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The Effect of Maternal Separation and Postnatal Oxytocin Administration on Social Recognition in Adolescent Female Mice

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Abstract

A vast body of literature indicates that exposing rodents to maternal separation during infancy results in long – term developmental changes in physiological, behavioral, and psychological domains. Because several previous studies have indicated that maternal separation may be detrimental to the processing and discrimination of social cues, the primary aim of this dissertation was to determine whether separating female infant mouse pups from their mother, but not siblings, for 3 hrs daily, during the first two weeks of life, influences social recognition during adolescence (Experiment 1). This dissertation also sought to evaluate whether postnatal administration of the oxytocin would influence social recognition or the acquisition of social odor preferences in adolescent control and maternally separated female mice (Experiment 2). The results of Experiment 1 showed that, in contrast to control subjects, maternally separated females showed diminished habituation to repeated presentations of a familiar conspecific and significant impairments in the ability to discriminate between a previously encountered and novel mouse during the dishabituation session. In Experiment 2, postnatal oxytocin injections did not substantially affect the expression of either social recognition behavior or social odor preferences by control-reared females during adolescence. By contrast, postnatal oxytocin administration improved the acquisition of social odor preferences, but did not recover social recognition behavior in MS females. The results of this dissertation suggest that altering the early social environment by means of the maternal separation procedure can disrupt the ability to recognize conspecific odor cues, which are fundamental components in establishing and maintaining social relationships later in life.

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Introduction

In mammals, a ubiquitous quality of the developmental process involves the accumulation of social experiences that gradually shape brain function and contribute to behavioral flexibility that extends across a variety of situations. For many species, this aspect of development is dependent upon olfactory cues that play a fundamental role in mediating social interactions by facilitating individual recognition and discrimination. This facet of olfactory-mediated communication, known as social olfaction, first occurs between the mother and offspring and goes on to provide the foundation for the multitude of affiliative relationships that are commonly part of the adult social world. The importance of the early olfactory-mediated experiences between the mother and offspring on long-term development has been repeatedly underscored by studies that have demonstrated how manipulations of the early social environment influence the appearance of normal displays of maternal caretaking, aggression, and social preferences and their neurochemical correlates later in life (Ferguson, Young, & Insel, 2002; Insel, 1997; Insel & Young, 2001; Lim & Young, 2006; Nelson & Panksepp, 1998). This introduction will begin with a selective review of several notable examples of social olfaction that occur throughout the course of development.

Olfactory Modulation of Social Behavior

The olfactory modulation of social interactions has been explored in several mammalian species, including rabbits, cats, dogs, sheep, pigs, and non-human primates (Barton, 2006; Curley & Keverne, 2005; Hepper, 1994; Kendrick, Levy, & Keverne, 1992; Kristensen, Jones, Schofield, White, & Wathes, 2001; Rosenblatt, 1983). Studies using these species have indicated that odor cues provide many apparent advantages in

facilitating communication between conspecifics, or members of one's own species (Doty, 1986). However, the most substantial source of empirical evidence in support of olfactory-mediated social behavior across a wide range of situations has been derived from studies using gerbils, hamsters, rats, and mice (Cornwell, 1976; Cornwell-Jones & Sobrian, 1977; Cornwell-Jones & Azar, 1982; Johnston & Petrulis, 1999; Muller-Schwarze, 2009; Pettijohn & Paterson, 1982; Thomas, Fonken, LeBlanc, & Cornwell, 2010; Veenema, Bredewold, & De Vries, 2012). The existing body of literature on rodent social behavior broadly indicates that odors are used both to discriminate between individual conspecifics and to communicate information about social relationships to fellow conspecifics. For example, Gheusi, Goodall, & Dantzer (1997) used a go/no-go odor sampling procedure to demonstrate that rats have the ability to individually discriminate the olfactory signature of a familiar live conspecific and its soiled bedding from the odor of a novel, unfamiliar stimulus rat.

One of the beneficial elements in using olfactory cues as a primary mode of communication is that such cues have been shown to play a significant role in directing behavior in situations where other forms of sensory input are difficult for animals to utilize. In rodents, this aspect of olfactory communication most notably begins to play a supporting role in survival and social learning during early postnatal experiences as pups gain familiarity with the scent of their mother and nest environment. Specifically, olfactory cues provide a critical sensory avenue through which pups can locate the nipple and nurse when the infant's eyes do not yet provide functional input for them rely on visual cues (Alberts, 2007; Bolles & Woods, 1964; Polan & Hofer, 1998; Wilson & Sullivan, 1994). Several studies have further underscored the important role of early

olfactory cues in nipple location and suckling behavior by showing increases in the latency to locate the nipple or failure to suckle following the disruption of olfactory input via lesions of the olfactory system (Risser & Slotnick, 1987; Singh & Tobach, 1975; Teicher, Shaywitz, & Lumia, 1984) or manipulation of the chemical substrates that help guide pups to the mother's ventral surface (Hofer, Shair, & Singh, 1976; Teicher & Blass, 1976). Rodent pups quickly establish an olfactory tether to the nest (Cornwell-Jones & Sobrian, 1977) and gain experience with the first in a lifetime of conspecific odors via the mother and siblings (Kojima & Alberts, 2009; Raineki, Pickenhagen, Roth, Babstock, McLean, Harley, et al., 2010). Behavioral preference for the odors found within the early social environment increases rapidly throughout the first two weeks of life as pups repeatedly encounter maternal and nest odors (Brunjes & Alberts, 1979; Maras & Petrulis, 2008). This early form of social memory is critical because it orients pups preferentially toward social odors that come to represent familiar sources of food and comfort.

In addition to aiding rodent pups in nipple location and nest orientation, early experiences with odor-oriented social cues in the nest have been shown to play a role in the long-term development of responses that rely on both social recognition and the acquisition of social odor preferences. For instance, affiliative responses and later life mating experiences, such as mate preferences, are two social contexts that have been most robustly linked to the influence of olfactory-mediated social interactions earlier in development (Brown, 1982; Mainardi, Marsan, & Pasquali, 1965; Shah, Oxley, Lovic, & Fleming, 2002). In turn, it has been demonstrated that experimental manipulations that aim to be disruptive to the mother-infant dyad, such as decreasing the quality and

frequency of interactions, will result in a non-normative shift in responses to social odors, which in at least one study has involved decreased preferences for familiar nest odors later in life (Thomas et al., 2010). Further, Saxton (2013) demonstrated that exposing rats to early life social stress increases the likelihood that they find their place in a subordinate position within social hierarchies, which are mediated by the ability to recognize and discriminate conspecific odors, beginning as early as adolescence. Thus, the quality and consistency of the early environment in which an animal is reared is crucial because it serves to mold long-term social memory abilities and odor preference behavior that will guide rodent responses toward or away from sources of sustenance, individual and group safety, and mates.

A final invaluable benefit of a reliance on odor cues during social interactions is that they can be diffusely spread about the environment to permit a more broad form of communication. The use of odors in this manner allows mature animals to effectively convey discriminatory sensory signals about health and safety, mating availability, aggression and territoriality, and social affiliations (Brennan et al., 1990; Gheusi et al., 1994; Carr, Loeb, & Dissinger, 1965; Lydell & Doty, 1972; Stern, 1970; reviewed in Keverne, 2005). For example, both rats and mice have been demonstrated to display increased attention (Valenta & Rigby, 1968) and activity (Mackay-Sim & Laing, 1980) when exposed to the odors of conspecifics that have recently been stressed. In a similar vain, aggressive behaviors in rats and mice involve a series of olfactory-mediated behaviors that contribute to stronger predispositions toward aggression during encounters with strange conspecifics (Archer, 1968; Ropartz, 1968), rapid increases in investigatory sniffing prior to aggressive displays (Banks, 1962; Mackay-Sim & Rose, 1981), and the

establishment of social hierarchies that involve odor-based discrimination (Lee & Brake, 1971; Ropartz, 1968). Additional evidence in support of the importance of odor discrimination and recognition in each of these social situations comes from studies that have demonstrated behavioral attenuation following lesions of the olfactory bulbs or chemical blockade of the olfactory stimuli (Da Vanzo, Sydow, & Garris, 1983; Ropartz, 1968). In general, the behavior that accompanies interindividual social communication appears to be largely driven by quick discrimination and the identification of the familiarity or the "strangeness" of each conspecifics odor during encounters. In support of this familiarity perspective, research has shown a marked reduction in aggressive responses by rats during a first physical encounter following pre-exposure to the odor of the 'stranger' for several days prior to testing (Mackintosh & Grant, 1966). These findings provide evidence to suggest that rodents can quickly learn the identity of another rat through exposure to odor alone in absence of any additional sensory cues that would be encountered during interaction with a live animal.

The Social Recognition Test Paradigm

Research into the many facets of mammalian odor-driven social behavior has shown that one of the most crucial elements in the success or failure of complex social relationships is the ability of animals to encode, retain, and refer to information related to previously encountered individuals with some degree of familiarity (Insel & Fernald, 2004). Several decades of studies on the neural and behavioral substrates underpinning the acquisition and expression of this familiarity, also referred to as social recognition, has revealed that it is also one of the most complicated aspects of the mammalian social experience (Bielsky & Young, 2004; Ligout & Porter, 2006).

For rodents, it is clear that odors provide the basis upon which social recognition is accomplished. During social encounters between two or more individuals a significant amount of time is spent engaged in mutual sniffing of the nasal, genital, anal, and glandular regions (Schloeth, 1956). Demonstrations of recognition by these means have been observed in both naturalistic and semi-natural settings in several species of rodents, including mice, rats, gerbils, hamsters, and guinea pigs (reviewed in Doty, 1986). Moreover, there is sufficient evidence to suggest that olfactory cues represent the identity of an individual animal by demonstrations of the ability to substitute urine or soiled bedding as social stimuli for live animals in recognition tests (Sawyer, Hengehold & Perez, 1984).

Beginning with the seminal work with rats by Thor and Holloway (1982), the behavioral correlates of social recognition have commonly been modeled by means of the habituation-dishabituation test. The demonstration of social recognition in this paradigm relies on the natural tendency by rodents to engage in investigatory sniffing of the anogenital region upon encountering another member of their species (for extensive reviews see Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009; Kendrick, 2006). If a rodent recognizes a familiar individual it will spend significantly less time investigating that individual during subsequent exposures (Thor & Holloway, 1982; Winslow, 2003). The most well validated version of the habituation-dishabituation paradigm involves the following two steps (also presented in schematic form in Figure 1):

1. The *habituation* stage, involves presenting a stimulus rodent (either once or repeatedly) to an experimental rodent that results in a reduction

in investigatory behavior over subsequently repeated presentations of the stimulus animal.

2. The *dishabituation* step, involves the presentation of a different (unfamiliar) conspecific to the experimental subject to test for the reinstatement of the initial level of social investigation.

In the habituation-dishabituation paradigm, the trial length during habituation is most commonly 2-5 min with an inter-trial interval (ITI) of 5-30 min across 3 or 4 exposure sessions. The latency to approach the stimulus animal, frequency of social investigation, and amount of time spent engaged in investigatory sniffing (also nose pokes if the animal is behind mesh wire) is observed and reductions in these behavioral markers are interpreted as social recognition. Because the exposures to the stimulus animal in the habituation stage are very brief, the maintenance of habituation behavior over the span of several hours has been suggested to measure short-term social memory (Thor & Holloway, 1982), while maintenance of habituation behavior over longer spans of 24-48 hr has been suggested to reflect long-term social memory (Bielsky & Young, 2004). Using this paradigm, short-term social memory has been demonstrated in adult males and females belonging to several species of rodent including rats, mice, gerbils, hamsters, and prairie voles (Dantzer, Bluthe, Koob, & Le Moal, 1987; Dluzen, Muracka, Engelmann, Ebner, & Landgraf, 2000; Gheusi, Blunthe, Goodall, & Dantzer, 1994; Thor & Holloway, 1982; Richter, Wolf, & Engelmann, 2005). In contrast, studies designed to the test the persistence of long-term social memory in the habituation-dishabituation paradigm have revealed that male rodents perform poorly (with no studies indicating habituation lasting >24 hr), while females require much less initial investigatory behavior

and demonstrate sustained memory over much longer intervals (longer than 24 hrs in rats) (Bielsky & Young, 2004; Bluthe & Dantzer, 1990; Thor, 1980). Although the mechanism behind this sexual dimorphism has yet to be elaborated, these sex differences are potentially a manifestation of behavior that reflects stronger activation of the neural circuitry along the limbic-olfactory tract in female rodents, which is likely to enhance social recognition capabilities.

Neuromodulation of Social Recognition

For rodents, the ability to appropriately respond to the presence of another conspecific or lingering odors that identify individuals depends upon the close anatomical association and interactive relationship between the olfactory system and regions of the brain that are responsible for neuroendocrine function, arousal/attention, learning and memory, many of which can be found within the hypothalamic-limbic-olfactory tract (Brennan, Kaba, & Keverne, 1990; Firestein, 2001; Lledo, Gheusi, & Vincent, 2005; Mucignat-Caretta, Radaelli, & Caretta, 2012).

Current evidence suggests that the neuropeptide that plays arguably the most widespread and important biological faciliatory role in many aspects of mammalian social behavior is oxytocin. Within the central nervous system, oxytocin serves as a sociobehavioral neuromodulator by stimulating dense pockets of receptors within the ventromedial hypothalamus, olfactory nuclei, extended amygdala (including the nucleus accumbens and ventral pallidum), lateral septum, bed nucleus of the stria terminalis, and lower brain stem (Freund-Mercier, Stoeckel, Palacios, Pazos, Reichhart, Porte, et al., 1987; Gimpl & Fahrenholz, 2001; Insel & Young, 2001). Aside from directly stimulating localized activity within specific structures of the brain and tracts that project into the

olfactory system, oxytocin also plays a neuroregulatory role by influencing the release of opioid peptides (e.g. dynorphin, enkephalin, and beta-endorphin), norepinephrine, and several other major hypothalamic neurohormones during social encounters, most notably among these being arginine vasopressin and the stress-response related glucocorticoids (Lim & Young, 2006; Nelson & Panksepp, 1998; Suh, Liu, Rasmussen, Gibbs, Steinberg, & Yen, 1986; Windle, Shanks, Lightman, & Ingram, 1997). In rodents, the analysis of a variety of social situations across the lifespan has demonstrated that gains in familiarity leading to odor-dependent recognition is, at least in part, reliant upon relatively widespread oxytocin-mediated neural activation (Lim & Young, 2006).

During the early postnatal period, social interaction between the mother and infant is oriented toward survival and caretaking. Suckling, physical contact, and the thermal stimulation involved in the social bonding process all rapidly activate the infant's central oxytocin neurons and increase the presence of the neuropeptide in the cerebrospinal fluid and blood plasma (Higashida, Lopatina, Yoshihara, Pichugina, Soumarokov, et al., 2010; Uvnäs-Moberg, Bruzelius, Alster, & Lundeberg, 1993). For pups, this caretaking behavior occurs in an environment that is dominated by the maternal odor and concurrent to an increase in circulating oxytocin that has been hypothesized to induce what has been labeled an opioid-independent "calming" response within the hypothalamic-limbic system of very young pups (Blass, Fillion, Weller, & Brunson, 1990), thus facilitating the conditional acquisition of the only required early olfactory-mediated social relationship, the mother-infant bond. Despite the limitations that a lack of pup mobility place on observing social odor recognition using the habituation-dishabituation paradigm, several studies have demonstrated that the preference for maternal/nest odors social odors

increase dramatically during the first two weeks of life culminating in reliable demonstrations of recognition for those socially-derived odors using free-choice social odor tests (Moriceau & Sullivan, 2004; Moriceau & Sullivan, 2005; Sobrian & Cornwell, 1977). In addition to behavioral correlates of social recognition, investigation of the neurobiological substrates involved in the acquisition of familiarity for maternal/nest odors has revealed that social odor recognition is dependent upon the degree to which the above mentioned physical stimuli induce the release of central oxytocin onto pockets of noradrenergic neurons along the limbic-olfactory tract at the outset of social encounters (Dluzen, Muraoka, Engelmann, Ebner, & Landgraf, 2000) and during social interaction directly between individuals or during exposure to social odors (Challis & Olson, 1988; Freund-Mercier & Richard, 1984; Marasco, Cornwell-Jones, & Sobrian, 1979; Stock & Uvnäs-Moberg, 1988; Wakerly, Clarke, & Summerlee, 1988).

In addition to the broad influence that oxytocin casts over early postnatal social behavior, studies using adolescent and adult rodents have revealed a continued reliance on the same neurophysiological circuitry upon reaching maturity (Cornwell-Jones, Decker, Gianulli, Wright, & McGaugh, 1990; Dluzen & Kreutzberg, 1993). Direct exposure to conspecific odors promotes the release of copious amounts of oxytocin into the mature rodent brain (Neumann, 2008). In turn, the presence of the neurohormone induces anatomical changes in the density of hypothalamic nuclei and the reciprocal connections between oxytocinergic neurons and the noradrenergic projections that play a critical role in activating the olfactory system during social encounters that involve discriminating between individuals or recognizing a familiar conspecific (Carter, 1998; Johnson, Ball, Coirini, Harbough, McEwen, & Insel, 1989; Smithson, Weiss, & Hatton,

1989; Yang & Hatton, 1987). In contrast to the normal circumstance of social recognition in mature rodents, it has also been demonstrated that disrupting the oxytocin-mediated noradrenergic input along the olfactory tract leads to impaired social recognition by rats in the habituation-dishabituation test situation (Griffin & Taylor, 1995).

In the mature rodent, the influence of the oxytocin-mediated neural networks on social recognition behavior have been further scrutinized to great effect using pharmacological manipulations (Choleris, et al., 2009, Ferguson, Aldag, Insel, & Young, 2001; Insel, 1992; Popik & van Ree, 1998). Systemic, intraperitoneal (IP) or intracerebraventricular (ICV) injections of low dosages of synthetic oxytocin prior to the initial interaction with another animal have been shown to enhance social recognition by adult male and female rats during both habituation-dishabituation and odor preference tests (Benelli, Bertolini, Poggioli, Menozzi, Basaglia, & Arletti, 1995; Engelmann, Ebner, Wotjak, & Landgraf, 1998; Popik, Vetulani, & van Ree, 1992). Higher, non-physiological dosages (> 4 mg/kg) impair the acquisition and expression of recognition behavior by both sexes (Benelli et al., 1995; Dantzer, et al., 1987; Popik & Vetulani, 1991). In addition, the ICV administration of an oxytocin antagonist immediately after the first encounter with a conspecific has been demonstrated to interfere with the formation of short-term social memory in female rats (Engelmann, et al., 1998), but facilitates recognition in males (Popik & Vetulani, 1991). The results of these apparently conflicting studies were rectified with the discoveries that the olfactory-tract-to-brain pathway that is required for rodent social recognition relies much more extensively on arginine vasopressin in male rodents (Tobin, Hashimoto, Wacker, Takayanagi,

Langnaese, Caquineau, et al., 2010), rather than the oxytocinergic control that has been repeatedly documented in female rodents (Bluthe & Dantzer, 1990; Larrazolo-Lopez, Kendrick, Aburto-Arciniega, Arriaga-Avila, Morimoto, Frias, & Guevara-Guzman, 2008).

