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MONOAMINE MODULATION OF SEPARATION-INDUCED ULTRASONIC
VOCALIZATIONS IN PREWEANLING F344 AND SD RATS

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts
in
Psychology:
General-Experimental

by
Cynthia Elizabeth Britt
March 2013

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ABSTRACT

Autism is a complex developmental disorder in which impairments of social interaction and communication are present. Currently, there are few effective treatments for treating deficits in social communication and the development of new therapies has been impeded by the lack of animal models. Thus, the purpose of this study was to determine if Fischer 344 (F344) rats, a strain which naturally shows deficits in social communication during adolescence, could be used as a model of the social communication deficits observed in autism. Specifically, we measured ultrasonic vocalizations (an early onset form of social interaction) to determine if preweanling F344 rats would show deficits in social communication when compared to Sprague Dawley rats (SD). We also investigated the role of monoamines in the social communication of F344 and SD rats by using selective monoaminergic compounds (i.e., atomoxetine, GBR-12909, and fluoxetine) to modulate monoamine activity. Monoamine activity in the striatum was also measured. On postnatal day (PD) 15, rats were injected 30 minutes prior to testing with 0.3, 1, or 3 mg/kg atomoxetine, 1.5, 5, or 15 mg/kg GBR-12909, or 3, 10, or 30 mg/kg fluoxetine, or vehicle. Ultrasonic vocalizations were then measured for 20 minutes followed

by an immediate measurement of rectal temperature.

Overall, F344 rats emitted more ultrasonic vocalizations regardless of drug treatment. Atomoxetine (1 and 3 mg/kg) increased the frequency of ultrasonic vocalizations compared to vehicle-treated rats. GBR-12909-induced ultrasonic vocalizations were altered by both strain and sex. In male rats, 1.5 mg/kg GBR-12909 increased ultrasonic vocalizations in F344 rats, but not in SD rats. In contrast, female rats exhibited a brief increase in vocalizations after the 5 mg/kg dose. Rats treated with fluoxetine (30 mg/kg) emitted fewer ultrasonic vocalizations than rats treated with the lowest dose of fluoxetine (3 mg/kg) or vehicle. The results indicated that the social communication of preweanling F344 rats and SD rats differed. Specifically, F344 rats emitted more ultrasonic vocalizations than did SD rats. This difference was not altered by changes in monoamine activity, because F344 rats and SD rats typically responded in a similar manner to the selective monoamine compounds. This finding was surprising because basal levels of dopamine and serotonin turnover are lower and norepinephrine levels are higher in F344 than SD rats. Overall, these data suggest that F344 rats maybe useful for assessing early onset

social communication deficits and the role of monoamines
in these behaviors warrants further investigation.

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CHAPTER ONE

INTRODUCTION

Delays in the development of social interaction and communication have been linked to a wide range of disorders, including anxiety, mood, and substance use disorders (Gillis, Callahan, & Romanczyk, 2011; Merikangas, Nakamura, & Kessler, 2009). These delays are particularly important for the diagnosis of pervasive developmental disorders (PDDs), such as autism, Asperger's disorder, Rett's disorder, and childhood disintegrative disorder (American Psychiatric Association, 2000; Levy, Mandell, & Schultz, 2009). In particular, the presence of social interaction deficits is considered to be the definitive diagnostic criteria for autism and distinguishes autism from other developmental psychiatric conditions (Constantino & Todd, 2003).

Autism

Overview

Autism is a complex neurodevelopmental disorder in which impairments of social interaction and communication are present and accompanied by restricted and repetitive behaviors (Corbett, Schupp, Levine, & Mendoza, 2009; Landa, Holman, & Garrett-Mayer, 2007). Symptoms gradually

begin after the age of six months and continue into adulthood (Rogers, 2009). Currently, no definitive biological markers exist for autism and diagnosis relies on the recognition of behavioral symptoms which can vary greatly from case to case (Liu, King, & Bearman, 2010).

Diagnosis of Autism

The Diagnostic and Statistical Manual of Mental Disorders, 4th ed., text revision (DSM-IV-TR) defines autism by the presence of two symptoms of social interaction impairments, at least one symptom of communication impairment, and at least one symptom of restricted and repetitive behavior (American Psychiatric Association, 2000). Repetitive patterns of behavior are defined as repetitive movements (stereotypy), arrangement of objects (compulsive behavior), resistance to change (sameness), unvarying routine for daily tasks (ritualistic behavior), limited focus, interest, or activity (restricted behavior), and self injury or injury to others (self-injury) (Dominick, Davis, Tager-Flusberg, & Folstein, 2007; Johnson, Myers, & Council on Children with Disabilities, 2007; Lam & Aman, 2007). No one pattern of repetitive behavior is specific to autism and symptoms vary widely (Bodfish, Symons, Parker, & Lewis, 2000).

The onset of symptoms in autism must be prior to the age of three (American Psychiatric Association, 2000). Diagnostic instruments commonly used are the Autism Diagnostic Interview-Revised (ADI-R) (parent interview) and the Autism Diagnostic Observation Schedule (ADOS), where children are observed during interaction with other children. The Childhood Autism Rating Scale (CARS) is widely used to assess the severity of autism (Volkmar, Chawarska, & Klin, 2005).

Socialization and Communication in Autism

The social deficits in autism distinguish this disorder from other developmental disorders (Rapin & Tuchman, 2008). Children with autism display impaired non-verbal behaviors such as eye contact and imitation (Levy, et al., 2009; Sigman, Dijamco, Gratier, & Rozga, 2004; Volkmar et al., 2005). Children with autism also display an absence of seeking shared enjoyment and interests with other children (Levy et al., 2009). The absence of these behaviors results in delayed development of peer interactions, few or no friendships, and little interaction with other children (Levy et al., 2009). Autistic children tend to form attachment relationships with their primary caregiver, but display less attachment security than non-affected children (Sigman et al., 2004).

