR binding, whereas mid L/G rats show very large levels of density as subordinates and low levels of binding as dominants.

Baseline Measures

Another important aim of this study was to determine if other characteristics present before group formation were predictive of social status or the characteristics we used to determine social status. The Thatcher-Britton (T-B) version of the open field test of anxiety incorporates a motivational component to determine whether an animal's desire to explore an object (any ethologically relevant item or substance) placed in the center of an open/unprotected arena will overcome their desire to avoid danger (open area). We conducted this test at PND 22 (before group formation) and 39. Another task that exclusively measures anxiety/fear (L/D box) was also performed before group formation. Behavioral results from the T-B test on PND 22 in the high and mid L/G conditions were predictive of eventual rank (Fig. 5). This finding is in relation to the amount of time

spent in the center portion of the T-B apparatus and not the latency to approach or time spent in contact or consuming the chocolate in the center of the arena. It is more perplexing that the directions of these significant effects are in opposition to each other. Rats from the mid L/G group that spent significantly more time in the center area came to occupy the dominant position in the group, while high L/G animals that spent significantly more time in the center became subordinate members of their group. The data used for these analyses were free of outliers and normally distributed in both conditions, which makes this result difficult to interpret. It can be argued though, that our measure of status based on competition for water and chocolate is valid since a clear direction of effect is lacking and the significant behavioral measure in the task was not linked to the motivational drive to explore or eat the chocolate as it was amount of time in the center of the arena that was predictive of social position. Having said this, it is possible something we have not detected in the analysis of this data is influencing social status formation.

Rank Effects

Effects of rank were seen in OTR binding density in a single brain area in each of the three group housed conditions (Fig. 6). In the mid L/G cohort significant OTR binding differences were seen in the BNST between both dominant and subordinate and dominant and meso animals. The differences in BNST OTR binding due to rank mirror the significant differences seen between these groups in the amount of time spent in the center of the T-B arena at PND 22 discussed above. Increased center time indicates attenuated expression of anxiety and the BNST has been implicated in mediating the expression of fear and anxiety (Veinante & Freund-Mercier 1997). Additionally, variations in OTR binding in the BNST due to differences in maternal care have not been present in male rats (Francis et al 2002b), hinting that the differences seen here, if associated with the behavioral differences seen at PND 22 may be genetic or caused by something other than maternal care while still in the presence of the litter.

A variation in OTR binding in the NAcc of high L/G animals was seen between dominant and subordinate rats with dominant animals displaying higher levels of OTR in this area. This finding replicates an earlier finding from chapter 3 in which dominant animals possessed larger amounts of OTR in the NAcc. Lastly, a significant difference in OTR binding density was seen between dominant and meso rats of the low L/G housing group in the amygdala. Meso rats display significantly greater levels than dominant rats. This finding does not coincide with any behavioral differences in this cohort in the present study. In fact, no differences in rank were seen in the low L/G housing group throughout the entire study. However, this finding closely resembles the difference in OTR amyg binding seen in meso rats in chapter 3. Combined with the NAcc results above, the current OTR binding results suggest that these differences as well as the differences seen in chapter 3 are indeed attributable to rank and replicable.

Several rank effects were seen in the pair housed cohort of animals (Fig. 7). Dominant animals consistently displayed significantly attenuated levels of anxiety

compared to subordinates. For instance, dominant animals approached the syringe in the puzzle task more quickly in the training and test trials within the home cage, emerged from the dark portion of the L/D box more quickly and remained in the illuminated portion of the box longer (PND 73) than subordinate animals. Ironically, and counter to my intuition, dominant animals engaged in significantly less social approach behavior during a social interaction task as adults and significantly more social withdrawal behavior than their subordinate counterparts. No rank differences in receptor binding emerged in pair housed animals. Perhaps this is attributable to the less social housing dynamic, i.e. two animals rather than three or four. It will be interesting to conduct a follow up experiment with larger groups of animals from different maternal care backgrounds to address this possibility.