In addition to its direct role as a hormonal regulator within the neurophysiology that mediates 'real-time' social responses, the release of oxytocin also plays a critical role in emotional and stress-coping responses that underlie a complex array of social interactions throughout the lifespan. The stress-reducing component of oxytocinergic activity exerts its influence by acting within hypothalamic-pituitary-adrenal (HPA) axis and by activating pockets of receptors found on non-neural tissues within the peripheral nervous system, such as those belonging to the adrenal and cardiovascular system (Freund-Mercier, Stoeckel, Palacios, Pazos, Reichhart, Porte, & Richard, 1987; Gimpl & Fahrenholz, 2001; Sofroniew, 1983). In rat pups, the resulting behavioral effect of oxytocinergic activation via tactual stimulation includes decreases in blood pressure (Lund, Lundeberg, Kurosawa, & Uvnäs-Moberg, 1999), the attenuation spontaneous motor activity (Uvnäs-Moberg, Alster, Lund, Lundeberg, Kurosawa, & Ahlenius, 1996), elevated nociceptive thresholds (Agren, Lundeberg, Uvnäs-Moberg, & Sato, 1995), and the prolongation to the pulsatile release of the neurohormone for several moments following social stimulation (Moos, Freund-Mercier, Guerne, Guerne, Stoeckel, & Richard, 1984). Furthermore, in both infant and mature rats, oxytocin has been demonstrated to inhibit activation of the stress-response network within the hypothalamic-pituitary-adrenal (HPA) axis at the outset of social encounters (reviewed in Windle, Shanks, Lightman, & Ingram, 1997; Neumann, Torner, & Wigger, 2000;

Neumann, Wigger, Torner, Holsboer, & Landgraf, 2000). The inhibitory action of oxytocin within the HPA axis is reflected by attenuation of hypothalamic secretions of corticotrophin releasing factor, reductions in circulating corticosterone, and suppression of anxiety-like behavior during encounters with conspecifics or conspecific odors (Insel & Winslow, 1991; Olausson, Uvnäs-Moberg, & Sohlstrom, 2003; Neumann, 2008; Sohlstrom, Carlsson, & Uvnäs-Moberg, 2000). At first glance, the collection of anxiolytic responses that are prompted by the release of oxytocin within the brain seem to be unrelated to social interaction. However, when viewed as a broader pattern of oxytocin-mediated neural influence that occurs in parallel to the induction of activity along the limbic - olfactory pathways within the brain and neuroendocrine system, it becomes increasingly clear that mammals have a highly tuned biological mechanism with a directive to potentially reduce stress responses and permit animals to engage in a wide variety of affiliative and investigatory behaviors that depend upon social discrimination and recognition (Onaka, 2004). For example, the complex social interactions that underpin maternal caretaking, aggression, mating behavior, and affiliative behavior all have been hypothesized to benefit from the influence of this basic neurobiological network involving reciprocal interaction between oxytocin projections from the hypothalamic region to pathways that travel along the limbic system and olfactory tract (Carter, 1992; Insel, 1992; Numan & Insel, 2003; Veenema & Neumann, 2008; for reviews see Nelson & Panksepp, 1998; Ross & Young, 2009).

Developmental Effects of Postnatal Oxytocin Administration

Because the oxytocin system is functional at birth and the neurohormone is abundant in the CNS and circulatory system throughout postnatal development (Altstein & Gainer, 1998) the long-term development of the neural network and the impact on

relevant behavioral outcomes can be easily studied using pharmacological manipulations. Although no studies to date have specifically investigated the potential developmental impact of postnatal oxytocin administration on social recognition, several studies have examined the influence on related neurophysiological and behavioral correlates. In studies performed using various species of infant rodents, the circulating peptide levels have been artificially elevated using SC injections or ICV infusion of synthetic oxytocin (*pitocin*). Central (ICV) infusions of oxytocin attenuate ultrasonic vocalizations in rat pups that have been isolated from their mother (Insel & Winslow, 1991), reduce plasma levels of corticosterone during isolation (Kramer, Cushing, & Carter, 2003), and decrease the time pups spend in contact with, or near their mother (Nelson & Panksepp, 1996), which lends credence to the previous suggestion that oxytocinergic activity promotes the possibility of gaining familiarity with a conspecific by playing the role of a stress-reducing compound during social interactions. Specifically, these studies support the proposed hypothesis that under normal circumstances an infant's social interaction with, and attachment to its mother is likely enhanced by an ample supply of exogenous oxytocin from the mother during the postnatal period (Keverne, Nevison, & Martel, 1997). Furthermore, these studies suggest that at this very young age oxytocin may also play a role in neurobiologically rewarding social interaction that contributes to recognition for maternal/nest cues, perhaps by interacting with the other social neuromodulators from the very first instances of social contact (Lim & Young, 2006; Nelson & Panksepp, 1998). The high levels of circulating oxytocin present when a pup is proximal and in direct contact with its mother quickly reduce perturbations within the stress physiology, which is in turn reinforcing to prosocial behavior during a time when

such experiences can be most beneficial in contributing to a healthy trajectory of development in the infant.

In addition to the possibility of acute benefits resulting from postnatal oxytocin administration, there is an ever-growing body of literature concerned with studying the potential long-term physiological and behavioral effects of exogenously manipulating the oxytocin system early in postnatal development. In adult female rodents, central (ICV) or peripheral (SC) injections of the synthetic peptide during postnatal development have been found to enhance neuronal activation within the hypothalamic nuclei (Cushing, Yamamoto, Carter, & Hoffman, 2003) and induce long-term increases in the expression of oxytocinergic neurons in the paraventricular and ventromedial nuclei of the hypothalamus, as well as in the amygdaloid nuclei (Kramer, Yoshida, Papademetriou, & Cushing, 2007; Yamamoto, Cushing, Kramer, Epperson, Hoffman & Carter, 2004). Additionally, several studies have shown that postnatal oxytocin administration may enhance long-term physiological development as a result of the organizational changes that occur within the CNS, specifically with respect to the hypothalamic nuclei. Voles and rats that have been exposed to postnatal oxytocin injections display increased body weight, plasma levels of cholecystokinin and growth rate into adulthood, while blood pressure and basal plasma corticosterone levels are significantly decreased in comparison to control subjects (Holst, Uvnäs-Moberg, & Petersson, 2002; Olausson, Uvnäs-Moberg, & Sohlstrom, 2003; Uvnäs-Moberg, Alster, Petersson, Sohlstrom, & Bjorkstrand, 1998). The few known behavioral studies that have sought to compliment the neurobiological and physiological research provide limited support for the hypothesis that long-term development benefits from early postnatal oxytocin administration. Uvnäs-Moberg et al.

(1998) found that postnatal administrations of oxytocin (SC injections) significantly increased the nociceptive threshold in 60-day-old female rats during the tail-flick test. In addition, Bales and Carter (2003) found that administering postnatal injections of oxytocin to female voles facilitated a more rapid onset in the mate-guarding component of pair bonding during adulthood. The suggestion that postnatal oxytocin administration enhances development in not only social function, but across many domains, was further extended by the demonstration that repeated early life injections of the neuropeptide were capable of remediating the deleterious impact of exposing pups to malnutrition on the neurobiological organization of the HPA axis and later life physiological reactivity to an acute stressor (Olausson, et al., 2003). Although no known studies have specifically sought to analyze the long-term effects of postnatal oxytocin administration on social recognition, each of the above studies has been instructive in increasing our understanding of the effects of postnatal oxytocin administration on physiological and behavioral development. Of specific interest to the present research is the potential long-term organizational effect that early oxytocin exposure may have on the developing brain. It is clear that this area is in need of additional research in order to understand the relationship between the early life flexibility of the oxytocin system and long-term developmental outcomes as it pertains to designing effective clinical interventions that may prove beneficial to children in atypical rearing experiences, such as those that involve disruptions to the early social environment. Given the potential broad-spectrum benefit of postnatal oxytocin exposure on development, it is also of exceptional interest to examine what role, if any, early life treatments with oxytocin would play in the development of social behaviors, such as social recognition abilities.

Current Direction Issues in Social Recognition and Oxytocin Research

It is clear that oxytocin is a neuropeptide that plays a facilitatory role in female rodent prosocial behaviors as they contribute to establishing social relationships and the gain in familiarity that leads to social recognition. It is secreted endogenously by young rodents either in the presence of, or contact with, their mother, is sensitive to the influence of postnatal events, and has been hypothesized to play a regulatory role in a variety of neuroendocrine responses that contribute to rodent social behavior. In turn, the sociality of the mother-infant relationship shapes the developmental morphology and physiology of the oxytocin system. The trajectory of this development is critical because in mature rodents, the success of recognizing a previously encountered conspecific is largely tethered to the competency of the central oxytocin system as it may provide some degree of oversight on the neurobiological mechanisms that might underlie social learning and memory. In this respect, the oxytocin system and the expression of social recognition behavior to which the neuropeptide contributes are excellent candidates to study following manipulations that seek to alter the quality of caretaking or disrupt the early social environment. In this same light, it would also be instructive to determine whether postnatal administration of oxytocin ameliorates any differences in social recognition that might be caused by altering the early social environment.

The Maternal Separation Paradigm

Animal models of maternal separation were originally developed as systems that could be used to characterize the patterns of behavior that follow interruptions to the mother-infant bonding process or exposure to poor quality maternal caretaking (i.e. neglect). Non-human primates were widely used as subjects at first because they display specific emotional attachment to the mother that behaviorally mimics human attachment

(Harlow, 1958; Harlow, 1962; Harlow, Dodsworth, & Harlow, 1965; Suomi, 1997). In recent years, the cost effectiveness and availability of more technically refined research protocols (i.e. gene targeting) have promoted the use of rodent species rather than non-human primates in studies investigating the influence of the early social environment. Furthermore, rats and mice are particularly well suited to study the potential impact of maternal separation on social behavior because they are highly gregarious animals and have been found to display evidence of the standard criteria used to define the infant-mother relationship in non-human primates and humans. Most importantly, these behaviors include recognition of and preference for the maternal caretaker (primarily by olfactorants) as well as a distressful reaction upon involuntary separation (Lehmann & Feldon, 2000; Lehmann, Camille, & Feldon, 2000; Raineki, Pickenhagen, Roth, Babstock, McLean, Harley, Lucion, & Sullivan, 2010; Schmidt, Oitzl, Levine, de Kloet, 2002). Furthermore, many similarities have been documented in the response of rat and mouse pups and species of primate infants to brief or prolonged periods of maternal separation (Francis, Caldji, Champagne, Plotsky, & Meaney, 1999; Pryce & Feldon, 2003).

The introduction of rodent species into the maternal separation model immediately expanded the scope of the paradigm to research a broader spectrum of interactions between early life adversity or variations in social experiences and long-term developmental outcomes. In the rodent model, the most coconmmon form of maternal separation is known as partial maternal separation and involves repeatedly separating pups from their mother for several hours (3-6 hr) per day during the first two weeks of life (Plotsky & Meaney, 1993; Wigger & Neumann, 1999). Additional forms of maternal

separation include subjecting rats or mice to repeated, brief (15 min) maternal separation, early deprivation (pups are separated from the dam and siblings for extended periods per day), and artificial rearing (pups are reared in isolation away from maternal or sibling stimuli) (Gutman & Nemeroff, 2002; Pryce & Feldon, 2003). In this paradigm, the forms of postnatal maternal separation are compared with animal facility reared pups as a control condition. In animal facility rearing, the dam and litter experience only the disturbance of routine animal husbandry (Pryce, Bettschen, Nanz-Bahr, & Feldon, 2003). In the mouse and rat, these different postnatal manipulations have yielded important insights in the effects of altering the nature of the early social environment on long-lasting changes in behavior, neuroendocrinology, neurochemistry, and cognitive functions in adulthood (Gutman & Nemeroff, 2003; Plotsky & Meaney, 1993; Pryce & Feldon, 2003).

Among the above-mentioned conditions, prolonged maternal separation (referred to as *maternal separation* throughout the remainder of the document) is the most widely used postnatal manipulation in this paradigm. In comparison to animal facility reared controls, rats and mice that have been exposed to maternal separation and tested in adulthood display reliable elevations in fear- and anxiety-related behavior (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000; Huot, Thrivikramen, Meaney, & Plotsky, 2001; Kalinichev, Easterling, Plotsky, & Holtzman, 2002; Wigger & Neumann, 1999), increased depressive-like responses (Aisa, Tordera, Lasheras, Del Rio, & Ramirez, 2007, Veenema, Blume, Niederle, Buwalda, & Neumann, 2006), and deficits in hippocampal-dependent memory, such as avoidance and spatial learning (Lehmann, Pryce, Bettschen, & Feldon, 1999; Oitzl, Workel, Fluttert, Frosch, & De Kloet, 2000). These behavioral

changes have long been linked to distinct changes in brain morphology and neurophysiology. Maternal separation impacts neuronal organization and elevates neuropeptide receptor mRNA (corticotrophin releasing factor) expression in many coriticolimbic areas of the brain including the hippocampus, forebrain, amygdala, and, most importantly, within the collection of structures that comprise the HPA axis (Champagne, 2009). In turn, the changes in adult brain morphology are correlated with lower tonic and enhanced activational states of the HPA system in response to stressors (Pryce & Feldon, 2003), which most notably influences efferent activational patterns in the locus coeruleus, forebrain, and amygdala (Ladd, Huot, Thrivikraman, Nemeroff, Meaney, & Plotsky, 2000; Plotsky, Thrivikraman, Nemeroff, Caldji, Sharma, & Meaney, 2005).

All of the above studies tend to ignore the important role of hypothalamic neurohormones while placing strong emphasis on the HPA axis as the starting point for evaluating the long-term impact of variations in the early social environment on the development of corticolimbic systems that regulate endocrine and emotional responses to stressors (Caldji, Diorio, & Meaney, 2003; Champagne & Meaney, 2006; Francis, Champagne, Liu, & Meaney, 1999), which will not be the focus of the research in this dissertation. The importance in the above studies is that they provide evidence of a potential neurobiological pathway through which maternal separation manifests in recently-documented changes in social behavior and the underlying neurophysiology in rodents. I have previously noted that oxytocin plays a critical regulatory role in this very same pathway and has even been putatively hypothesized to play an important role in shaping the developmental morphology of the limbic-HPA axis. Throughout

development social contact stimulates the oxytocinergic system, which in turn produces behavioral calming by attenuating the release of stress hormones into the brain and circulation (Neumann, Kromer, Toschi, & Ebner, 2000). Further, in adult animals that have been exposed to stressors, ICV administration of oxytocin into the paraventricular nucleus of the hypothalamus reduces the pulsatory release of all primary stress hormones (Windle, et al., 2004) and ameliorates the long-term deleterious effects of early adversity if administered during postnatal development (Olausson et al., 2003). Given the degree of known interaction between the limbic-HPA axis and oxytocinergic systems, it would be reasonable to suggest that maternal separation impacts the development of the oxytocin system and in doing so may influence its regulatory control over complex social behaviors. Although any hypothesis about the specific direction of the interaction between the HPA axis and oxytocin system in maternally separated animals would be speculative, a critical first step will be demonstrating that maternal separation impacts aspects of behavior that have been shown to be mediated by activation within the central oxytocin system.

Maternal Separation Effects on Social Behavior

The maternal separation procedure alters the context of interactions between the mother and offspring in the postnatal social environment. In addition, this early life manipulation has been demonstrated to have neurobiological consequences in areas that significantly overlap with the neural circuitry involved in complex social behavior, such as social recognition. Despite the existence of such an intriguing area of research that could expand the clinical relevance of the maternal separation paradigm, there has been very little research on the interaction between maternal separation and oxytocin-mediated social behavior.

A small body of literature has begun to document the potential impact that maternal separation may have on the long-term development of the neurobiology and behavior involved in social interaction. Maternal separation has been demonstrated to induce both hypo- and hyper-normal aggressive responses in adolescent and adult males and alters the behavioral expression of maternal aggression in postpartum females (Boccia & Pedersen, 2001; Veenema & Neumann, 2009; Veenema, Bredewold, & Neumann, 2007; Veenema, Blume, Niederle, Buwalda, & Neumann, 2006). In addition to these behavioral manifestations, maternal separation also induces changes in the underlying neurobiology on which these behaviors depend. Studies on neuropeptidergic immunoreactivity have shown that maternal separation significantly increases the activation of vasopressinergic nuclei in the male rat hypothalamus, while the changes in female agonistic responses are associated with a selective reduction in oxytocinergic release and binding in the paraventricular nucleus of the hypothalamus (Veenema et al., 2007; Veenema & Neumann, 2008; Veenema, 2009). It is worth noting that the maternal separation procedure does not appear to significantly influence the development of the noradrenergic system (Hennessy, Tamborski, Schiml, Lucot, 1989), which was previously noted to be a key element in the action of oxytocin on olfactory-mediated social behaviors. The results of these studies were particularly useful in establishing the initial relationship between maternal separation and alterations in later life neuropeptidergic activation and provide evidence about the mechanism that might lead to deficits in social function. Of particular interest for the current research is the reduction in oxytocin activity in the hypothalamic nuclei of adult females because this functional

change could potentially interfere with other, non-aggressive, social behaviors, mainly conspecific recognition.

Additional research on the development of social behavior in maternally separated rodents has involved the examination of social odor preferences. A beneficial aspect of assessing responses to odor-related social cues is that the neural circuitry that underlies the expression of olfactory preference behavior has been well mapped in several species (Moriceau & Sullivan, 2004; Raineki et al., 2010) and is known to interact with the oxytocin system (Ross & Young, 2009; Yu, Kaba, Okutani, Takahashi, Higuchi, 1996). Thomas, Fonken, LeBlanc, and Cornwell (2010) used this approach to investigate the short-term impact of maternal separation in the responses to social odors in infant mice. The results of this study revealed that at 10- and 14-days of age maternal separation did not yet disrupt the ability of mice to acquire an odor preference for their home nest bedding. In contrast, a previous study found that adolescent (aged 29, 39, and 49 days) female mice that were exposed to maternal separation displayed a complete disruption in their ability to acquire an olfactory preference for the social odors present in their post-weanling environment (Thomas, Fonken, Boyd, LeBlanc, & Cornwell, 2008). Although the impact of two studies on social odor preferences alone is not substantial, when viewed collectively with the outcomes of research on agonistic responses an interesting pattern emerges that begs further investigation. First, the behavioral observations in this literature suggest that maternally separated rodents are potentially deficient in the domain of social recognition. The failure by mice to acquire a preference for a social odor that they persistently encounter in their postnatal environment and the demonstration of abnormal responses during aggressive interaction supports this hypothesis. Additionally,

the presence of decreased oxytocin binding in the hypothalamic nuclei in female rodents provides particularly strong neurobiological support for the hypothesis that they will display some disruption in social recognition abilities. Second, there appears to be a delay in the expression of atypical social behavior that may reflect upon the development of the underlying neural systems responsible for social interaction. Because the supply of endogenous oxytocin is privy to an exogenous supplement via the mother's milk during the first 2-3 weeks of life, it is possible that the weaning experience and transition away from the maternal supplement of oxytocin plays a role in the delayed appearance of atypical patterns of social behavior. Any interpretation of this pattern is purely speculative until it is determined the extent to which social recognition deficits are present in maternally separated rodents. However, an empirical demonstration of poor social recognition (as suggested above) would implicate oxytocin as a neuropeptide of interest in future developmental studies.

Dissertation Aims and Hypotheses

There is an abundance of literature that has documented the important role that the early social environment plays in long-term physiological and psychological health (Als, Duffy, McAnulty, Rivkin, Vajapeyam, Mulkern, et al., 2004; Heim & Nemeroff, 1999; Heim, Newport, Wagner, Wilson, Miller, & Nemeroff, 2002). However, our understanding of the impact that interactions between an infant and its mother have on patterns of social behavior later in life is limited. The basis for many aspects of social interaction throughout the lifespan relies on the determination of familiarity within one's species, whether it be the mother during infancy or a friend/foe in adulthood. In rodents, successful social recognition in this context is mediated by olfaction and is dependent on

complex neurochemical action by norepinephrine and oxytocin within the olfactory system and hypothalamic-limbic structures in the brain. Because both social olfaction and hypothalamic-limbic organization are demonstrated to be sensitive to manipulations of the early environment, it is possible that significant disruptions to the mother-infant relationship could have lasting effects on social recognition that extend beyond the abnormal responses to social odors previously shown in pre-adolescent mice (Thomas et al., 2008).

The primary aim of this dissertation was to explore how disrupting the consistency of the early social environment via maternal separation will impact social recognition in adolescent female mice. In addition, to my knowledge there are no known studies that have examined whether the expression of later life social recognition behavior by maternally separated rodents could be modified by an intervention designed to reduce the impact of maternal separation on the neurobiological systems that underlie social recognition, such as early postnatal oxytocin administration. Therefore, the secondary aim of this dissertation was to conduct a preliminary investigation into the long-term influence of early postnatal oxytocin administration on social recognition and social odor preferences in control and maternally separated female mice. The experiments described in the following sections were designed to address the following questions:

1. Does maternal separation affect the long-term development of social recognition in adolescent female mice?

2. Does the administration of oxytocin during early postnatal development (daily, SC injections during PND 1-14) influence social recognition or the development of a preference for familiar social odors in mice that have experienced no manipulation of the early rearing environment?

3. Does the administration of oxytocin during early postnatal development (daily, SC injections during PND 1-14) influence social recognition or the development of a preference for familiar social odors in maternally separated adolescent female mice?

Hypothesis 1 (Experiment 1)

I hypothesized that subjecting infant female mice to maternal separation during the first two weeks of life would significantly impair social recognition upon reaching adolescence. Specifically, I predicted that in a test of social recognition maternally separated female mice that are repeatedly exposed to a familiar conspecific (MS-FAM condition) would display significantly greater screen investigation time and a greater frequency of investigative behaviors than control subjects (AFR-FAM), which is indicative of disrupted social recognition in the habituation-dishabituation test. Additionally, I predicted that, relative to control subjects (AFR-NOV), maternally separated female mice (MS-NOV) that are exposed to a familiar conspecific during a sequence of 3 exposure sessions, followed by the introduction to a novel conspecific during a fourth exposure session would fail to display an increase in screen investigation time and investigatory behavior that normally characterize dishabituation.

Hypothesis 2 and 3 (Experiment 2)

Although no previous studies have sought to directly investigate the impact of early postnatal oxytocin administration in control-reared rats or mice the known pattern of oxytocinergic system development and social recognition behavior in rodents supports the hypothesis that oxytocin administration during the early postnatal period could potentially strengthen social odor preferences and enhance social recognition abilities (habituation and dishabituation). Specifically, I predicted that, compared to saline-injected control subjects (AFR-SAL), oxytocin-injected control subjects (AFR-OXT) would display a significantly greater preference for familiar nest odors. Additionally, I predicted that, relative to saline-injected control subjects that are repeatedly exposed to a familiar conspecific (AFR-FAM/SAL), oxytocin-inected control subjects (AFR-FAM/OXT) would display significantly greater screen investigation time and a higher frequency of investigative behaviors, which is an indicator of enhanced social recognition behavior in the habituation-dishabituation test. Further, I predicted that, relative to saline-injected control subjects (AFR-NOV/SAL), oxytocin-injected control mice (AFR-NOV/OXT) that are exposed to a familiar conspecific during a sequence of 3 exposure sessions, followed by the introduction to a novel conspecific during a fourth exposure session would display greater increases in screen investigation time and investigatory behavior that typify dishabituation.

In addition, it was hypothesized that early postnatal oxytocin administration would reestablish the preference for familiar social odors significantly and improve, if not fully restore, social recognition behavior and during adolescence. Specifically, I predicted that, compared to saline-injected maternally-separated subjects (MS-SAL), oxytocin-injected maternally-separated subjects (MS-OXT) would display a significantly

greater preference for familiar nest odors. Additionally, I predicted that, relative to saline-injected maternally-separated subjects that are repeatedly exposed to a familiar conspecific (MS-FAM/SAL), oxytocin-injected maternally-separated subjects (MS-FAM/OXT) would display significantly greater screen investigation time and an increased frequency of investigative behaviors, which is an indicator of recovered social recognition behavior in the habituation-dishabituation test. Moreover, I predicted that, relative to saline-injected maternally-separated subjects (MS-NOV/SAL), oxytocin-injected maternally-separated mice (MS-NOV/OXT) that are exposed to a familiar conspecific during a sequence of 3 exposure sessions, followed by the introduction to a novel conspecific during a fourth exposure session would display a re-emergence of elevations in screen investigation time and investigatory behavior that characterizes dishabituation. Furthermore, it was predicted that the pattern of habituation and dishabituation behavior displayed by oxytocin-injected maternally separated subjects (FAM and NOV) would parallel that of control subjects (FAM and NOV) with respect to screen investigation time and frequency of investigative behavior.

General Methods and Procedures

Subjects

The subjects used in this dissertation were 324 female mice (CD-1 strain) born from 54 litters. All litters were bred from stock originally acquired from Charles River Laboratories (St. Constant, Quebec) and housed in the vivarium at the Institute for Sensory Research (Syracuse University, Syracuse, NY). Pregnant females were checked daily and if litters were found, the day of birth was designated postnatal day (PND) 0. On the day after birth (PND 1), litters were sexed, culled to 10 pups (6 females, 4 males), and randomly assigned to one of two rearing conditions that were used in completing the

experiments in this dissertation: 1) animal facility reared (AFR; the control condition) or 2) maternal separation (MS). Although this dissertation will focus on the behavioral development of female mice, males must be retained in each litter as part of the culling process because it has been demonstrated that litters consisting of only female or male offspring elicit patterns of maternal care that substantially differs from the care received by mixed sex litters (Alleva, Caprioli, & Laviola, 1989). All litters of pups were housed with their dams in large polypropylene cages that were lined with Aspen hardwood shavings. Pups were weaned from the dam on PND 21 and housed in same sex groups for the remainder of the experiment. All subjects in this research were maintained in a temperature (23□) and humidity (53%) controlled vivarium on a 12:12 hr light:dark cycle (lights on at 05:00 hr) with *ad libitum* access to food and water. All mice used in this dissertation were housed and tested in accordance with the ethical and welfare guidelines established by the Syracuse University Institutional Animal Care and Use Committee and the National Institutes of Health (1985).

Rearing Conditions

During PND 1-14, litters were either AFR or exposed to daily MS. With the exception of removing pups from the nest for routine animal husbandry purposes (e.g. regular bedding material changes), pups in litters assigned to the AFR condition were undisturbed from the natural rearing experience. Litters belonging to the MS condition were separated from their mother, but not littermates, for 3 hr per day during PND 1-14. The 3 hr separation period was used in this dissertation because it has been demonstrated to be a consistent disruption of the mother-infant bonding experience without transitioning into maternal deprivation (Pryce and Feldon, 2003) and has previously been validated to alter responses to social odors (Thomas et al., 2010). All separation

procedures took place during the dark phase of the light cycle and began at 17:00 hr. At the beginning of MS, the dam was removed from the home cage and placed in a small polypropylene holding cage that was lined with Aspen hardwood shavings. The dam remained in the vivarium while litters were transported to the laboratory in the home cage. Once in the laboratory, litters were transferred to small polypropylene separation cages for the duration of their 3 hr separation period. The cages that housed the MS litters during the separation period were lined with fresh, unscented hardwood shavings (750 ml per cage) and were not changed throughout the 14 days of the MS procedure. The bedding material was not changed in order to prevent continually exposing pups to repeated neonatal novelty, which has been reported to contribute to enhanced later life social recognition in rats (Tang, Reeb, Romeo, & McEwen, 2003; Tang & Reeb, 2004). During MS, litters were maintained at approximately nest temperature (28° +/- 2°), in a dark, soundproof and ventilated room within the laboratory. Upon completion of the separation period, the MS pups were returned to their home cage, followed by reunion with the dam in the vivarium.

Social Recognition Test

Test apparatus. The test apparatus that was used to examine social recognition memory in this dissertation was based on the design used by Cornwell-Jones and Bolles (1983) to investigate social investigation in the male rat. The apparatus (see Figure 2) consists of a Plexiglas start box (15 x 20 x 20 cm) that is connected to a recognition arena (a large polypropylene cage; 20 x 28 x 20 cm) via a Plexiglas tunnel (8 x 8 cm). A second Plexiglas tunnel (8 x 8 cm) is located directly opposite from entry tunnel from the start box and is separated from the recognition arena by a wire mesh screen. This second Plexiglas tunnel contained the stimulus animals during recognition testing. A source of

positive airflow was connected to the stimulus receptacle and air flowed through the tunnel, across the recognition arena, and toward the test subject in the start box. The floor of the recognition arena was lined with 1.5 liters of clean, unscented hardwood shavings that were changed prior to the introduction of each new test subject.

Test procedure. On PND 49, female mouse social recognition was examined using the habituation-dishabituation procedure. Six adolescent females from each litter were used to complete social recognition testing. Three females from each litter were randomly selected to serve as test subjects. The remaining three females in each litter were used as stimulus animals during observations of subjects from unrelated litters. Prior to the beginning of the first observation session, the mouse to be used as a test subject was removed from its home cage and weighed. Following the collection of the test subject's weight, females from unrelated litters that had been assigned to serve as stimulus animals were weighed and a female within the range of +/- 3 grams of the test subject was matched for subsequent behavioral testing. The test subject and stimulus animal were then transferred to small polypropylene holding cages and transported into the laboratory to await pre-trial habituation.

As in previous investigations of social behavior, the procedure used in this study involved either repeatedly exposing a mouse to a familiar conspecific across a series of 4 exposure sessions or a sequence of 3 exposure sessions with a familiar conspecific and 1 exposure session with a novel conspecific. To avoid the potential influence of a dominance-related hierarchy on social behaviors (Moura, Meirelles, & Xavier, 2010), MS/MS and AFR/AFR pairs of mice were weight matched. The weights of test subjects were measured prior to conducting any trials in the social recognition apparatus. Testing

the MS/MS or AFR/AFR pair in a neutral cage and reducing the within-pair weight difference was intended to minimize aggression, which can be motivated by territorial instinct in the home cage or in the presence of territorial odors.

Social recognition testing involved exposing a test mouse to another mouse for 5 min exposure sessions (S). Prior to the beginning of the testing period, a habituation session was used to allow the test subject to adapt to the neutral testing environment. During habituation, the test subject was initially placed in the start box and permitted to freely explore the entire apparatus for the length of one trial. Following the habituation session, the test subject was immediately returned to the start box, the matched adolescent female was placed in the stimulus receptacle behind the wire mesh screen, and the first exposure session (S1) commenced. Intertrial intervals (ITIs) of 10 min separated the S's to maximize habituation and improve discrimination between individual conspecifics (Tang & Reeb, 2004). During the ITIs, both the test subject and stimulus animal were returned to their respective holding cages and placed in separate rooms within the laboratory. After the ITI, the animals were brought back to the test apparatus for the next trial. There were a total of four exposure sessions (S1-S4). A general methodological timeline for the habituation-dishabituation procedure used in these experiments can be viewed in Figure 4.

Three habituation-dishabituation conditions were used in this dissertation. Subjects that were assigned to the Familiar (FAM) condition were exposed to the same conspecific during S1-S4. Subjects assigned to the Novel (NOV) condition were exposed to the same conspecific during S1-S3 and to a novel conspecific during S4. Subjects assigned to the None (NO) condition were administered a trial in the test apparatus, but not paired with

another conspecific during S1-S4. One subject per litter was assigned to each dishabituation condition and *N*'s for each condition in the design are presented within each of the individual experiments described below. A potential confounding factor when investigating social recognition using the habituation-dishabituation paradigm is generalized social fatigue. In this case, the animal serving as the test subject becomes satiated to any social stimuli and no longer discriminates between a familiar and novel conspecific (Thor & Holloway, 1982). Test subjects in the NOV condition permit the examination of this potential confound. A significant increase in the amount of time investigating the stimulus receptacle by mice in the NOV group and significant differences in the social investigative behaviors between subjects in the three dishabituation conditions provides the basis to exclude social fatigue as a confound during interpretations of the behavioral correlates that are hypothesized to represent social recognition in rodents (Thor & Holloway, 1982).

All behavioral testing was videotaped under red light illumination for off-line analysis of social recognition behavior. Social recognition behavior was analyzed using three raters. Two raters were blind to the treatment conditions of the animals at the time of testing. Inter-rater reliability was calculated among all three raters using a correlation coefficient and was demonstrated to be $r = 0.84$. The behavioral measures recorded included the total time spent engaged in screen investigation and investigatory behavior frequency. The total time (measured in seconds) with a minimum of 0 and a maximum of 300 of social investigation was continuously recorded for each test subject during the four trials and was defined as the female being proximally oriented toward the stimulus animal (the tip of the nose within ~1 cm) or in direct contact with the wire mesh screen.

In addition, investigatory behaviors were defined as instances of sniffing, nosing, biting, or climbing onto the wire screen. The frequencies of these behaviors were measured during sixty 5-sec video segments for each of the 5 min exposure sessions. If the behavior was present at any time during the 5 sec duration, an occurrence of one was counted for a minimum score of 0 and a maximum score of 60.

Olfactory Preference Test

An odor preference test was used as an additional behavioral measure in the second and third experiment to explore the effect of postnatal oxytocin administration on responses to social cues. Olfactory preference testing was conducted on PND 48 using two randomly selected females from each litter (*one oxytocin-administered female and one saline-administered female*). The odor preference test is a two-odor free-choice situation that is conducted under red-light illumination. The apparatus consists of a Plexiglas frame (29 x 22 x 10 cm) with a wire mesh floor, which permit mice olfactory, but not gustatory or tactile contact with wood shavings in two right triangle compartments below the screen (see Figure 3 for a diagram of the odor preference apparatus). One right triangle compartment contained a socially derived odor (familiar nest bedding from the test subjects home cage) and the other compartment contained clean hardwood shavings. The orientation of the odor presentations was counter-balanced across test subjects. A center triangle without shavings separated the two right triangle odor compartments.

Prior to testing, one liter of each type of shavings was placed in each compartment. At the beginning of each trial, subjects were individually placed at the midline of the screen floor and observed for the course of one trial (180 s). Following

testing, the apparatus was cleaned with a diluted soap solution, rinsed, and thoroughly dried. Odor preference testing was videotaped under red light illumination for off-line behavioral analysis. Odor preference behavior was analyzed using three raters. Two raters were blind to the treatment conditions of the animals at the time of testing. Inter-rater reliability was calculated among all three raters using a correlation coefficient and was demonstrated to be $r = 0.81$. The behavioral measures recorded for each animal included amount of time spent over each type of shavings and the amount of time spent over the center triangle. Additionally, a difference score was calculated for each subject by subtracting the time spent over the clean hardwood shavings from the time spent over the nest bedding. Difference scores that were significantly greater than zero indicated a preference for the social cue over the natural hardwood shavings.

Experiment 1. Does maternal separation affect the long-term development of social recognition abilities in adolescent female mice?

A growing body of literature suggests that manipulations of the early life environment can have a powerful influence on the ability to recognize and appropriately respond to social cues throughout the course of development. Tang and Reeb (2005) demonstrated that rearing rats in circumstances that enrich the early social environment, such as repeated exposure to neonatal novelty, enhances recognition for social stimuli later in life. By contrast, research using an opposing early life treatment, MS, has indicated that even moderate disruptions of the early social environment negatively impacts long-term physiological and behavioral development (Pryce & Feldon, 2003), including the expression of social responses (Zimmerberg & Sageser, 2011). Thomas and colleagues (2008) found that in comparison to control subjects, female mice that were exposed to early life MS failed to develop a preference for familiar social odors

during adolescence. Additionally, upon reaching adulthood maternally separated female rats and mice display marked abnormalities in maternal aggression in the resident-intruder paradigm and significant reductions in the expression of brain oxytocin receptors within the hypothalamic-limbic pathways that play a critical role in olfactory-mediated social behaviors (Veenema, 2009; Veenema et al., 2007). Collectively, these results suggest that MS results in domain-specific disruptions to conspecific recognition. However, no studies with rodent species have directly assessed later life social recognition abilities following exposure to MS. I hypothesize that subjecting female mice to MS will negatively influence social recognition abilities to the extent that, in comparison to AFR-FAM subjects, MS-FAM mice will display a lower magnitude reduction in screen investigation time and investigative behavior frequency across the four exposure sessions that comprise the habituation procedure. Additionally, in contrast to the dishabituation response that is expected in females assigned to AFR-NOV, mice in the MS-NOV condition will display a smaller magnitude elevation in screen investigation and investigatory behavior upon introduction of the novel stimulus animal during the fourth exposure session.

Method

Subjects and Procedures

The subjects in this experiment were 108 adolescent female mice birthed from 18 litters of mice (9 litters per rearing condition). Litters of pups were randomly assigned to either AFR or MS on PND 1 and were then reared according to the procedures detailed in the general methodology. Fifty-four adolescent females from AFR and MS litters (n = 27 per rearing condition) served as test subjects, while the remaining fifty-four female

littermates were used as stimulus animals. A schematic of the design for social recognition testing for each AFR and MS combination can be found in Table 1.

Statistical Analyses

There are a variety of techniques available to evaluate social recognition outcomes, but the data analytic approach in this experiment was designed to follow a common conservative approach that has previously been used to examine social recognition in rodents (Ferguson, Young, & Insel, 2002; Spiteri & Agmo, 2009; Tang, Reeb, Romeo, & McEwen, 2003). Six 2 x 4 (Rearing condition x Exposure session) mixed ANOVAs were used to analyze the behavioral correlates of social recognition across the FAM, NOV, and NO dishabituation conditions. ANOVAs were conducted separately for each dishabituation condition. The between-subjects factor used in each analysis was rearing condition. Exposure session was the within-subjects factor in each analysis. The response variables used in each analysis were the following: 1) time spent investigating the wire screen in the social arena, and 2) frequency of social investigatory behaviors. Partial eta-squared (η_p) was calculated as a measure of effect size for significant main effects or the interaction of the factors used in the ANOVA. Post hoc analysis of main effects for exposure session were conducted using Tukey's HSD procedure. Additionally, post hoc analysis of interaction effects between rearing condition and exposure session involved polynomial trend analysis (linear for habituation by FAM subjects and quadratic for dishabituation by NOV subjects) to test for simple effects within each rearing condition. Tukey's HSD was then used to compare mean differences between exposure sessions within rearing condition. Post hoc evaluation of interaction effects also included the calculation of unpaired Student's t-tests to examine

rearing condition differences at each level of exposure session. Significant comparisons using Student's t-test were accompanied by the calculation of Cohen's *d* as a conservative measure of effect size..

Results

Screen Investigation Time

A 2 x 4 mixed ANOVA was used to evaluate the mean duration of time (s) AFR-FAM and MS-FAM subjects investigated the wire screen during the social recognition test. AFR-FAM females spent significantly less time than MS-FAM females investigating the wire screen across all exposure sessions, $F(1,16) = 19.61$, $p < 0.001$, $\eta_p = 0.55$. The results of the ANOVA also showed a main effect of exposure session, $F(3,48) = 12.27$, $p < 0.001$, $\eta_p = 0.43$. In addition to the presence of significant main effects, the ANOVA revealed the presence of a significant rearing condition by exposure session interaction, $F(3,48) = 3.73$, $p < 0.05$, $\eta_p = 0.19$. See Figure 5 for mean investigation times for all rearing conditions.

Post hoc analysis of the interaction between rearing condition and exposure session revealed the presence of a habituation response by AFR-FAM subjects, $F(1,8) = 46.82$, $p < 0.001$, $\eta_p = 0.85$. Pairwise comparisons between exposure sessions indicated that investigation time during session 4 was significantly reduced in comparison session 1, 2, and 3 (all p's < 0.05). In contrast, subjects in the MS-FAM condition did not display habituation to the social stimulus, $F(1,8) = 4.45$, $p > 0.05$. Additional post hoc comparisons between rearing conditions at each level of exposure session also indicated that mean investigation time by AFR-FAM females did not significantly differ from MS-FAM females during exposure session 1 or 2 (both p's > 0.05). However, during

exposure session 3, $t(16) = 3.72$, $p < 0.01$, $d = 1.86$ and exposure session 4 $t(16) = 5.00$, $p < 0.001$, $d = 2.50$ AFR-FAM females displayed a significantly greater habituation response than MS-FAM females.

A 2 x 4 mixed ANOVA was used to evaluate the mean duration of time (s) AFR-NOV and MS-NOV subjects investigated the wire screen during the social recognition test. AFR-NOV females spent significantly less time than MS-NOV females investigating the wire screen, $F(1,16) = 4.51$, $p < 0.05$, $\eta_p = 0.22$. The results of the ANOVA also showed a main effect of exposure session, $F(3,48) = 16.47$, $p < 0.001$, $\eta_p = 0.51$. The ANOVA also revealed the presence of a significant rearing condition by exposure session interaction, $F(3,48) = 4.73$, $p < 0.01$, $\eta_p = 0.23$.