4.5 Conclusion

A priori predictors of eventual social rank were not present for pair housed animals, but L/G predicted social rank in high L/G group housed rats. Amount of time spent in the center portion of the T-B arena predicted social rank in high and mid L/G groups, but in opposite directions. Many differences in OTR binding found in chapter 3 were replicated in the present study indicating validity of the present findings and several OTR binding differences coincided with behavioral differences indicating a possible role for these neurotransmitters in the establishment and/or maintenance of social position. Effects of rank were not seen collapsing across all group-housing conditions nor were any differences in AVP receptor binding revealed. For potential confounds in this study and others please see Chapter 5.

4.6 Figures

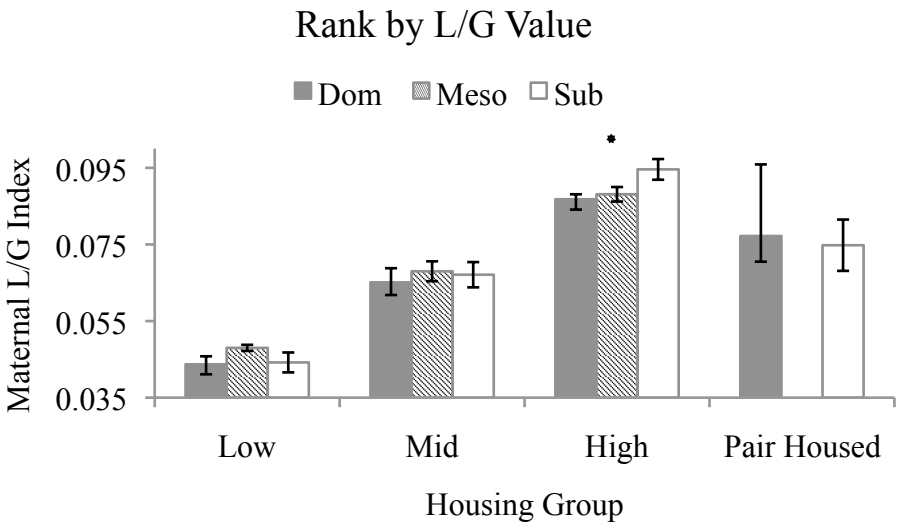
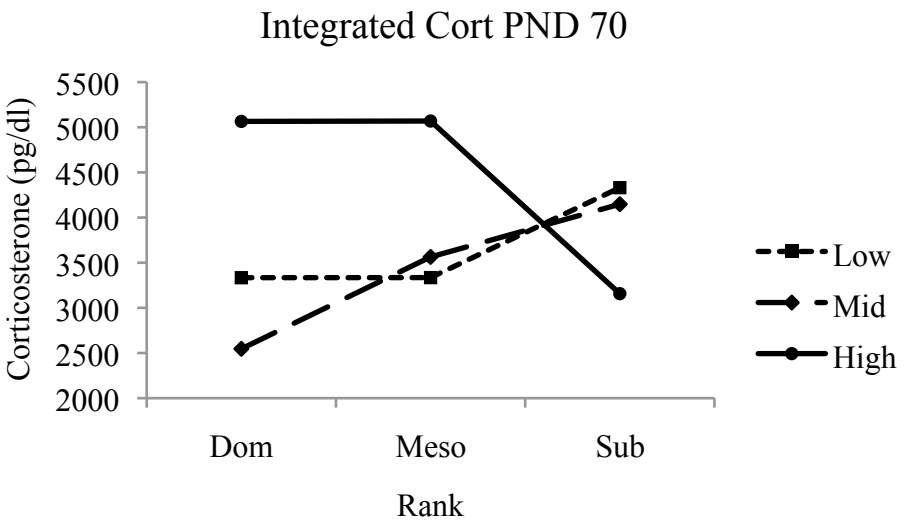


Figure 1.
A main effect of maternal L/G behavior across ranks was not seen, nor was L/G a significant predictor of rank in pair housed animals mismatched on L/G background. However, L/G did significantly predict rank among the high L/G housing condition. *p<.05

a.



b.