Post hoc analysis of the interaction between rearing condition and exposure session indicated that AFR-NOV females displayed significant changes in screen investigation time across exposure sessions, $F(1,8) = 81.01$, $p < 0.001$, $\eta_p = 0.91$. Habituation to the social stimulus by AFR-NOV females was demonstrated by reductions in mean investigation time between exposure session 1 and sessions 2 and 3 (both p's < 0.001). AFR-NOV females also displayed the predicted dishabituation response as indicated by increased mean investigation time between exposure session 3 and 4, $p < 0.01$. In contrast to subjects in the AFR-NOV group, MS-NOV females did not display a significant change in screen investigation across exposure sessions (i.e. absence of habituation or dishabituation), $F(1,8) = 3.79$, $p > 0.05$. Further analysis of the interaction effect revealed that during exposure session 2, $t(16) = 11.26$, $p < 0.001$, $d = 5.63$ and exposure session 3, $t(16) = 3.79$, $p < 0.01$, $d = 1.90$ AFR-NOV subjects spent significantly less time than MS-NOV subjects investigating the stimulus animal.

A 2 x 4 mixed ANOVA was used to evaluate the mean duration of time (s) AFR-NO and MS-NO subjects investigated the wire screen during the social recognition test. The results indicated that AFR-NO females and MS-NO females did not significantly differ, $F(1,16) = 0.028$, $p > 0.05$. The duration of time spent investigating the wire screen did not significantly vary across exposure sessions, $F(3,16) = 0.45$, $p > 0.05$, and did not interact with rearing condition, $F(3,48) = 0.01$, $p > 0.05$.

Investigatory Behavior

A 2 x 4 mixed ANOVA was conducted to examine mean differences in investigatory behavior by AFR-FAM and MS-FAM subjects. The results indicated that AFR-FAM and MS-FAM females did not significantly differ in the overall frequency of investigatory behaviors during the social recognition test, $F(1,16) = 2.21$, $p > 0.05$. The results of the ANOVA did show a main effect of exposure session, $F(3,48) = 9.26$, $p < 0.001$, $\eta_p = 0.37$. Additionally, exposure session was found to significantly interact with rearing condition, $F(3,48) = 3.56$, $p < 0.05$, $\eta_p = 0.18$. See Figure 6 for comparisons of overall mean investigatory behavior for all conditions.

Exploration of the interaction between exposure session and rearing condition showed that the investigatory behavior displayed by AFR-FAM females did not significantly differ across exposure sessions, $F(1,8) = 1.02$, $p > 0.05$. In contrast, MS-FAM subjects displayed significant changes in investigatory behavior across exposure sessions, $F(1,8) = 21.26$, $p < 0.01$, $\eta_p = 0.73$. MS-FAM females displayed an increase in investigatory behavior between exposure session 1 and 3, $p < 0.05$, and then a significant decrease in investigation between exposure session 3 and 4, $p < 0.01$.

A 2 x 4 mixed ANOVA was conducted to examine mean differences in investigatory behavior by AFR-NOV and MS-NOV subjects. The results indicated that AFR-NOV subjects engaged in more frequent social investigation than MS-NOV mice, $F(1,16) = 24.61$, $p < 0.001$, $\eta_p = 0.61$. The results of the ANOVA also showed a main effect of exposure session, $F(3,48) = 14.65$, $p < 0.001$, $\eta_p = 0.48$. Additionally, exposure session and rearing condition significantly interacted, $F(3,48) = 5.59$, $p < 0.05$, $\eta_p = 0.26$.

Exploration of the interaction between exposure session and rearing condition showed a significant change in displays of investigative behavior by AFR-NOV females across exposure sessions, $F(1,8) = 7.39$, $p < 0.05$, $\eta_p = 0.48$. However, pairwise comparisons revealed that the pattern was not characteristic of a habituation response as AFR-NOV subjects engaged in significantly more investigation behavior during session 3 than 2, $p < 0.01$. Further, displays of investigative behavior by AFR-NOV subjects during exposure session 4 were only significantly greater than during session 1 and 2 (both p's < 0.001), which does not provide conclusive support for a dishabituation response. In contrast to AFR-NOV subjects, MS-reared females did not display significant changes in investigative behavior across exposure sessions, $F(1,8) = 1.69$, $p > 0.05$. Further evaluation of the interaction effect revealed that during exposure session 3 ($t(16) = 3.24$, $p < 0.01$, $d = 1.62$) and 4 ($t(16) = 6.14$, $p < 0.001$, $d = 3.07$) AFR-NOV females more frequently engaged in investigatory behavior than their MS counterparts, which indicates a between condition difference in the magnitude of change in investigatory behavior during the transition from the habituation step to the dishabituation session.

A 2 x 4 mixed ANOVA was conducted to examine mean differences in prosocial investigative behavior by AFR-NO and MS-NO subjects. AFR-NO and MS-NO subjects did not significantly differ from one another in displays of investigative behavior across exposure sessions, $F(1,16) = 4.51$, $p > 0.05$. The main effect of exposure session was not significant, $F(3,48) = 2.21$, $p > 0.05$. Despite the absence of main effects for each, exposure session and rearing condition significantly interacted with one another $F(3,48) = 5.26$, $p < 0.01$, $\eta_p = 0.25$.

Post hoc analysis of the interaction effect indicated that the frequency of social investigation by AFR-NO changed over the course of social recognition testing, $F(3,24) = 5.33$, $p < 0.01$, $\eta_p = 0.40$. AFR-NO females displayed a significant increase in social investigation between exposure session 2 and 3 ($p < 0.05$) and a significant decrease in investigatory behavior between session 3 and 4 ($p < 0.05$). Additional post hoc evaluation further revealed that the frequency of investigatory behavior by MS-NO females changed across exposure session, $F(3,24) = 3.06$, $p < 0.05$, $\eta_p = 0.28$. Tukey's HSD procedure did not reveal any significant pairwise differences between exposure sessions for MS-NO females (all p's > 0.05).

Discussion

The early social environment is dominated by the mother-infant bonding process and provides the initial context in which developing mammals begin their acquisition of social abilities. These early experiences play a critical role in promoting the development the neurobiological substrates that are involved in successful social recognition. In turn, the development of this neural circuitry serves as a dominant force in establishing lifelong predispositions toward behavioral competence in several domains of sociality

that depend upon the recognition of individual conspecifics. Thus, a significant disturbance of the early social setting has the potential to exert a deleterious impact on how mature individuals navigate a complex social environment and gain familiarity with conspecifics during bonding experiences. This experiment examined the hypothesis that disrupting the early social environment of female mice by subjecting them to MS during the first two weeks of life would significantly alter social recognition abilities later in life during adolescence.

As was predicted, early life MS led to an attenuation of social recognition by adolescent female mice. During the initial exposure session, S1, females belonging to both rearing conditions spent approximately the same amount of time investigating the previously unfamiliar conspecific. Mice belonging to the AFR-FAM condition displayed a clear trend of habituation to the stimulus animal as indicated by diminishing social investigation across the four exposure sessions. In contrast, MS-FAM subjects did not display behavior indicative of habituation to the presence of the familiar stimulus animal. The divergence in habituation responses between the two rearing conditions was further substantiated by the fact that AFR-FAM mice spent significantly less time than MS-FAM mice interacting with the social stimulus during S3 and S4. The decrease in investigation time across exposure sessions by AFR-FAM, but not MS-FAM, subjects provides strong behavioral evidence of familiarity to the presence of a consistent social stimulus. The demonstration of significant differences between rearing conditions in social recognition were reified by the absence of similar observations within the NO condition. The observed differences in social recognition between MS and AFR females were genuinely driven by the presence of another animal and not simply spontaneous differences in

investigation. The finding of a gain in familiarity following repeated brief exposures to a conspecific is in agreement with previous demonstrations that such behavior is a reliable characteristic of social recognition in juvenile and adult rodents (Bluthe & Dantzer, Tang & Reeb, 2004; Tang, Reeb, Romeo, & McEwen, 2003; Thor & Holloway, 1982; reviewed in Bielsky & Young, 2004; Gheusi, et al., 1994). During the same interval, there was a significant elevation in prosocial behavior by MS females, which further supports the prediction that MS would serve as an impedance in the habituation response that underlies the function of social memory.

A critical aspect to interpreting the behavioral correlates of social recognition in rodent studies involves a dishabituation response to the presentation of a novel conspecific after gaining familiarity with one animal. In this experiment, AFR-NOV females displayed a pattern of stimulus dishabituation that has previously been demonstrated in semi-natural settings and by control subjects in several studies on rodent social recognition (Burman & Mendl, 1999; Gheusi, et al., 1994; Tang, et al., 2003; Thor & Holloway, 1982; Winslow, 2003). Following the display of an appropriate gain in familiarity during S1-S3 that paralleled the behavior of AFR-FAM subjects, AFR-NOV mice went on to display a strong dishabituation response during S4. Relative to females in the AFR-FAM group, littermates assigned to the AFR-NOV group displayed an appropriate re-initiation of social interest when exposed to a stimulus animal with which they had no previous experience. These findings are consistent with displays of dishabituation in previous studies on rodent behavior (e.g. Tang & Reeb, 2004) and are an important indication that the control subjects in this experiment were definitively capable of establishing a memory for one adolescent female during several brief social

encounters and then could display discrimination upon the introduction of a new individual during S4.

In contrast to the more common presentation of social dishabituation responses by AFR females, MS proved detrimental to distinguishing a familiar from unfamiliar individual during an encounter on S4. Like the MS-FAM subjects, females in the MS-NOV condition did not habituate during S1-S3. Furthermore, unlike the social dishabituation observed in subjects belonging to the AFR-NOV condition, females in the MS-NOV group did not dishabituate upon being presented with a novel female during S4. This finding leads to the suggestion that, despite repeated interactions with the same stimulus animal by subjects in the MS-FAM and MS-NOV group, neither attained a high a degree of familiarity with that subject over the course of several exposure sessions.

The MS-induced alteration to the amount of time females spent investigating the stimulus animal was also accompanied by moderate, but inconsistent, changes in the frequency of prosocial behaviors during the pairings. AFR-FAM subjects did not display substantial changes in investigatory behavior across S1-S4, while the MS-FAM subjects were found to sample less frequently between S1-S3 and then increase their sampling between S3 and S4. This pattern of responding seems to provide behavioral corroboration to a general lack of familiarity and confusion about the presence of a consistent stimulus animal by MS females. This finding is also consistent with a need for greater behavioral activation to acquire social information during repeated encounters with a conspecific. AFR-NOV subjects were more behaviorally active than their MS counterparts throughout the four exposure sessions. The higher frequency of sampling by AFR-NOV is indicative of more interest in the stimulus animal and was ultimately

carried by a substantial increase in prosocial behavior upon presenting the novel stimulus animal during S4. The analysis of this measure indicated that across all exposure sessions there was a broad-spectrum fluctuation in the expression of prosocial behaviors by MS females, while the AFR females behavior was more predictable in the context of gaining familiarity with a conspecific and then shifting that familiarity when a novel subject was introduced. Although this finding appears modest when viewed alone, it becomes more significant as a supporting characteristic of the decreased gain in familiarity that was observed by MS females on the investigation time measure. This general characteristic of inconsistent investigatory behavior and poor conspecific discrimination by MS mice further supports the rearing group differences that were observed in analyzing the amount of time spent investigating the stimulus animal.

The present results provide a valuable contribution to the corpus of studies that have detailed the profound impact that the early social environment can have on the development of a wide range of later life social behaviors, including conspecific recognition and social memory (Champagne & Curley, 2011; Cushing & Kramer, 2005; Tang, et al., 2003). Tang and colleagues (Tang & Reeb, 2004; Tang, et al., 2003) showed that employing rearing procedures designed to enrich the early environment and social experiences enhance later life social recognition and individual discrimination. By contrast, the present results provide a clear demonstration that altering the early social environment in the opposing direction by restricting contact between the mother and infant impairs social recognition memory in adolescent female mice. These findings using the MS model expand upon a growing depth of literature that suggests disrupting social contact in the early environment is detrimental to how individuals identify and

respond to social cues (Thomas et al., 2008), engage in social communication and displays of aggression at maturity (Boccia & Pedersen, 2001; Veenema & Neumann, 2009; Veenema, et al., 2007; Veenema, et al., 2006), and socioemotionally respond to other conspecifics (Pryce & Feldon, 2003).

One plausible underlying explanation for the behavioral differences between AFR and MS females in displays social recognition is dysfunction within the limbic system circuitry. However, Kogan and colleagues (2000) demonstrated that such a failure over a brief ITI on the part of MS females is not likely to have arisen solely from limbic disruption because hippocampal lesions only began to influence the strength of social memories at ITI's greater than 30 min. Instead, a small, but growing, body of literature (Ferguson, et al., 2000; Lim & Young, 2006; Nelson & Panksepp, 1998; Winslow & Insel, 2002) suggest that the current data are more likely a behavioral manifestation of dysfunctional connectivity between the oxytocin system and the other components of the neuromodulatory network that is known to initiate motivated prosociality and promote olfactory-mediated social recognition memory at the outset of social engagements and over shorter ITI's. Intentional disruption of this early life stimulation by exposing infant rodents to the MS procedure contributes to decreased central oxytocinergic immunoreactivity (Lukas, Bredewold, Neumann, & Veenema, 2009; Veenema, et al., 2007) and poor production (Dief, Sharara, & Ibrahim, 2008) of the neurohormone requisite in stimulating the limbic circuitry during social encounters. The decreased presence of oxytocinergic neuron bodies within the hypothalamic-limbic tract may ultimately lead to an underwhelming solicitation of noradrenergic activity during social encounters. In turn, these neurobiological deficits offer a potential explanatory

mechanism for the variety of sociobehavioral dysfunctions including altered aggressive responses and preferences for social odors (Thomas, et al., 2008; Veenema et al., 2007) that have recently been reported. Based on the previous neurophysiological and behavioral findings, it is reasonable to suggest that the current observations provide additional insight into the ramifications of an oxytocinergic system that is not appropriately stimulated by interactions with conspecifics, leading to failures in social recognition by adolescent MS female mice.

In summary, mounting evidence suggests that the most parsimonious interpretation of the poor gain in familiarity and the increased levels of investigative behavior by MS females in this study is that their early life social experience perhaps does not adequately prepare them to engage in the information seeking process that leads to successes during social interaction and species-specific individual recognition. The research of Veenema and colleagues (Lukas, et al., 2010; Veenema, 2009; Veenema & Neumann, 2008; Veenema, et al., 2007) has pursued the hypothesis that in female rodents an underdeveloped or poorly functioning oxytocin system strongly contributes to social detriments, such as the ability to establish an initial memory for a conspecific, which then contributes to impaired maintenance of that memory over time. Because oxytocin has been identified by several lines of research as a critical hormone in forming the neuroendocrine basis of social recognition and memory (Bielsky & Young, 2004; Carter, 2003; Ferguson, Young, & Insel, 2002; Neumann, 2008) it will be critical to better understand how this system is primed by variations in early life experiences that stimulate this social neurobiology. In this case, one of the current suggestions is that MS leads to an oxytocinergic system that can "turn on" enough to gather some information,

but due to poor systemic development it does not successfully interact with other regions that are necessary in the behavioral displays of having formed robust social memories. The subsequent experiment in this dissertation will involve a preliminary exploration of how early postnatal oxytocin administration might shape the development of social memory and cue recognition by adolescent female mice.

Experiment 2 – Does the administration of oxytocin during early postnatal development (using daily, SC injections during PND 1-14) influence social recognition or the development of a preference for familiar social odors in AFR and MS female mice?

Consistent maternal contact exerts a powerful influence over the development of the central oxytocin system by promoting neural organization and regulating the release of oxytocin in rodent pups during exposures to social stimuli (Uvnäs-Moberg, Bruzelius, Alster, & Lundeberg, 1993). In contrast to the beneficial effect of consistent maternal caretaking, maternal separation has been consistently demonstrated to exert a powerful and sometimes deleterious impact on the consistency of interactions between the infant and mother and later life neurobehavioral outcomes (Francis & Kuhar, 2008; Pryce & Feldon, 2003; Ladd et al., 2000). For example, Thomas and colleagues (2010) previously demonstrated that maternal separation modifies the early acquisition of preferences for maternally-derived social odors, which has been hypothesized to be at least partially dependent on neural activation within the oxytocin system as a by-product of the social dyad that results from the mother-infant bonding process (Douglas, 2010). In addition, Veenema and colleagues (2007) provided much needed insight into the impact of MS on the neurobiology that underlies social interaction by demonstrating that this early life manipulation reduces basal and post-social encounter oxytocin immunoreactivity in the hypothalamic nuclei of mature female mice. Together with the results from the first

experiment in this dissertation, a corpus of literature has begun to provide evidence that MS contributes to a trait-like impairment in sociobehavioral domains (Thomas et al., 2008; Veenema, 2009; Veenema et al., 2007).

Further evaluation of the developmental impact of early life oxytocinergic activity and its long-term influence on the brain and behavior has most recently involved the use of psychopharmacological techniques, such as postnatal oxytocin administration. Despite the existence of studies designed to investigate the long-term effects of postnatal oxytocin administration on several domains of development, no studies have sought to characterize its potential impact on responses to species-specific social cues, including olfactory-derived stimuli and social recognition abilities, in AFR or MS rodents.

The previous experiment in this dissertation provided evidence that early experiences can substantially influence the later life expression of oxytocin-mediated social responses by MS mice. Therefore, this experiment tested the hypothesis that repeated, postnatal administrations of oxytocin (1 mg/kg) during the first two weeks of life would, 1) enhance the already well validated preference for familiar social odors in AFR (AFR-OXT) mice, 2) contribute to improved development of the preference for social odors derived from group housing conditions by MS mice (MS-OXT), 3) improve social recognition abilities in AFR mice (AFR-FAM/OXT and AFR-NOV/OXT), as measured by the habituation-dishabituation paradigm, and 4) remediate behavioral displays of social recognition by MS mice (MS-FAM/OXT and MS-NOV/OXT) in the habituation-dishabituation paradigm.

Method

Subjects and Procedures

The subjects in this experiment were 216 adolescent females birthed from 36 litters of mice (18 litters per rearing condition). All females used in this experiment were randomly assigned to either AFR or MS on PND 1 and were then reared according to procedures described in the general methodology. The only significant alteration from either the general methodology or experiment 1 in rearing procedure that was introduced in this experiment involved the daily, postnatal administration of oxytocin or physiological saline. A split-litter design was used to randomly assign 3 females per litter (n=108) to receive daily, subcutaneous (SC) injections of oxytocin and 3 females per litter (n=108) to receive daily, SC injections of an equivalent volume of saline. At weaning (PND 21), all animals were group housed with like condition females and maintained according to the procedures detailed in the general methodology. The experimental design and litter assignments can be viewed in Table 2 for AFR subjects and for MS subjects.

Olfactory preference testing was conducted on PND 48 for all subjects. Prior to the start of testing two adolescent females were randomly selected from each litter, with one belonging to the oxytocin-injected condition (n = 9) and one belonging to the saline-injected condition (n = 9). Olfactory preference testing was conducted as detailed in the general methodology and nest bedding material served as the social odor stimulus and clean shavings as the non-social odor. The bedding material that served as the social odor was extracted from the nesting cage on the day of behavioral observations and the test apparatus was cleaned with a diluted soap solution and thoroughly dried between each test subject.

Social recognition testing began on PND 49 and was conducted in accordance with the procedures outlined in the general methods section. The subjects that were used as stimulus animals in this experiment were unrelated, uninjected female mice of the same age and similar weight. One test subject per injection condition per litter was assigned to each dishabituation presentation category.

Oxytocin Administration

Drugs. Postnatal oxytocin-treated (OXT) mice were injected, subcutaneously with oxytocin (Bachem, California, United States). Oxytocin was dissolved in physiological saline and injected into pups in a concentration of 1 mg/kg. The dosage used in the present experiment was selected following previous demonstrations that this concentration is effective in inducing long-term physiological and behavioral effects in rats (Petersson & Uvnas-Moberg, 2008; Uvnas-Moberg, Alster, Petersson, Sohlstrom, & Bjorkstrand, 1998). The control subjects in this experiment were administered SC injections of physiological saline (SAL; 0.9% NaCl) in the same volume administered to OXT mice.