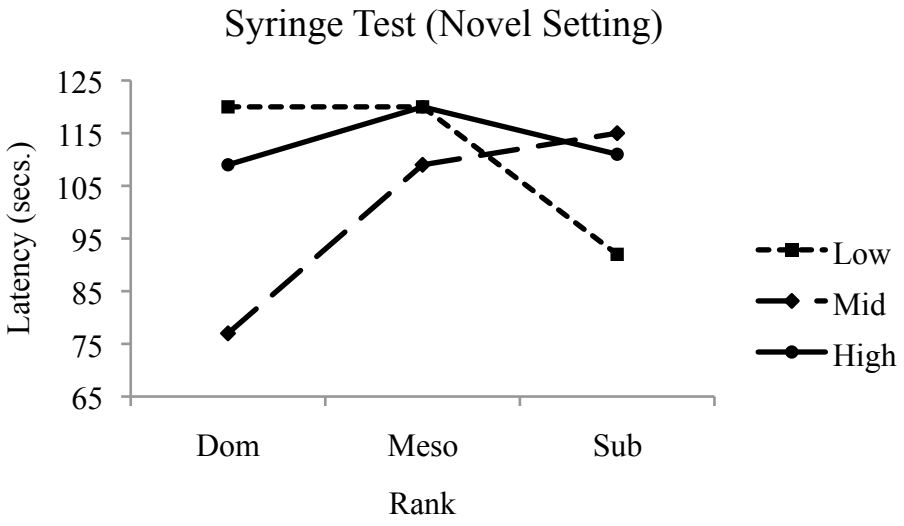
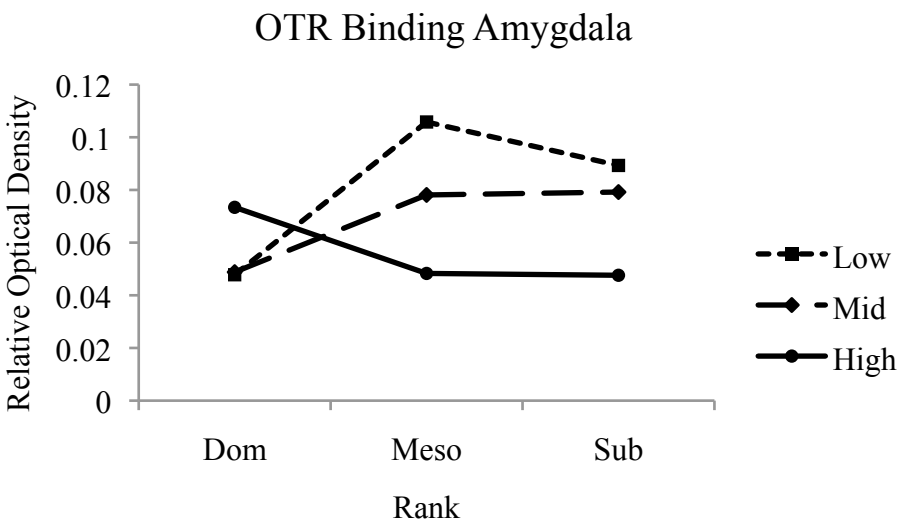


Figure 2.
Significant Interactions between social rank and L/G-based housing groups were seen in stress reactivity to restraint stress at PND 70 (a), and latency to approach a syringe puzzle in a novel/unfamiliar cage (b).

a.



b.

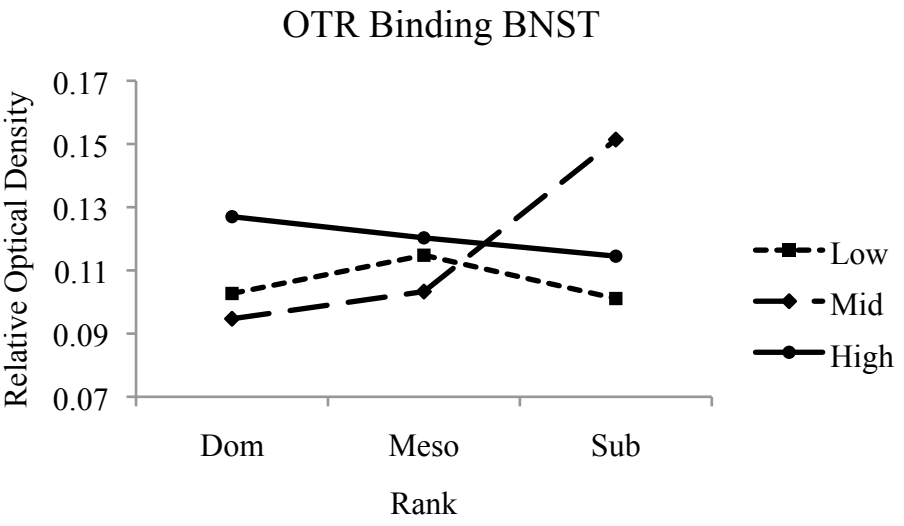


Figure 3.

Significant oxytocin receptor binding interactions found between social rank and L/G-based housing groups in the amygdala (a) and bed nucleus of the stria terminalis (BNST) (b).

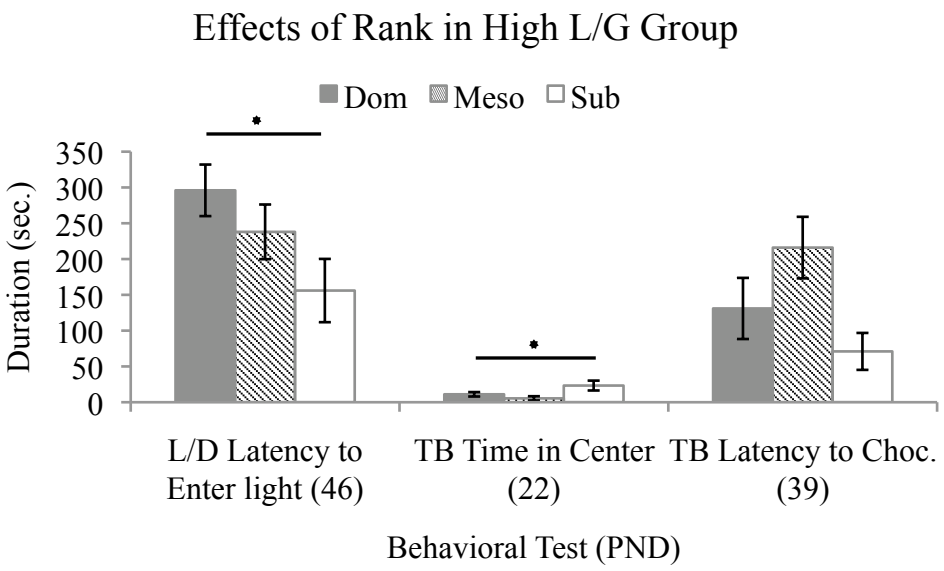


Figure 4.

Rank effects found in the high L/G-based housing group in the latency to enter an illuminated portion of the light/dark box at PND 46, time spent in the center of a Thatcher-Britton arena at PND 22 (before group formation) and latency to approach a mini chocolate chip sitting in the center of the arena at PND 39. *p<.05

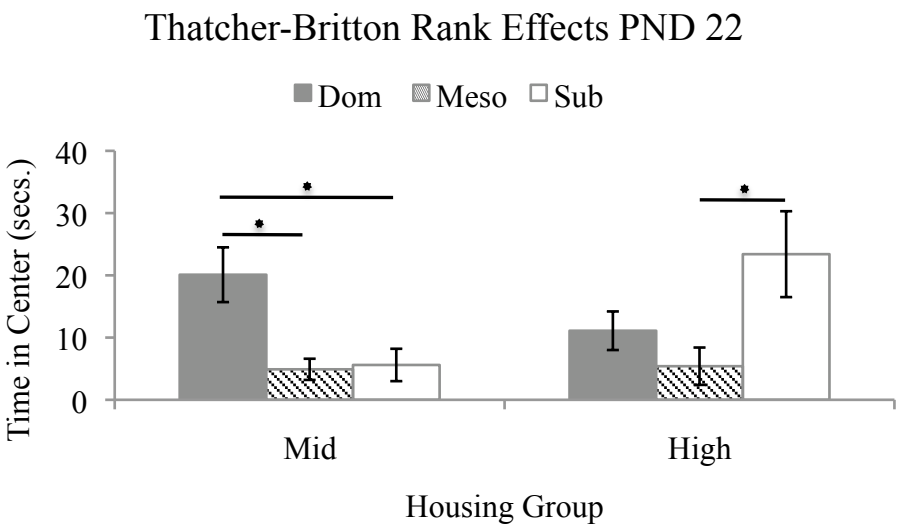


Figure 5.