Postnatal Oxytocin Treatment. Following the split-litter design, three female pups were randomly selected to receive SC injections of oxytocin and the three remaining female pups in the litter were injected with the physiological saline. In order to avoid introducing an experimental confound into the design of this study, litters of pups were transferred from the vivarium to the laboratory while remaining in the home cage with the mother, were only briefly removed for the injection procedure, and then immediately returned to the nest. SC injections of either oxytocin or saline were administered daily, at 17:00 hr during PND 1-14. For all pups, injections were administered to the nape of each

animal's neck or over several sites on the animals back in instances when injection volumes were larger than 0.25 ml. The use of oxytocin- and saline-treated mice produced a modified experimental design by creating the following four groups that would then be randomly assigned to a dishabituation condition:

1. Animal facility-reared/oxytocin-treated (AFR-OXT): n = 54 females
2. Animal facility-reared/saline-injected (AFR-SAL): n = 54 females
3. Maternally separated/oxytocin-treated (MS-OXT): n = 54 females
4. Maternally separated/saline-injected (MS-SAL): n = 54 females

Statistical Analyses

Olfactory Preference Test. In this experiment, olfactory preference data was analyzed using four 2 x 2 (Rearing condition x Injection condition) ANOVA's. The independent factors that were used in each analysis included rearing condition (MS or AFR) and injection condition (OXT or SAL). The response variables used during this data analysis included the time spent over familiar nest bedding, time spent over the clean shavings, time spent over the center triangle, and a difference score (computed by subtracting the time spent over the clean shavings from the time spent over the nest bedding). Additionally, the analysis of the difference scores involved conducting single sample *t*-tests for each injection condition against a hypothetical zero to provide a statistical indicator of preference for the socially-derived odor. Mean difference scores that were observed to be significantly greater than zero are indicative of a preference for the social cue over the odor of the natural shavings.

Social Recognition. Six 2 x 2 x 4 (Rearing condition x Injection condition x Exposure session) mixed ANOVAs were used to analyze behavioral indices of social recognition across the FAM, NOV, and NO. The between-subjects factors used in each analysis were rearing condition and injection condition. Exposure session was the within-subjects factor. The response variables used in the analyses were the following: 1) time spent investigating the wire screen in the social arena, and 2) frequency of prosocial investigatory behaviors. Partial eta-squared (η_p) was calculated as a measure of effect size for significant main effects or the interaction of the factors used in the ANOVA. Post hoc analysis of main effects for exposure session were conducted using Tukey's HSD procedure. Additionally, post hoc analysis of interaction effects between rearing condition and exposure session involved polynomial trend analysis (linear for habituation by FAM subjects and quadratic for dishabituation by NOV subjects) to test for simple effects within each rearing condition. Tukey's HSD was then used to compare mean differences between exposure sessions within rearing condition. Post hoc evaluation of interaction effects also included the calculation of unpaired Student's t-tests to examine rearing condition differences at each level of exposure session. Significant comparisons using Student's t-test were accompanied by the calculation of Cohen's *d* as a conservative measure of effect size.

Results

Social odor preferences

Results of the 2 x 2 ANOVA's for the amount of time spent investigating the familiar nest bedding showed that AFR females spent significantly more time than MS females investigating the familiar bedding material, $F(1,32) = 41.07$, $p < 0.001$, $\eta_p = 0.56$.

The main effect for injection condition and interaction between the two factors was not significant, both p's > 0.05.

Results of the 2 x 2 ANOVA's for the amount of time spent investigating the clean shavings showed that AFR females spent significantly less time than MS females investigating the fresh bedding material, $F(1,32) = 16.30$, $p < 0.001$, $\eta_p = 0.34$. The main effect for injection condition and interaction between the two factors were not significant, both p's > 0.05.

Results of the 2 x 2 ANOVA's for the amount of time spent in the center triangle of the odor preference apparatus indicated that MS females spent significantly more time than AFR subjects in the central area of the test apparatus, $F(1,32) = 12.84$, $p < 0.001$, $\eta_p = 0.29$. The main effect for injection condition and interaction between the two factors were not significant, both p's > 0.05.

Mean time spent investigating the familiar nest bedding, fresh shavings, and the central area of the test apparatus can be found in Table 3.

Results of the 2 x 2 ANOVA's for mean difference scores (familiar bedding time - fresh bedding time) indicated that MS females had significantly reduced difference scores in comparison to AFR subjects, $F(1,32) = 46.56$, $p < 0.001$, $\eta_p = 0.59$. Additionally, the ANOVA results showed that mean differences scores were significantly greater for oxytocin-injected mice compared to those calculated for saline-injected mice, $F(1,32) = 4.12$, $p < 0.05$, $\eta_p = 0.12$. Rearing condition and injection condition did not significantly interact with one another, $p > 0.05$. Despite the finding of no interaction between rearing condition and drug condition, informal observation of Figure 7 suggests that the rearing

conditions were differentially impacted by oxytocin-administration, therefore simple main effects were evaluated. The single-sample t-tests indicated that AFR mice belonging to both the oxytocin-injected ($t(8) = 10.62$, $p < 0.001$, $d = 7.51$) and saline-injected ($t(8) = 8.89$, $p < 0.001$, $d = 6.29$) groups displayed a behavioral preference for their home nest shavings. Additionally, single-sample t-tests indicated the MS-OXT mice also preferred their home nest shavings over clean bedding material, $t(8) = 4.79$, $p < 0.001$, $d = 3.39$. However, females in the MS-SAL condition did not display preferential behavior for their home nest shavings, $t(8) = 0.91$, $p > 0.05$. Mean difference scores for each injection condition are presented in Figure 7.

Social Recognition

Screen Investigation Time. A 2 x 2 x 4 mixed ANOVA was used to evaluate the mean duration of time (s) AFR-OXT/SAL and MS-OXT/SAL subjects in the FAM dishabituation condition investigated the wire screen during the social recognition test. In comparison to MS females, AFR subjects spent significantly less time investigating the wire screen across all exposure sessions, $F(1,32) = 65.66$, $p < 0.001$, $\eta_p = 0.67$, which is consistent with the results of Experiment 1. The ANOVA results indicated the presence of a main effect of exposure session, $F(3,96) = 30.00$, $p < 0.01$, $\eta_p = 0.48$. Exposure session was also shown to significantly interact with rearing condition, $F(3,96) = 6.20$, $p < 0.01$, $\eta_p = 0.16$. The results of the ANOVA showed that no other main effects or interactions were significant, all p's > 0.05. See Figure 8 for mean investigation time comparisons by dishabituation condition (FAM, NOV and NO) and injection condition (SAL and OXT) across exposure session.

Analysis of the interaction effect between rearing condition and exposure session revealed that, irrespective of injection condition, there was a habituation response across exposure session by AFR-FAM subjects, $F(1,17) = 32.87$, $p < 0.001$, $\eta_p = 0.66$. Mean screen investigation time by AFR-FAM mice during exposure session 1 was significantly greater than during session 2 thru 4 (all *p's* < 0.05). Additionally, irrespective of injection condition, females in the MS-FAM condition displayed a change in response to the social stimulus, $F(1,17) = 13.72$, $p < 0.01$, $\eta_p = 0.45$. Mean screen investigation time by MS-FAM subjects during exposure session 1 was significantly greater than during session 2 thru 4 (all *p's* < 0.05). Pairwise comparisons were also conducted between rearing conditions (irrespective of injection condition) at each level of exposure session and indicated that mean investigation time by AFR-FAM females did not significantly differ from MS-FAM females during exposure session 1, $p > 0.05$). However, during exposure session 2, $t(16) = 5.17$, $p < 0.001$, $d = 2.59$, exposure session 3, $t(16) = 8.76$, $p < 0.001$, $d = 4.38$, and exposure session 4, $t(16) = 9.08$, $p < 0.001$, $d = 4.54$. AFR-FAM females displayed significantly greater reductions in mean investigation time in comparison MS-FAM females. See figure 8 for mean investigation time comparisons between AFR-OXT, AFR-SAL, MS-OXT, and MS-SAL subjects within the FAM condition. In summary, the interaction between rearing condition and exposure session seems to be driven by the greater magnitude of habituation displayed by the AFR-FAM animals. As in Experiment 1, AFR-FAM animals show substantial habituation, but in contrast to Experiment 1 MS-FAM animals also displayed at least some level of habituation between exposure session 1 and 2, though not to the degree as AFR-FAM animals.

A 2 x 2 x 4 mixed ANOVA was used to evaluate the mean duration of time (s) AFR-OXT/SAL and MS-OXT/SAL subjects in the NOV dishabituation condition investigated the wire screen during the social recognition test. The results indicated that in comparison MS females, AFR subjects spent significantly less time investigating the wire screen across all exposure sessions, $F(1,32) = 11.63$, $p < 0.001$, $\eta_p = 0.26$, which is consistent with Experiment 1. The ANOVA results further revealed the presence of a main effect of exposure session, $F(3,96) = 13.20$, $p < 0.001$, $\eta_p = 0.30$. Exposure session was also shown to significantly interact with rearing condition, $F(3,96) = 8.20$, $p < 0.001$, $\eta_p = 0.20$. No other main effects or interactions were significant, p's > 0.05. See Figure 8 (AFR subjects) and 8 (MS subjects) for mean investigation time comparisons by dishabituation condition (FAM, NOV and NO) and injection condition (SAL and OXT) across exposure session

Analysis of the interaction effect between rearing condition and exposure session revealed that, irrespective of injection condition, there was a characteristic habituation-dishabituation response by AFR-NOV subjects, $F(1,17) = 19.40$, $p < 0.001$, $\eta_p = 0.66$. Mean screen investigation time by AFR-NOV mice during exposure session 1 was significantly greater than during session 2 and 3 (all p's < 0.05), but did not differ from investigation time during session 4, $p > 0.05$. Additionally, mean screen investigation time during exposure session 2 and 3 was shown to be significantly less than during session 4, both p's < 0.01. In contrast, irrespective of injection condition, females in the MS condition did not show a significant change in screen investigation that would indicate the presence of a habituation-dishabituation response, $F(1,17) = 0.17$, $p > 0.05$. Pairwise comparisons further revealed that during exposure session 2, $t(16) = 5.83$, $p <$

0.001, $d = 2.92$, and exposure session 3, $t(16) = 5.04$, $p < 0.001$, $d = 2.52$ AFR females displayed significantly reduced mean investigation time in comparison MS females. See figure 8 for mean investigation time comparisons between AFR-OXT, AFR-SAL, MS-OXT, and MS-SAL subjects within the NOV condition.

A 2 x 2 x 4 mixed ANOVA was used to evaluate the mean duration of time (s) AFR-OXT/SAL and MS-OXT/SAL subjects in the NO dishabituation condition investigated the wire screen during the social recognition test. There were no main effects and no interactions, all p's > 0.05. See Figure 8 (AFR subjects) and 8 (MS subjects) for mean investigation time comparisons by dishabituation condition (FAM, NOV and NO) and injection condition (SAL and OXT) across exposure session.

Investigatory Behavior. A 2 x 2 x 4 mixed ANOVA was conducted to examine mean differences in the frequency of investigatory behavior by AFR-OXT/SAL and MS-OXT/SAL subjects within the FAM condition. The results indicated that MS females displayed significantly more investigatory behavior than AFR subjects, $F(1,32) = 8.48$, $p < 0.01$, $\eta_p = 0.21$. Additionally, saline-injected mice engaged in more investigatory behavior than oxytocin-injected mice, $F(1,32) = 13.30$, $p < 0.001$, $\eta_p = 0.29$. Finally, rearing condition and injection condition significantly interacted, $F(1,32) = 6.30$, $p < 0.05$, $\eta_p = 0.16$. The ANOVA results also revealed a significant effect of exposure session, $F(3,96) = 5.98$, $p < 0.001$, $\eta_p = 0.16$. All other main effects and interactions were not significant, $p > 0.05$. See Figure 9 (AFR subjects) and 9 (MS subjects) for mean investigatory behavior comparisons by dishabituation condition (FAM, NOV and NO) and injection condition (SAL and OXT) across exposure session.

The results of the Tukey's HSD procedure across exposure session indicated that mean frequency of investigatory behavior during session 1 was significantly greater than during session 2, 3, and 4, all p's < 0.05. Student's t-tests were used to further evaluate the interaction between rearing and injection condition within the FAM group. Subjects in the AFR-OXT, AFR-SAL, and MS-OXT did not differ from one another in displays of social investigatory behaviors, all p's > 0.05. However, MS-SAL subjects displayed significantly more investigatory behavior than AFR-SAL subjects, $t(34) = 3.83$, $p < 0.001$, $d = 1.31$ and MS-OXT mice, $t(34) = 4.35$, $p < 0.01$, $d = 1.49$. See figure 9 for mean investigatory behavior comparisons between AFR-OXT, AFR-SAL, MS-OXT, and MS-SAL subjects within the FAM condition.

A 2 x 2 x 4 mixed ANOVA was conducted to examine mean differences in investigatory behavior by AFR-OXT/SAL and MS-OXT/SAL subjects within the NOV condition. The results indicated that, irrespective of injection condition, MS females displayed significantly more investigatory behavior than AFR subjects, $F(1,32) = 17.63$, $p < 0.001$, $\eta_p = 0.36$. The ANOVA results also showed that the within-subjects main effect for exposure session was significant, $F(1,32) = 8.86$, $p < 0.001$, $\eta_p = 0.22$. All other main effects and interactions were not significant, $p > 0.05$. See Figure 9 (AFR subjects) and 16b (MS subjects) for mean investigatory behavior comparisons by dishabituation condition (FAM, NOV and NO) and injection condition (SAL and OXT) across exposure session.

The results of the Tukey's HSD procedure across exposure session indicated that mean frequency of investigatory behavior during session 2 was significantly decreased in comparison to the frequency of behavior displayed during session 1, 3, and 4, all p's <

0.05. See figure 9 for mean investigatory behavior comparisons between AFR-OXT, AFR-SAL, MS-OXT, and MS-SAL subjects within the NOV condition.

A 2 x 2 x 4 mixed ANOVA was conducted to examine mean differences in investigatory behavior by AFR-OXT/SAL and MS-OXT/SAL subjects within the NO condition. The results indicated that, MS females displayed significantly more investigatory behavior than AFR subjects, $F(1,32) = 6.42$, $p < 0.05$, $\eta_p = 0.17$. Additionally, saline-injected mice engaged in more investigatory behavior than oxytocin-injected mice, $F(1,32) = 9.90$ $p < 0.01$, $\eta_p = 0.24$. Finally, rearing condition and injection condition significantly interacted, $F(1,32) = 6.55$, $p < 0.05$, $\eta_p = 0.17$. The ANOVA results also revealed a significant interaction between rearing condition and exposure session, $F(3,96) = 3.35$, $p < 0.001$, $\eta_p = 0.10$. All other main effects and interactions were shown to be not significant, $p > 0.05$. See Figure 9 (AFR subjects) and 9 (MS subjects) for mean investigative behavior comparisons by dishabituation condition (FAM, NOV and NO) and injection condition (SAL and OXT) across exposure session.

A one-way ANOVA was conducted on each rearing condition to examine the trend in investigatory behavior across exposure session and did not reveal significant within-condition changes, both p's > 0.05. However, between rearing condition comparisons at each level of exposure session indicated that during exposure session 2, $t(34) = 3.17$, $p < 0.01$, $d = 1.09$, and exposure session 4, $t(34) = 2.81$, $p < 0.001$, $d = 0.96$ MS females displayed significantly greater prosocial investigatory behavior than AFR subjects. In summary, the MS-SAL group deviated from all others by displaying a greater frequency of investigatory behavior across the exposure sessions. The aberrant behavior of this group was responsible for both the interaction between exposure session and rearing

condition and also the interaction between rearing condition and injection condition. See figure 9 for mean investigatory behavior comparisons between AFR-OXT, AFR-SAL, MS-OXT, and MS-SAL subjects within the NO condition.

Discussion

Maternal caretaking is pivotal in shaping the organization and function of the oxytocin system as well as how it will ultimately contribute to socially-oriented responses (Francis, Young, Meaney, & Insel, 2002; Meaney, 2001; Pryce, Ruedi-Bettschen, Dettling, & Feldon, 2002) which in turn are corrupted should the anxiolytic dynamic of the mother-infant relationship be disrupted (Hofer, 1994; Pryce & Feldon, 2003). Previous studies (Thomas et al., 2008; Veenema, 2009; Veenema et al., 2007) and the results of the first experiment in this document elaborate upon the detrimental effect that MS can have on the expression of a wide-range of oxytocin-dependent social behaviors in adolescence and adulthood as well. Veenema and colleagues (2007) went on to reveal that MS is likely to exert its effect on social behaviors by impeding growth of the oxytocinergic nuclei within the paraventricular region of the hypothalamus early in the development of female rodents. Because several studies (Holst, Uvnäs-Moberg, & Petersson, 2002; Insel & Winslow, 1991; Kramer, et al., 2003; Olausson, et al., 2003; Uvnäs-Moberg, et al., 1998) have previously identified postnatal oxytocin administration as a potential benefit to physiological and behavioral development, this experiment sought to examine whether the administration of oxytocin during early postnatal development (using daily, SC injections during PND 1-14) would enhance the development of a preference for familiar social odors or social recognition in AFR mice. This experiment also examined the hypothesis that the administration of oxytocin during early postnatal development (using daily, SC injections during PND 1-14) would rescue

the development of preferences for familiar social odors and social recognition memory in maternally separated female mice. Overall, the results of this experiment replicate Experiment 1. MS mice display impaired social odor preferences and disrupted habituation-dishabituation responses. However, with one exception, early postnatal administration had no effect on olfactory preference or social behavior.

The prediction that postnatal oxytocin administration would enhance how AFR mice would respond to social odors was not supported by the present data. Although females in both the OXT and SAL condition displayed the expected preference for the odor of social nest bedding, the group means did not significantly differ from one another. This finding indicates that, although previous studies (Lim & Young, 2006; Nelson & Panksepp, 1996; Nelson & Panksepp, 1998) have demonstrated that early life oxytocin administration facilitates the acquisition of preferences for maternal odors during the preweanling period, this added benefit is not observed later in life. Observations of their behavior while in the test apparatus showed that AFR females belonging to both injection conditions spent a majority of their time investigating the odor that had attained familiarity by virtue of the post-weaning group housing circumstances. Both of these findings are consistent with previous research using this particular mouse model showing that adolescent female mice acquire preferences for the social odors associated with group housing conditions (Thomas, et al., 2008). The broader application of this result is that it corresponds with the demonstration of rapid preference acquisition for social odors in several other species of adolescent and adult rodents (Eidson, Maras, Epperson, & Petrulis, 2007; Fortier, Erskine, & Tamarin, 1996; Galef & Laland, 2005).

Overall, postnatal oxytocin administration did not significantly improve preferential responding to odors of a social origin by MS mice. In comparison to MS-SAL females, MS-OXT subjects did not demonstrate significantly greater interest in the familiar odor derived from their home nest bedding. Evaluation of the difference scores revealed that females belonging to the MS-SAL condition did not prefer the odor of nest bedding over the odor of clean shavings. This finding corroborates the results of a previous study indicating that MS impairs the acquisition of preferences for social nest odors during the post-weanling period of development (Thomas et al., 2008). Difference scores for AFR mice demonstrated a strong preference for familiar social bedding over clean shavings. The single finding indicating that postnatal oxytocin administration could potentially benefit long-term behavioral development following disruptions to mother-infant bonding and may buffer the detrimental effect of disordered social bonding was the re-emergence of the behavioral preference for social odors by adolescent MS-OXT females.

Although a small body of literature exists detailing the physical and behavioral impact of postnatal oxytocin administration in rodents, only one other study to date has shown that early treatment with the neuropeptide could potentially ameliorate the stress-induced effects of adverse early experiences. In their study, Olausson, Uvnäs-Moberg, and Sohlstrom (2003) found that repeated administration of oxytocin early in life alleviated the effects of neonatal undernutrition by increasing body weight and reducing blood pressure and plasma corticosterone concentrations to levels observed in adult rats that had not been exposed to malnourishment. While Olausson and colleagues (2003) examined recovery growth following a physiological insult to development, the present findings expand the literature base by demonstrating that postnatal oxytocin

administration may alleviate the effects of significant early social-psychological stressors that act on the hypothalamic-limbic system structures as well.