Significant rank effects seen in the Thatcher-Britton task at PND 22 before groups were formed. Time in center significantly predicted future rank, though in opposite directions, in the two (mid and high) housing conditions. *p<.05

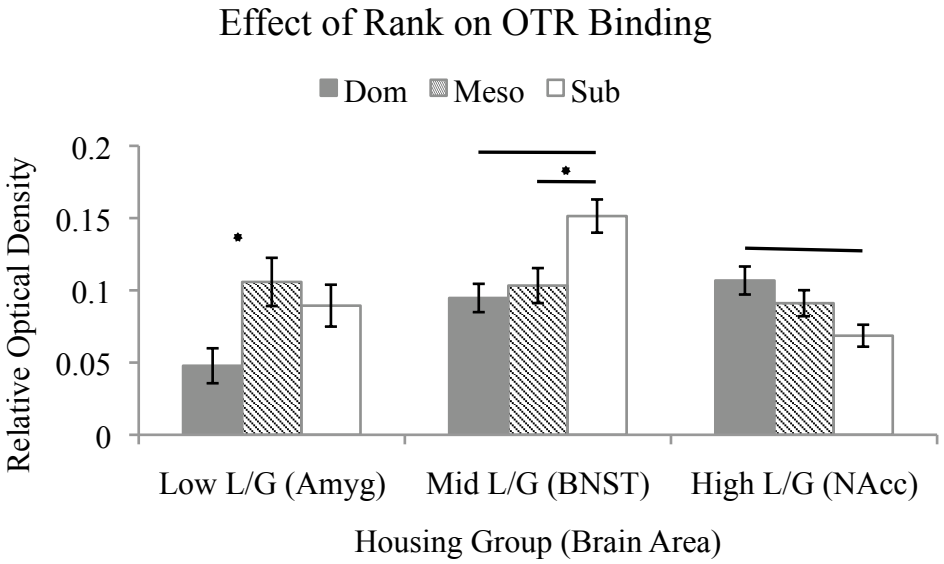


Figure 6.

Rank effects of oxytocin receptor binding density among L/G-based housing groups. Effects were seen in the amygdala of the low L/G group, in the BNST of the mid L/G group and in the nucleus accumbens of the high L/G group. *p<.05

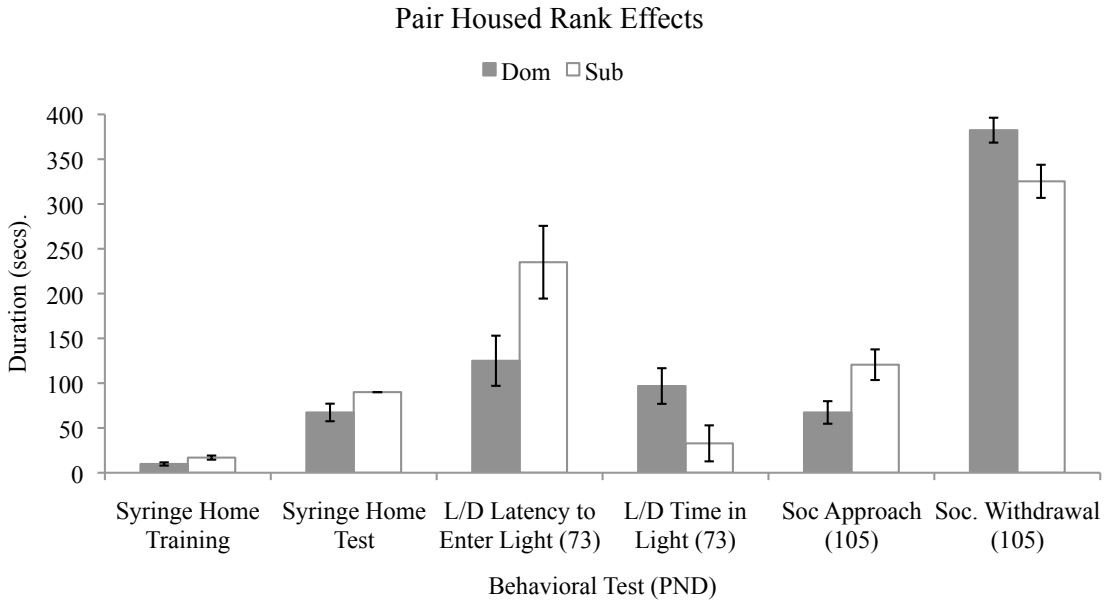


Figure 7.