Overall, MS disrupted the pattern of social recognition. Within the AFR group, AFR-OXT and AFR-SAL females collectively displayed social habituation (FAM) and dishabituation (NOV) that was inline with the findings in Experiment 1. MS animals displayed limited habituation in the FAM but not the NOV conditions. Postnatal oxytocin administration did not explicitly influence the amount of time that AFR or MS subjects spent investigating the stimulus animals during assessments of social recognition. Although these results are not in agreement with the hypothesis that postnatal oxytocin administration could impact social recognition memory, these data further affirm that adolescent female mice can adeptly form social memories on par with those demonstrated in rats and prairie voles (Bluthe & Dantzer, Tang & Reeb, 2004; Engelmann, et al., 1998; Tang, et al., 2003; also reviewed in Bielsky & Young, 2004).

In summary, although the results of this experiment did not support the predicted influence of postnatal oxytocin administration on social recognition the outcome did support to the findings for AFR and MS females in the first experiment. Specifically, the data from both experiments show that AFR females can readily acquire social memories that are retained across several exposures to a familiar conspecific. Moreover, the results show that AFR females are also capable of shifting their response set upon an encounter with a novel conspecific. In contrast, MS females displayed a more dysfunctional pattern of social recognition behavior that involved poorer gain in familiarity and an inability to shift interest when a novel conspecific was introduced to the test situation. Despite the absence of results that demonstrate an impact of postnatal oxytocin injections on social

recognition memory, it does contribute to a growing base of literature by providing an instance in which exposing infant rodents to exogenous oxytocin does not significantly impact two later life behaviors that are characteristically involved in establishing social relationships.

General Discussion

For mammals, early postnatal development is characterized by social interactions that are nonselective and almost entirely under the mother's control. It is these early social interactions between a mother and its infant that profoundly modulate the remainder of development in a wide range of physiological and behavioral domains (Broad, et al., 2006; Champagne & Curley, 2009; Hofer, 1994). However, if a significant disruption, such as maternal separation, is imposed upon the mother-infant bonding experience, developmental outcomes are generally poor and include significant deficits in areas such as socioemotional responding and cognitive function have been (Champagne & Curley, 2009; Cirulli, Berry, & Alleva, 2003; Pryce & Feldon, 2003; Pryce, et al., 2002). More recently, researchers have become increasingly interested in providing more detail on how postnatal manipulations to the early social environment influence social interactions and preferences that underlie social relationships at various intervals throughout the lifespan (Boccia & Pedersen, 2001; Thomas, et al., 2008; Veenema & Neumann, 2009; Veenema, et al., 2007; Veenema, et al., 2006). The data in this dissertation broadly corroborate the outcomes of previous studies and provide a further exemplification of the long-term deleterious impact of MS. The specific contribution of this work is the finding that social recognition in adolescence is impaired by MS in early life.

A handful of studies have shown that maternal separation affects the expression of several selective social behaviors in female rodents, including displays of aggression and social odor preferences (Boccia & Pederson, 2001; Thomas et al., 2008; Veenema & Neumann, 2008; Veenema, et al., 2007). The present study provides evidence that MS may induce a more broad behavioral failure that revolves around poor recognition of species-specific social cues and the discrimination of familiar from novel individuals. The current data provide a plausible mechanism to explain displays of erratic maternal aggression in mice and rats (Boccia & Pederson, 2001; Veenema & Neumann, 2008; Veenema, et al., 2007) and disruptions in the acquisition of preferences for social odors by mice (Thomas et al., 2008). Individuals that have been reared in environments characterized by a deficiency in social contact may simply establish a behavioral prototype of stress-induced social neophobia rather than attempts by an individual to become familiar with other members or odors belonging to its species (Witt, Winslow, & Insel, 1992). However, further research is necessary to better understand how the social recognition deficits found in maternal separated animal's impact additional aspects of information processing and social interaction, such as those involved in kin recognition and the individual discrimination that underlies mate-selection and affiliative hierarchies.

The neurobiological substrate for social behavior in rodents is oxytocin. It has become increasingly clear that throughout development this nonapeptide provides a significant portion of the neuromodulatory foundation for widespread neural and behavioral activation when individuals encounter another conspecific (i.e. social recognition) in a social setting (Ferguson et al., 2000; Ferguson et al., 2002; Bielsky and Young, 2004; Carter, 1998, 2003; Ferguson et al., 2002; Winslow and Insel, 2004).

There is also ample evidence to suggest that the organization and function of the oxytocin system is sensitive to variations in early social stimulation, such as maternal caretaking (Francis, Young, Meaney, & Insel, 2002) and artificial manipulation via exogenous oxytocin treatment (Lim & Young, 2006; Neumann, 2008). Seemingly in contrast to this literature, the results of Experiment 2 showed that daily postnatal oxytocin treatment during the first two weeks of life did not significantly influence social odor preferences or social recognition at adolescence in AFR and MS females. Previous studies have demonstrated that postnatal oxytocin treatment can have life-long effects on physiological parameters in several species of rodents (Insel & Shapiro, 1992; Uvnäs-Moberg, et al., 1993; Uvnäs-Moberg, et al., 1998; Yamamoto et al., 2004) and social behaviors in prairie voles (Bales & Carter, 2003a; Cushing, et al., 2005; Witt, Carter, & Walton, 1990). It is possible that postnatal oxytocin treatment may not exert an impact on behavioral outcomes at the injection dosage used in this dissertation. Furthermore, it would have been beneficial to verify that physiologically relevant dosages of oxytocin crossed the blood-brain barrier to exert an impact on social recognition behavior. These and other potential explanations provide an important avenue for future research.

The postnatal oxytocin system has also displayed sensitivity to early environments in which the social contact is lacking or disrupted. Veenema, Bredewold, and Neumann (2007) showed that MS decreased oxytocin immunoreactivity within the female rat paraventricular hypothalamus. Experiment 2 showed that postnatal oxytocin administration recovered preference acquisition for social odors in adolescent female mice. The fact that one facet of prosociality was recovered as a result of the postnatal oxytocin treatment was a boon for the value of the outcome in experiment 2. Social odor

preferences and recognition are reliant on oxytocin-facilitated (and other neuromodulators) activation of the olfactory system and medial amygdala (Dluzen et al., 1998; Ferguson, et al., 2001; McEwen, 2004). Because one major aspect of sociobehavioral function was recovered this study suggests that postnatal oxytocin treatment may played a preservative role in one specific aspect sociobehavioral function in MS females. One potential route to explore in future studies involves better understanding the anxiolytic properties of oxytocin administration in maternally separated rodents and whether the fact that treatment was administered just prior to separation ameliorated the stress and negative biobehavioral consequences of the experimental procedure. In addition, future studies should be directed at mapping the broader organizational influence of both endogenously and exogenously supplied oxytocin. Such studies will play a critical role in the interpretation of the accumulating behavioral data from studies using genetically modified animals and experimental manipulations of the early environment alike. To that extent, a deeper understanding of the neuromodulatory role of oxytocin with areas such as the hypothalamic-limbic circuitry and estrogen receptor areas in the periphery would aid in the design behavioral assays that would optimally detect changes in social behavior that result from experimental manipulations. Most recently, a study using transgenic mice has shown that the CA2 region within the hippocampus is crucial in displays of social memory (Hitti & Siegelbaum, 2014). It would be reasonable to begin an exploration of how the central oxytocinergic system interacts with the CA2 region to contribute to gains in familiarity during social encounters. Additionally, a previous study by Choleris, Gustafsson, Korach, Muglia, Pfaff, and Ogawa (2003) indicated that social recognition relies on the

interaction between oxytocin and estrogen receptor α in female rodents. Kramer and colleagues (2007) went on to demonstrate that neonatal oxytocin administration had a long-term influence on the expression of estrogen receptor α, but not oxytocin neurons, in adult female rats. Thus, while the present results provide one avenue to pursue as an intervention for early insults to sociobehavioral development, it is clearly not as simple as just targeting the oxytocin system in relief of problematic social function.

In conclusion, this dissertation has provided a successful expansion to the knowledge base on the powerful effect that postnatal experiences have on social maturation in mammals. The interactions between a mother and infant truly do appear to not only promote the organization of the neurobiological foundation upon which future olfactory-mediated social discourse relies, but also has the potential to establish patterns of prototypical behavior at a young age. In addition, the outcome of this dissertation provides some preliminary insight into a tangible early postnatal treatment that might ameliorate the effects of early adversity. Ultimately, the value of the research in this dissertation lies in the ability to explain the results of previous studies by Thomas et al., (2008) and from the Veenema group (Lukas, et al., 2010; Veenema, & Neumann, 2008; Veenema, & Neumann, 2009; Veenema, et al., 2007), while providing innumerable routes for future research in several diverse areas that all possess the potential to better understand human social relationships.

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Appendix A. Acronyms

PND = postnatal day

AFR = animal facility reared

MS = maternally separated

S = exposure session

ITI = intertrial interval

FAM = familiar mouse

NOV = novel mouse

NO = no mouse

OXT = oxytocin-treated

SAL = saline-treated

AFR-OXT = oxytocin-treated animal facility reared mice

AFR-SAL = saline-treated animal facility reared mice

MS-OXT = oxytocin-treated maternally separated mice

MS-SAL = saline-treated maternally separated mice

Table 1. Design schematic for social recognition testing in Experiment 1.

Rearing Condition	***Hab/Dishab Treatment***	***Code***	***Trial 1***	***Trial 2***	***Trial 3***	***Trial 4***	***n***
AFR	***FAM***	*AFR-FAM*	Novel	Familiar	Familiar	Familiar	**9**
AFR	***NOV***	*AFR-NOV*	Novel	Familiar	Familiar	Novel	**9**
AFR	***NO***	*AFR-NO*	None	None	None	None	9
MS	***FAM***	*MS-FAM*	Novel	Familiar	Familiar	Familiar	9
MS	***NOV***	*MS-NOV*	Novel	Familiar	Familiar	Novel	9
MS	***NO***	*MS-NO*	None	None	None	None	9

***N* = 54**

Table 2. Design schematic for social recognition testing in Experiment 2.

Rearing Cond	*Dishab Treatment*	*Injection Cond*	*Code*	*Exp Sess 1*	*Exp Sess 2*	*Exp Sess 3*	*Exp Sess 4*	*n*
AFR	**FAM**	**OXT**	AFR-FAM/OXT	Novel	Familiar	Familiar	Familiar	**9**
AFR	**FAM**	**SAL**	AFR-FAM/SAL	Novel	Familiar	Familiar	Familiar	**9**
AFR	**NOV**	**OXT**	AFR-NOV/OXT	Novel	Familiar	Familiar	Novel	**9**
AFR	**NOV**	**SAL**	AFR-NOV/SAL	Novel	Familiar	Familiar	Novel	**9**
AFR	**NO**	**OXT**	AFR-NO/OXT	None	None	None	None	**9**
AFR	**NO**	**SAL**	AFR-NO/SAL	None	None	None	None	**9**
MS	**FAM**	**OXT**	MS-FAM/OXT	Novel	Familiar	Familiar	Familiar	**9**
MS	**FAM**	**SAL**	MS-FAM/SAL	Novel	Familiar	Familiar	Familiar	**9**
MS	**NOV**	**OXT**	MS-NOV/OXT	Novel	Familiar	Familiar	Novel	**9**
MS	**NOV**	**SAL**	MS-NOV/SAL	Novel	Familiar	Familiar	Novel	**9**
MS	**NO**	**OXT**	MS-NO/OXT	None	None	None	None	**9**
MS	**NO**	**SAL**	MS-NO/SAL	None	None	None	None	**9**

N = 108

Table 3. Group means and 95% C.I.'s for familiar vs. fresh bedding material.

Condition	Familiar odor time	Fresh odor time	Central area	Difference scores
AFR—SAL	112.58 [101.27, 123.90]	36.85 [29.16, 44.55]	30.56 [20.78, 40.35]	75.73 [59.03, 92.43]
AFR—OXT	111.47 [103.96, 118.99]	31.79 [24.03, 39.55]	36.74 [32.57, 40.91]	79.68 [64.98, 94.38]
MS—SAL	62.25 [48.11, 76.39]	54.43 [45.07, 63.78]	63.33 [46.30, 80.35]	7.82 [-9.06, 24.70]
MS—OXT	83.58 [69.87, 97.29]	46.58 [40.27, 52.89]	49.84 [34.80, 64.88]	36.99 [21.85, 52.14]

Figure Captions

Figure 1. A schematic representation of the habituation-dishabituation paradigm. In step 1 an experimental animal is pre-exposed to a conspecific (*A*) either once or a number of times prior to being exposed (Step 2) to either a novel (B) or the same, familiar (A) conspecific (adapted from Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009).

Figure 2. Diagram of the social recognition test apparatus (adapted from Cornwell-Jones & Bollers, 1983).

Figure 3. Diagram of the olfactory preference test apparatus (Thomas et al., 2008).

Figure 4. A schematic design of the habituation-dishabituation procedure that was used for each experiment in this dissertation.

Figure 5. Mean time spent investigating the wire screen by rearing condition (AFR and MS) and dishabituation condition (FAM, NOV, and NO) during exposure session 1-4 in the test of social recognition. n = 9 for each condition at each exposure session.

Figure 6. Mean investigatory behavior by rearing condition (AFR and MS) and dishabituation condition (FAM, NOV, and NO) during exposure session 1-4 in the test of social recognition. n = 9 for each condition at each exposure session.

Figure 7. AFR and MS group means for difference scores (social nest bedding –fresh shavings time) by injection condition (OXT or SAL) mice on PND 48. n = 9 for each injection condition. * Indicates a significant preference for social nest bedding, $p < 0.05$.

Figure 8. Mean time spent investigating the wire screen by rearing condition (AFR and MS), dishabituation condition (FAM, NOV, and NO) and injection condition (OXT and SAL) during

exposure session 1-4 in the test of social recognition. n = 9 for each condition at each exposure session.

Figure 9. Mean investigatory behavior displayed subjects shown across rearing condition (AFR and MS), dishabituation condition (FAM, NOV, and NO) and injection condition (OXT and SAL) during exposure session 1-4 in the test of social recognition. n = 9 for each condition at each exposure session.

Figure 1.

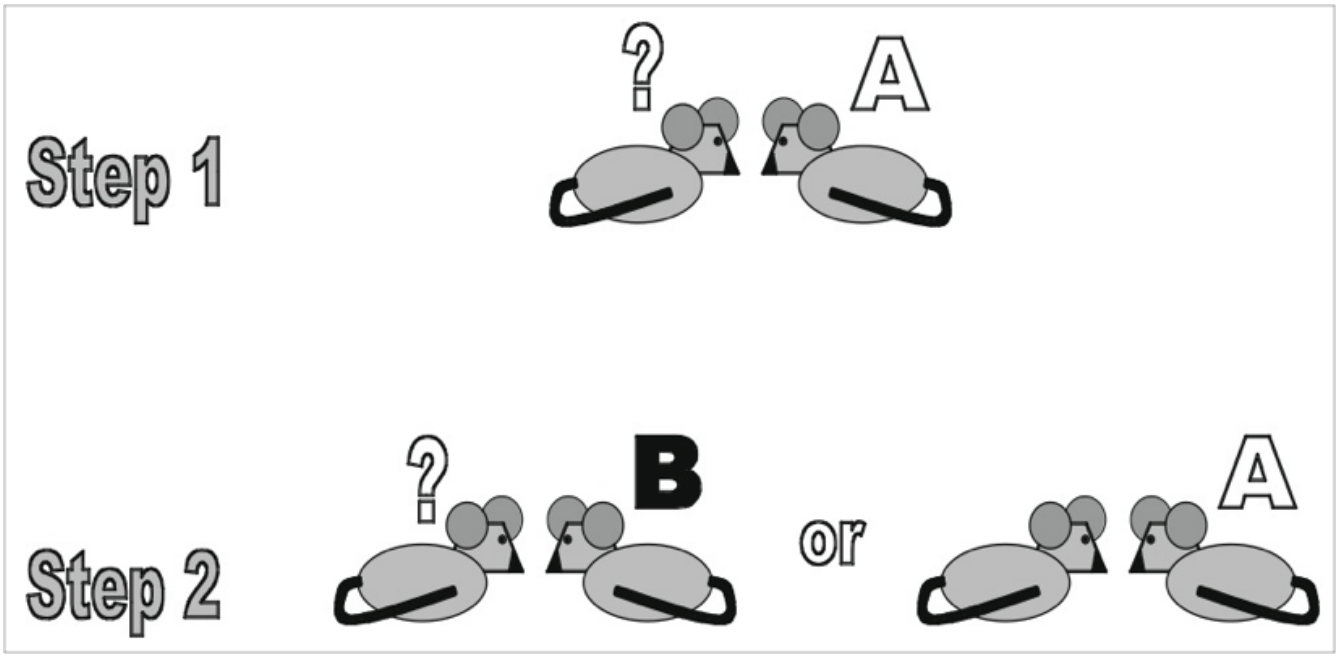


Figure 2.

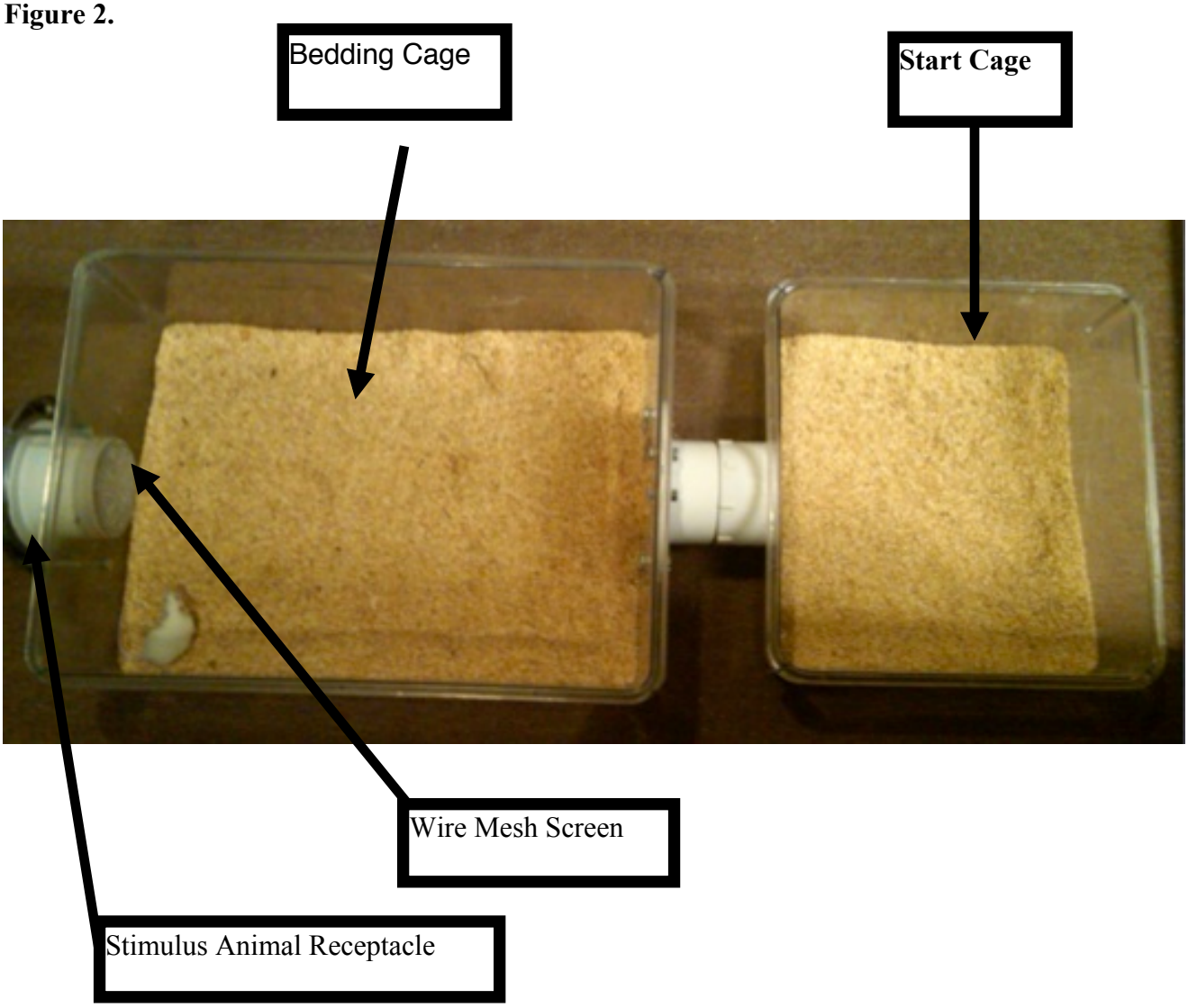

Bedding Cage
Start Cage
Wire Mesh Screen
Stimulus Animal Receptacle

Figure 3.

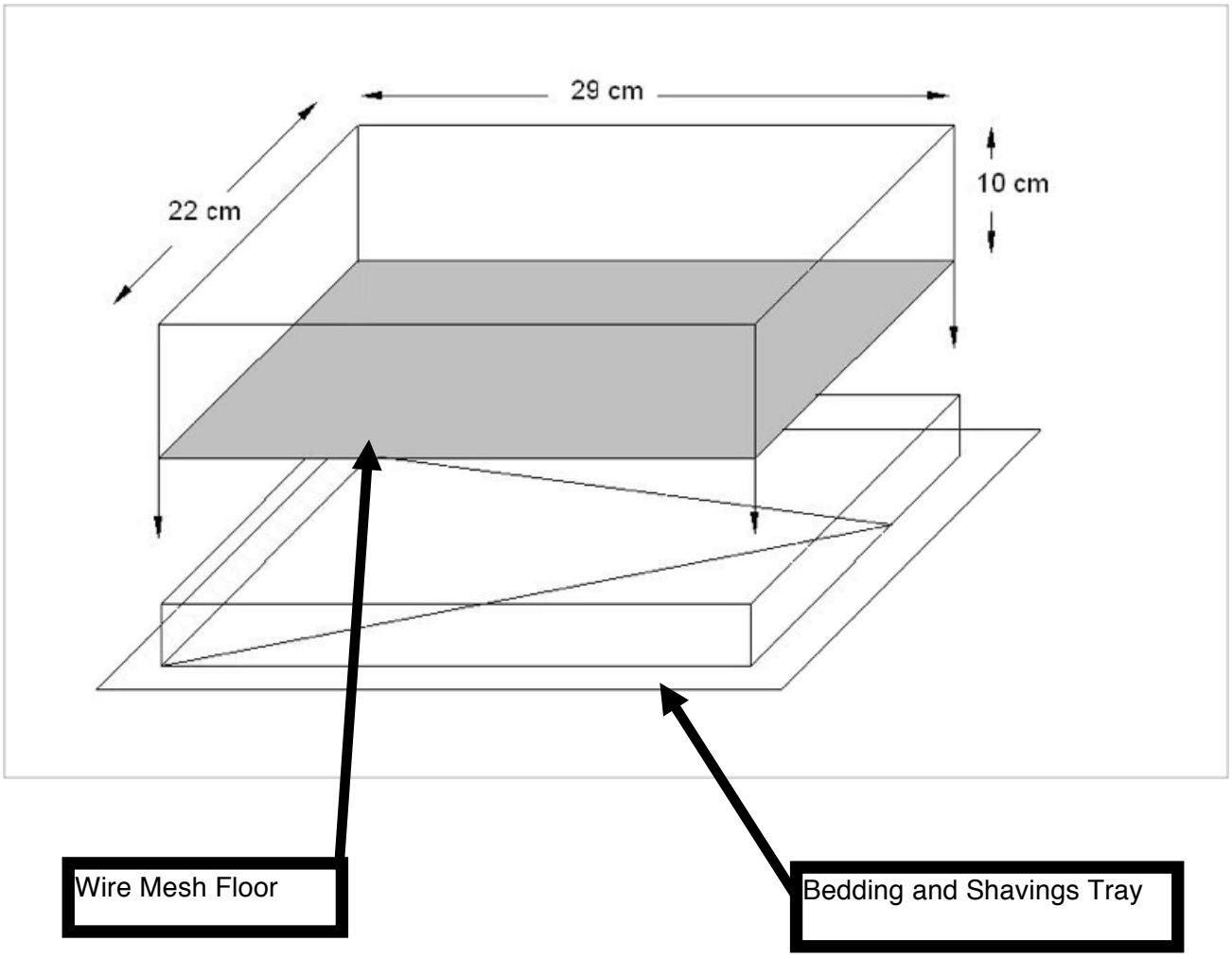


Figure 4.

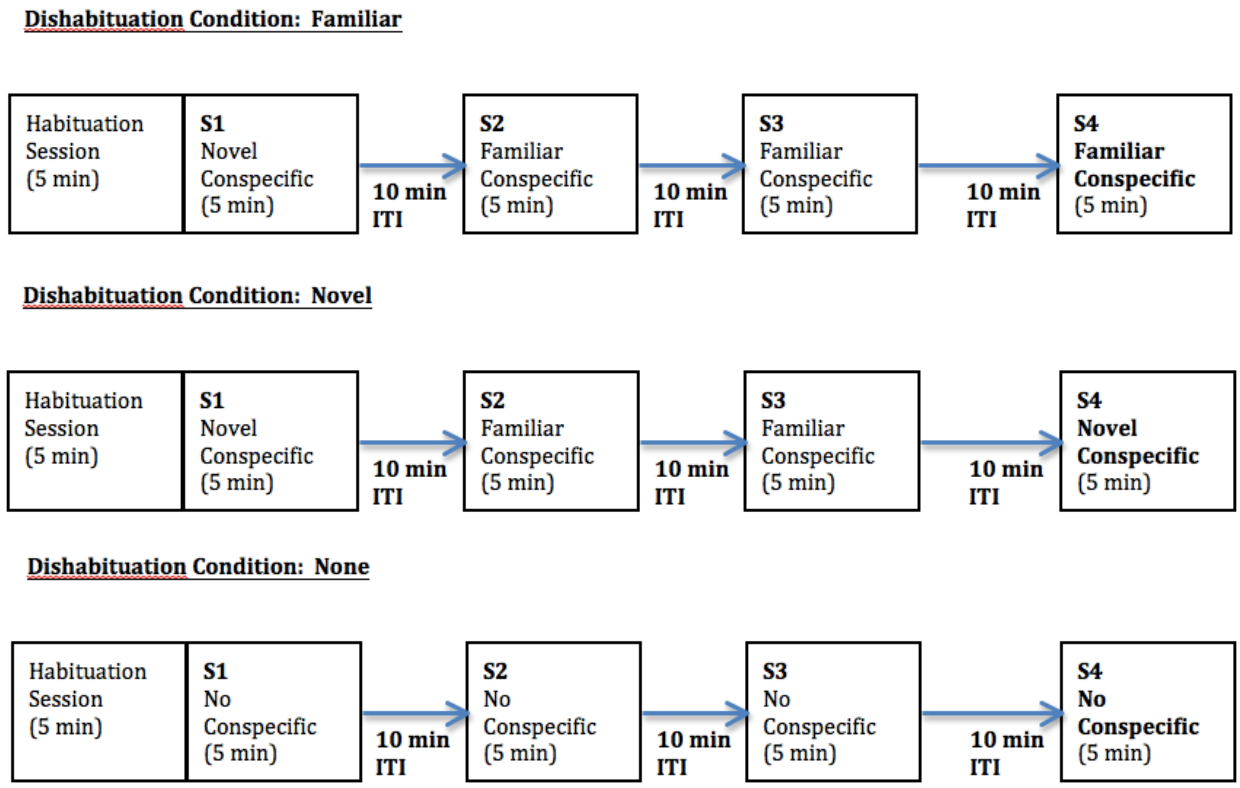
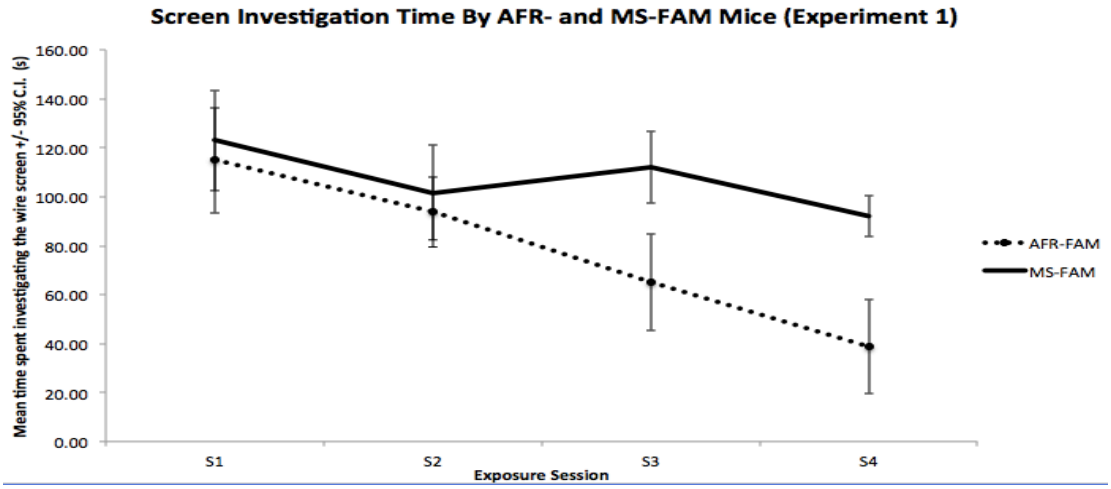
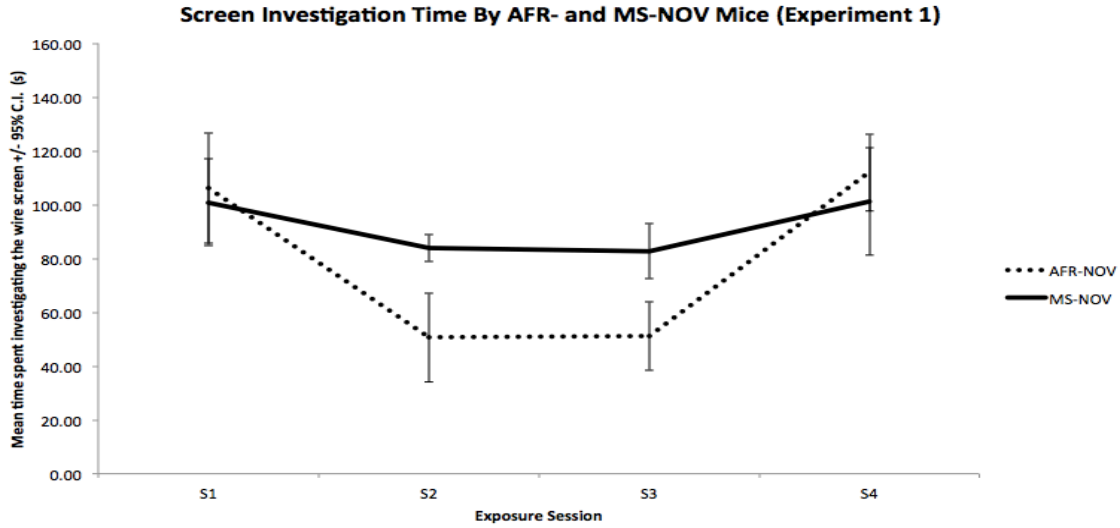

Dishabituation Condition: Familiar
Habituation Session (5 min)
S1 Novel Conspecific (5 min)
10 min ITI
S2 Familiar Conspecific (5 min)
10 min ITI
S3 Familiar Conspecific (5 min)
10 min ITI
S4 Familiar Conspecific (5 min)
Dishabituation Condition: Novel
Habituation Session (5 min)
S1 Novel Conspecific (5 min)
10 min ITI
S2 Familiar Conspecific (5 min)
10 min ITI
S3 Familiar Conspecific (5 min)
10 min ITI
S4 Novel Conspecific (5 min)
Dishabituation Condition: None
Habituation Session (5 min)
S1 No Conspecific (5 min)
10 min ITI
S2 No Conspecific (5 min)
10 min ITI
S3 No Conspecific (5 min)
10 min ITI
S4 No Conspecific (5 min)

Figure 5.


Screen Investigation Time By AFR- and MS-FAM Mice (Experiment 1)
Mean time spent investigating the wire screen +/- 95% C.I. (s)
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-FAM
MS-FAM


Screen Investigation Time By AFR- and MS-NOV Mice (Experiment 1)
Mean time spent investigating the wire screen +/- 95% C.I. (s)
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-NOV
MS-NOV

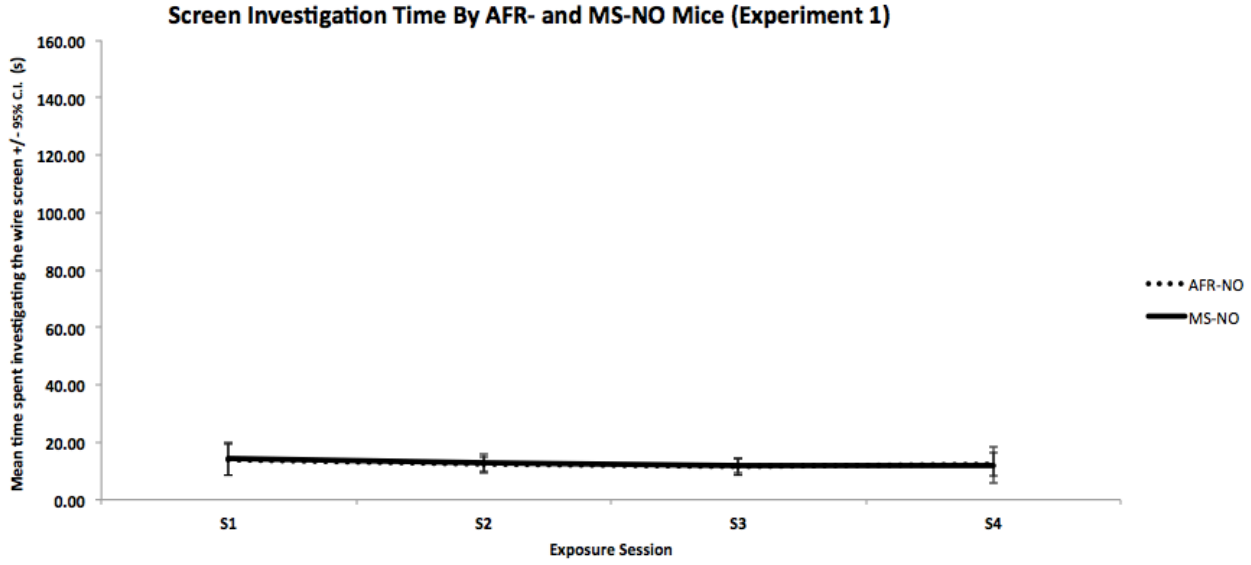

Screen Investigation Time By AFR- and MS-NO Mice (Experiment 1)
Mean time spent investigating the wire screen +/- 95% C.I. (s)
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-NO
MS-NO

Figure 6.

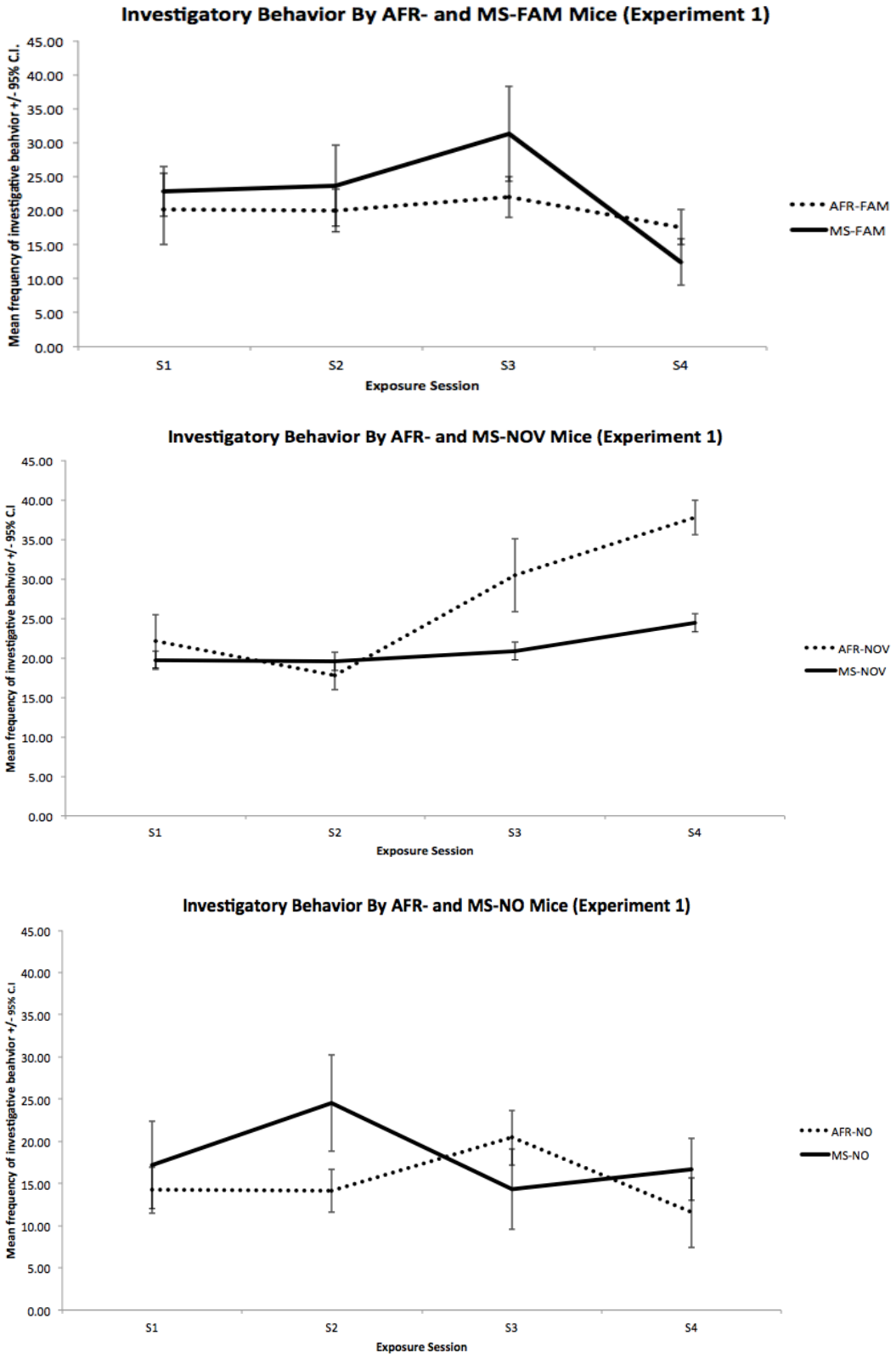

Investigatory Behavior By AFR- and MS-FAM Mice (Experiment 1)
Mean frequency of investigative beahvior +/- 95% C.I.
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-FAM
MS-FAM
Investigatory Behavior By AFR- and MS-NOV Mice (Experiment 1)
Mean frequency of investigative beahvior +/- 95% C.I
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-NOV
MS-NOV
Investigatory Behavior By AFR- and MS-NO Mice (Experiment 1)
Mean frequency of investigative beahvior +/- 95% C.I
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-NO
MS-NO

Figure 7.