Significant rank effects seen in the pair housed group, which was mismatched on level of maternal care received before group formation. Rank effects are consistent in terms of anxiety related behavior across the syringe puzzle training and test tasks in the home cage and measures of behavior in the light/dark box at PND 73. Anxiety profiles reverse according to rank in the social interaction task. All differences are significant at the .05 level.

Chapter 5

Conclusions

This dissertation was intended to explore the impact of early environment on social status and to isolate the effects of psychosocial factors attributable to social status in the hopes of unveiling neurobiological mechanisms that could be generalized to humans. Several theories concerning inequality of resources and challenging environments exist from which the negative consequences associated with lower social status can be explored. Though, these factors undeniably contribute to health disparities between social strata there is still an aspect of social status that is unaccounted for. It is apparent to me that the psychological impact of possessing a particular status within society differs as a function of that status and how one perceives it and themselves in it. I believe this aspect of status contributes to the health/SES disparity and is what I attempted to isolate in laboratory rats.

While this aspect of social status may be easier to study in humans that are capable of responding verbally to questions you might ask, it is more likely that finding neural correlates attributable solely to the experience of occupying a particular status would be found in an organism whose entire existence can be controlled and catalogued. Additionally, their characteristics, environment and experiences can be matched to truly isolate a particular variable.

To this end we devised a method of group housing in which we could assess social ranks under conditions that would be more ethologically similar to human sociality than current paradigms used to study rank differences.

Chapter 1.

As my ultimate goal was to assess the impact of maternal care differences on social behavior it seemed reasonable to see if differences in social behavior could be achieved and measured in animals whose early environment had been perturbed. MS was used instead of natural variances in maternal care because its effects are shown to be similar without undergoing the intense observational period required to assess maternal care profiles. The study resulted in differences between MS and control animals in approach and withdrawal behaviors toward conspecifics showing that early life differences in maternal care do affect social behavior in a measurable manner.

Chapter 2.

This study hoped to recreate the graded pattern of effects between social ranks and outcome measures seen in human SES research in animals that did not differ in any appreciable way before group formation. We matched these animals on weight, sex, and maternal care indices and assured that littermates were not housed together. All animals occupied large cages meant for guinea pigs, which should have ensured that every animal could access food, water and bedding at any given time. However, there may be other variables responsible for the differences revealed in this study that we did not account for, but every effort was made to make animals as

similar as possible before group formation. Results showed that a stable hierarchy did emerge under these conditions and graded rank effects were realized. Furthermore, neural correlates of rank were seen in several brain areas measured that coincided with behavioral phenotypes across ranks. These findings may inform human research as to brain areas and neurotransmitter systems that may be attractive targets for intervention research.

Chapter 3.

Since our novel animal model of social rank resulted in effects that resembled the human population we were able to finally manipulate the variable of maternal care to determine its effect on social rank formation. Groups of pair housed animals mismatched on the level of maternal care received did not differentiate into ranks predictable by maternal care. Maternal care did predict adult social rank in the group of animals matched on high levels of maternal care showing that small variations in maternal care can have a large impact in certain contexts while large variations in maternal care in another context may have no effect at all. Additionally, neural differences found in the previous experiment were replicated in some groups adding validity to their role in social status.

As this animal model is new there are potential pitfalls that we may have overlooked that will present themselves in the future. At present though, this model remains a valuable tool with which to study status relationships.

Future directions

The above paradigm can be used to explore the impact of many social and environmental variables on hierarchy formation and social mobility, perhaps making the discovery of practical interventions to improve the health of differentially ranked individuals possible. Limitations can be placed on resources for all, one, or any other number of animals within a group to determine the extent to which that resource has an appreciable impact on rank formation or stability. The same can be applied to social and environmental variables. Possibilities are constrained only by the ingenuity and creativity of future researchers.

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