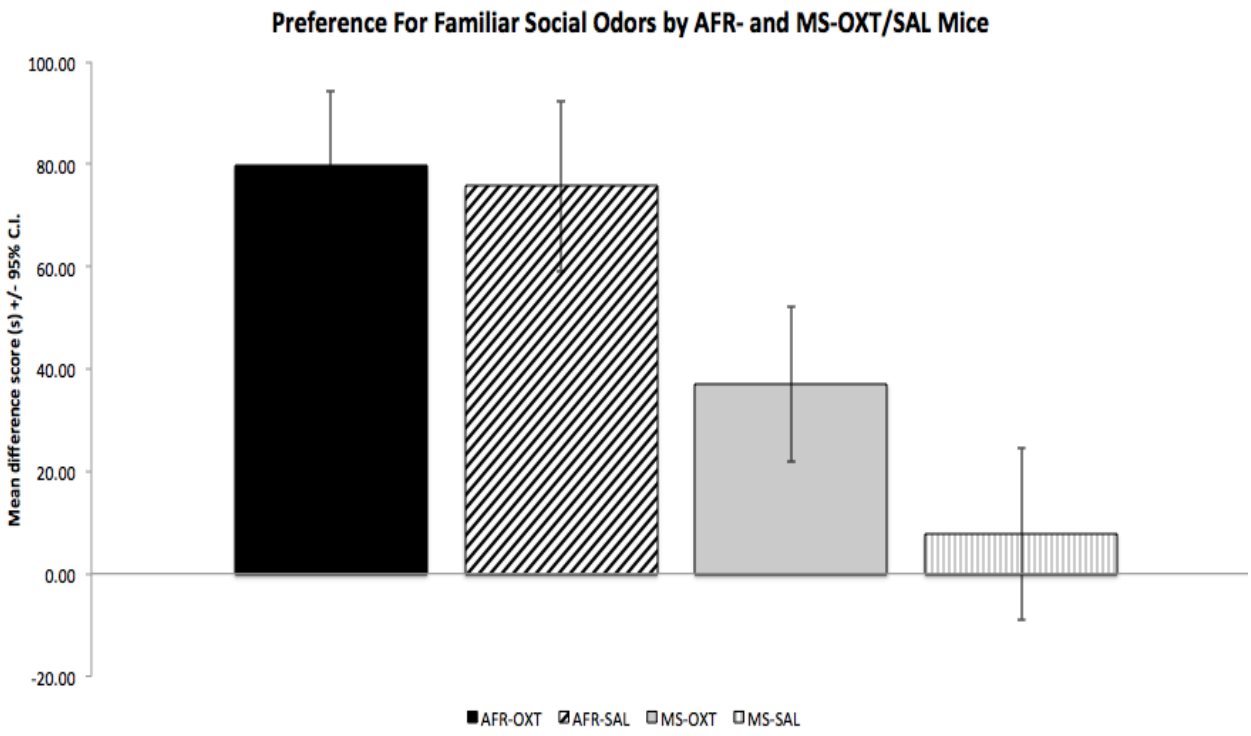
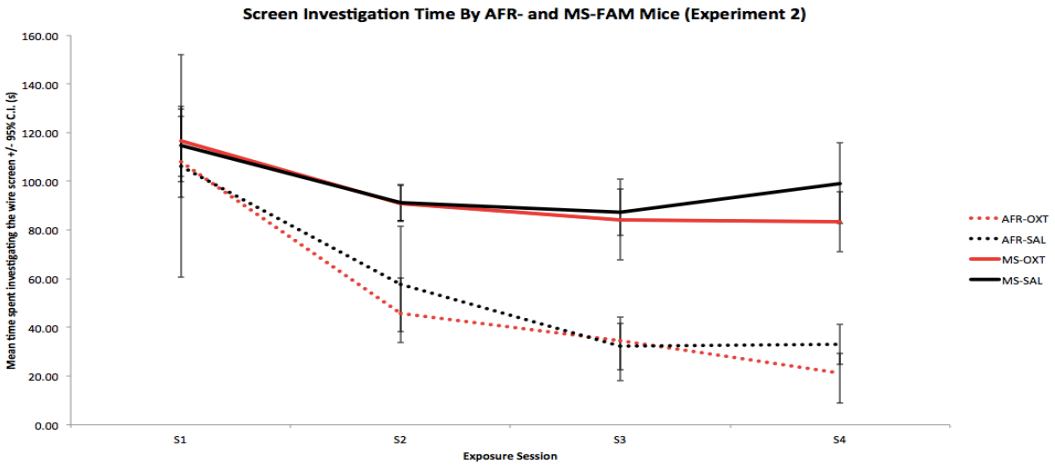
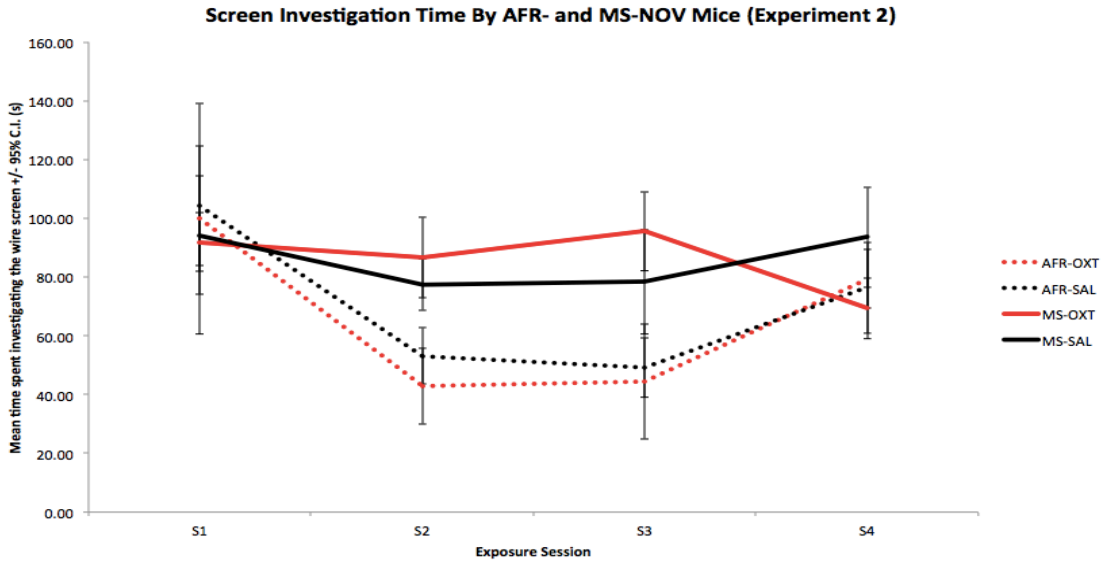

Preference For Familiar Social Odors by AFR- and MS-OXT/SAL Mice
Mean difference score (s) +/- 95% C.I.
100.00
80.00
60.00
40.00
20.00
0.00
-20.00
AFR-OXT
AFR-SAL
MS-OXT
MS-SAL

Figure 8.


Screen Investigation Time By AFR- and MS-FAM Mice (Experiment 2)
Mean time spent investigating the wire screen +/- 95% C.I. (s)
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-OXT
AFR-SAL
MS-OXT
MS-SAL


Screen Investigation Time By AFR- and MS-NOV Mice (Experiment 2)
Mean time spent investigating the wire screen +/- 95% C.I. (s)
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-OXT
AFR-SAL
MS-OXT
MS-SAL

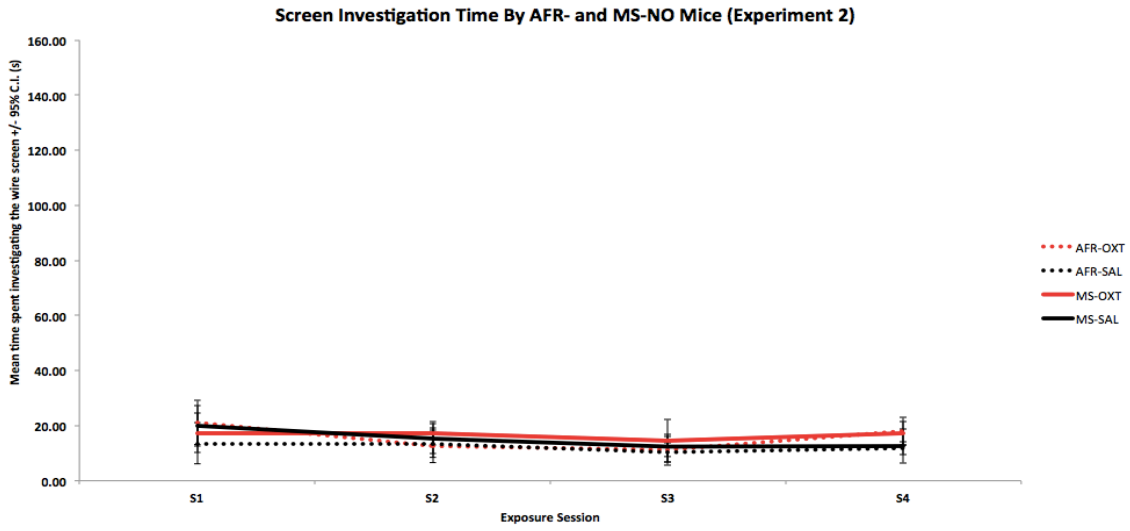
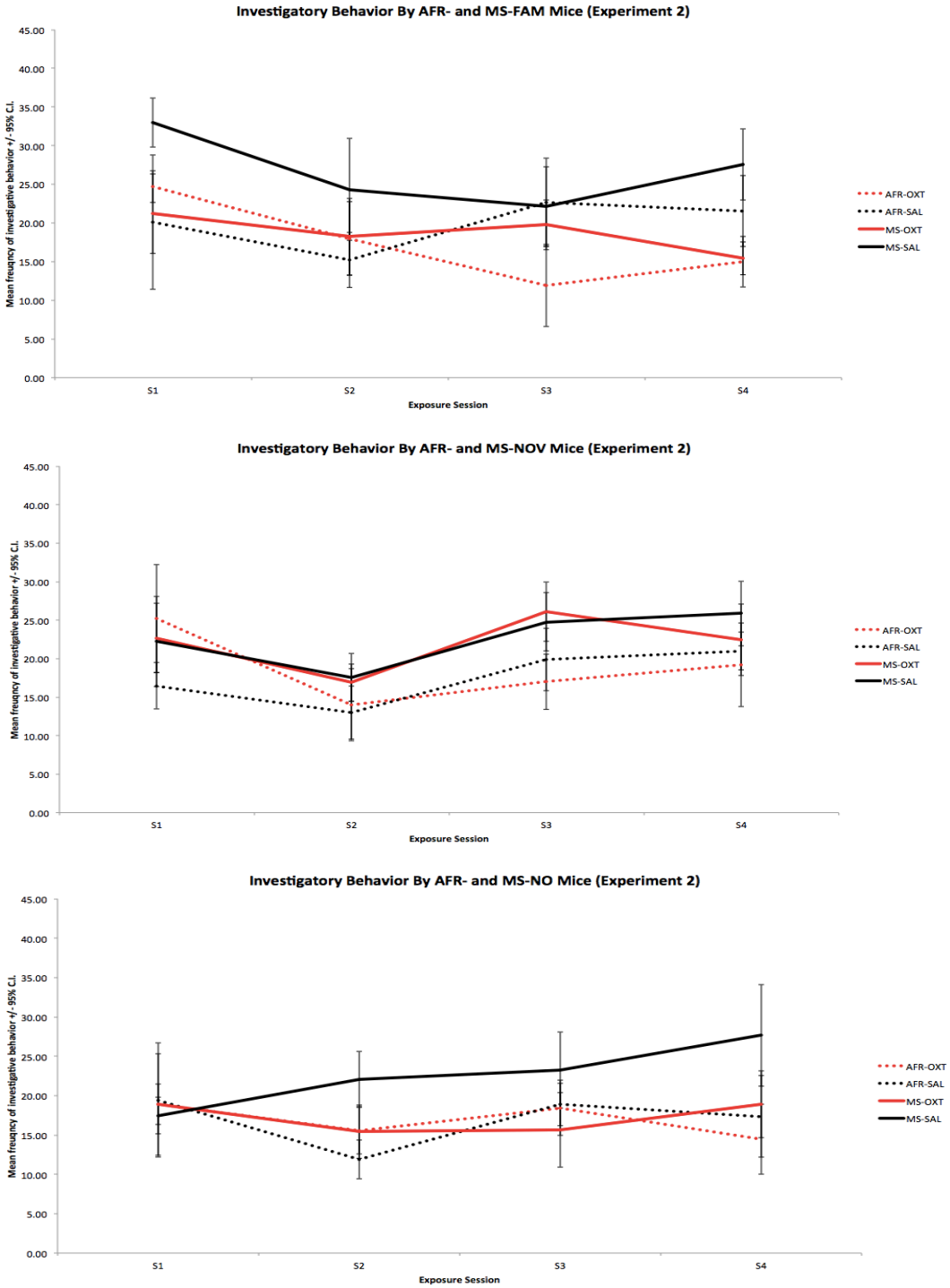

Screen Investigation Time By AFR- and MS-NO Mice (Experiment 2)
Mean time spent investigating the wire screen +/- 95% C.I. (s)
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-OXT
AFR-SAL
MS-OXT
MS-SAL

Figure 9.


Investigatory Behavior By AFR- and MS-FAM Mice (Experiment 2)
Mean freuqncy of investigative behavior +/- 95% C.I.
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-OXT
AFR-SAL
MS-OXT
MS-SAL
Investigatory Behavior By AFR- and MS-NOV Mice (Experiment 2)
Mean freuqncy of investigative behavior +/- 95% C.I.
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-OXT
AFR-SAL
MS-OXT
MS-SAL
Investigatory Behavior By AFR- and MS-NO Mice (Experiment 2)
Mean freuqncy of investigative behavior +/- 95% C.I.
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
S1
S2
S3
S4
Exposure Session
AFR-OXT
AFR-SAL
MS-OXT
MS-SAL

Biographical Sketch

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CURRENT EMPLOYMENT

Employer: SUNY - Cayuga Community College
Department: Psychology
Rank: Assistant Professor

EDUCATION

Ph.D. in Cognition, Brain, and Behavior, Syracuse University, Syracuse, NY, May 2014
Dissertation: *The Effects of Maternal Separation and Postnatal Oxytocin Injections on Adolescent Female Mouse Social Recognition.*
Advisor: Amy H. Criss

M.S. in Experimental Psychology, Syracuse University, Syracuse, NY, May 2008
Thesis: *Maternal Separation Alters Social Olfaction in Adolescent Female CD-1 Mice.*
Advisor: Catherine A. Cornwell

B.S. in Psychology, Coastal Carolina University, Conway, SC, May 2005
Summa Cum Laude

PROFESSIONAL ORGANIZATION MEMBERSHIP

International Society for Developmental psychobiology (ISDP)
International Society for Behavioral Neuroscience (ISBN)
Society for Neuroscience (SfN)
Psi-Chi National Honor Society in Psychology

PRESENTATIONS, PUBLICATIONS and Grants

PUBLICATIONS AND ACCEPTED MANUSCRIPTS

Thomas, N.R., Fonken, L.K., LeBlanc, M.L., & Cornwell, C.A. (2010). Maternal separation alters social odor preference development in infant mice. *Journal of Comparative Psychology, 124,* 295-301.
Thomas, N.R. (2009). Comparative psychology as an effective supplement to undergraduate core psychology courses. *Teaching of Psychology, 36*, 200-204.

MANUSCRIPTS IN PREPARATION/SUBMITTED

Thomas, N.R. The impact of learning communities on student persistence and completion in introductory psychology: A community college analysis. *In preparation.*

Thomas, N.R. Increasing student success in introductory psychology through the use of open educational resources (OER's). *In preparation.*

Thomas, N.R, Boyd, A., LeBlanc, M., Fonken, L.K., & Cornwell, C.A. Long-term maternal separation alters the olfactory preferences of adolescent female mice for nest and botanical odors. *In preparation*.

POSTERS PRESENTED

Thomas, N.R., & Cornwell, C.A. (2012). Maternal separation and postnatal oxytocin administration alter social recognition memory in adolescent female mice. Poster and Seminar Talk presented at the 21st Annual Meeting of the International Behavioral Neuroscience Society, Hawaii.

Thomas, N.R., LeBlanc, M.E., Boyd, A., & Cornwell, C.A. (2009). Maternal separation alters social odor preference in developing female mice. Poster presented at the 39th Annual Meeting of the Society for Neuroscience, Chicago.

Thomas, N.R., Boyd, A., LeBlanc, M., Fonken, L.K., & Cornwell, C.A. (2008). Maternal separation alters preference for familiar odors in adolescent female CD-1 mice. Poster presented at the 38th Annual Meeting of the Society for Neuroscience, Washington, D.C.

Thomas, N.R., Leister, K.L., Shine, K.H., Kelly, M., Cornwell, C.A. (2007). *Social Olfaction in Infant Maternally Separated CD-1 Mice*. Poster presented at the 37th Annual Meeting of the Society for Neuroscience, San Diego, CA.

Cornwell, C.A., **Thomas, N.R.**, & Leister, K.L. (2007). *Stress reactivity in maternally separated adolescent mice*. Poster presented at the 16th Annual Meeting of the International Behavioral Neuroscience Society, Rio de Janeiro, Brazil.

Thomas, N.R., Leister, K.L., & Cornwell, C.A. (2006). *Sex specific effects of maternal separation on stress reactivity and odor preferences in CD-1 mice*. Poster presented at the 39th Annual Meeting of the International Society for Developmental Psychobiology, Atlanta, GA.

Thomas, N.R. (2005) *Effect of fluoxetine on learning and anxiety in perinatal rat pups*. Oral presentation at the 30th Annual Meeting of the Carolinas Psychology Conference, Raleigh, NC.

Thomas, N.R. (2005). *Effect of fluoxetine on spatial memory in perinatal rat pups*. Poster presented at the 2nd Annual Celebration of Inquiry Conference, Myrtle Beach, SC.

GRANTS

Fall 2009 Syracuse University Allport Research Grant, Boyd, A., LeBlanc, M.E., **Thomas, N.R.** & Cornwell, C.A. The development of social odor preferences in facility-reared and maternally separated infant mice. **$500**

Spring 2009 Syracuse University Allport Research Grant, Rubin, K., Bell, M., **Thomas, N.R.** & Cornwell, C.A. The influence of maternal separation on maternal behavior and social odor preferences of infant mice. **$500**

Fall 2008 Syracuse University Allport Research Grant, **Thomas, N.R.**, Boyd, A., LeBlanc, M., Fonken, L.K., **Thomas, N.R.,** & Cornwell, C.A. Development of odor-guided behavior in infant maternally separated CD-1 mice. **$601**

Spring 2008 Syracuse University Allport Research Grant, Boyd, A., LeBlanc, M., Fonken, L.K., **Thomas, N.R,** & Cornwell, C.A. Social olfaction in female adolescent maternally separated CD-1 Mice, II. **$922**

Fall 2007 Syracuse University Research & Creative Project Grant, **Thomas, N.R.** The development of odor preferences and behavioral stress reactivity in adolescent CD-1 mice. $**1,900**

Spring 2007 Syracuse University Allport Research Grant, Leister, K.M., **Thomas, N.R.**, & Cornwell, C.A. Social olfaction in adolescent maternally separated CD-1 mice. $**600**

RELEVANT EXPERIENCE

2008 – 2010 Graduate Research Assistant*, "Development of an Oral Insulin Delivery System in the STZ – rat"* Department of Chemistry/Department of Exercise Science, Syracuse University
Mentors: Dr. Robert Doyle and Dr. Timothy Fairchild.

2005 – 2010 Graduate Research Assistant, *"The Influence of Maternal Separation on the Development of Olfactory Preferences and Social Behavior"*, Behavioral Neuroscience Laboratory, Center for Health and Behavior, Syracuse University,
Mentor: Dr. Catherine Cornwell.

2006 – 2007 Graduate Research Assistant, Department of Chemistry, Syracuse University,
Mentor: Dr. Robert Doyle

2005 Research Assistant, Psychology Department, Coastal Carolina University, Conway, SC.
Mentors: Dr. Linda Palm and Dr. Bernard Albiniak

TEACHING COMPETENCY

Introductory Psychology, Personality Psychology, Child Psychology, Adolescent Psychology, Lifespan Development, Death and Dying, Biological Psychology, Health Psychology, Gerontology, Psychology of Adjustment, Comparative Psychology, Sensation and Perception, Developmental Psychobiology, Statistical Methods, and Research Methodology

PROFESSIONAL DISTINCTIONS/HONORS

SUNY – Cayuga Community College SGO Faculty Member of the Year, 2012-13
Psi Chi / Erlbaum Award in Cognitive Science, 2008
Syracuse University Gardner Fellow, 2008/2009
Syracuse University Teaching Fellow, 2006/2007, 2007/2008, 2008/2009, 2009/2010