Communication deficits are also apparent by the first year and include delays in babbling, gestures, and lack of response to other children and peers (Noens, van Berckelaer-Onnes, Verpoorten, & van Duijn, 2006). Autistic children by the age of two or three express fewer words and word combinations, and are more likely to engage in repetition of words than non-affected children (Landa, 2007; Tager-Flusberg & Caronna, 2007). A lack of attention is also evident in autistic children which coincides with difficulty in imaginative play and development of symbols for language (Landa, 2007; Tager-Flusberg & Caronna, 2007).

Neurobiology of Autism

Neuroimaging and neuropathological procedures have been used to study the morphological brain abnormalities in autism (Pardo & Eberhart, 2007). This type of investigation has revealed that autistic infants have a reduced brain size at birth followed by a large increase in head circumference (Courchesne, 2004; Courchesne, Redcay, & Kennedy, 2004). Abnormal patterns of brain overgrowth in areas of the frontal lobe, cerebellum, and limbic structures between two and four years of age have also been noted (Courchesne, 2004; Courchesne & Pierce, 2005). Importantly many of the brain areas showing

abnormal growth have also been linked to the development of social communication and motor abilities (Dawson, Munson, Estes, Osterling, McPartland, Toth, Carver, & Abbott, 2002).

In addition to neuroanatomical structures, many neurochemicals have been studied in autism. In particular, the monoamines, serotonin and to a lesser extent dopamine and norepinephrine, have been the focus of investigation. Hyperserotonemia (high platelet levels of serotonin) is one of the most replicable findings in biological psychiatry (Janušonis, 2008) and occurs in one third of prepubertal children with autism (Janušonis, 2008). Interestingly, research on the serotonin transporter gene (SLC6A4), which encodes for both the platelet and neuronal transport of serotonin, has been linked to a deficiency in serotonin activity in autistic children (Lam, Aman, & Arnold, 2006). Specifically, it is believed that the serotonin transporter in some autism patients is overactive and leads to a decrease in synaptic serotonin activity (Lam et al., 2006). While most research has focused on serotonin, dopamine and norepinephrine have also been implicated in autism. For example, CSF levels of the dopamine metabolite, homovanillic acid (HVA), is elevated in autistic children, who exhibit high levels of

locomotor activity and severe stereotypies (Gillberg & Svennerholm, 1987; Narayan, Srinath, Anderson, & Meundi, 1992). In addition, elevated plasma norepinephrine levels have been found in autistic children (Lam et al., 2006).

CHAPTER TWO
MONOAMINES: OVERVIEW

Serotonin

Serotonin is an indolamine neurotransmitter involved in the regulation of mood, eating, body temperature, arousal, pain sensitivity, sexual behavior, and hormone release (Bauman & Kemper, 1994). Additionally, serotonin dysfunction is involved in many psychiatric disorders, including depression, anxiety, obsessive-compulsive behaviors, and autism (Best, Nijhout, & Reed, 2010; Dayan & Huys, 2008). Cell bodies of serotonergic neurons are found in nine clusters, most of which are located in the raphe nucleus in the pons and medulla, which send projections to the cerebral cortex (Carlson, 2001).

The synthesis of serotonin begins with the hydroxylation of tryptophan by tryptophan hydroxylase (TPH) to create 5-hydroxytryptophan (5-HTP) (Lam et al., 2006). This reaction is the rate-limiting step in the formation of serotonin (Lam et al., 2006; Neckameyer, Coleman, Eadie, & Goodwin, 2007). After tryptophan is converted into 5-HTP, it is decarboxylated by 5-HTP decarboxylase into serotonin (Lam et al., 2006). Once serotonin is formed, it is moved into vesicles by the

vesicular monoamine transporter (VMAT) (Elsworth & Roth, 1997). When an action potential is generated on a serotonin neuron, voltage-gated calcium channels (VGCC) on the terminal button are activated, which allows an influx of calcium into the cytosol. The influx of calcium triggers the process of exocytosis, which causes vesicles filled with serotonin to fuse with the membrane and allows serotonin to be released into the synaptic cleft (Best et al., 2010; Elsworth & Roth, 1997).

In the synaptic cleft, free floating serotonin is pumped back into the cytosol of the presynaptic neuron where it is catabolized (Best et al., 2010). The removal of serotonin from the synaptic cleft is accompanied by serotonin reuptake transporters (SERT) (Best et al., 2010). Serotonin then undergoes metabolism through the process of oxidation in the presence of monoamine oxidase (MAO) (Best et al., 2010). The action of MAO converts serotonin to 5-hydroxyindole acetaldehyde which, in turn, is readily metabolized by an isoform of aldehyde dehydrogenase (ALDH2) located in mitochondria. The product of this metabolic is 5-hydroxyindole acetic acid (5-HIAA), which is the major metabolite of serotonin (Best et al., 2010).

Serotonin Receptors

Serotonin receptors consist of seven families ($5HT_{1-7}$), and are classified according to their structure, pharmacology, and signal transduction characteristics (Murrin, Sanders, & Bylund, 2007). Some of the serotonin receptor families contain multiple subtypes (i.e., $1A$, $1B$, $1D$, $1E$, $1F$, $2A$, $2B$, $2C$, $5A$, $5B$) (Murrin et al., 2007). The $5HT_3$ receptor is a ligand-gated ion channel, whereas the other receptors (i.e., $5HT_1$, $5HT_2$, $5HT_4$, $5HT_5$, $5HT_6$, $5HT_7$) are G protein-coupled receptors (Hoyer, Hannon, Martin, 2002; Murrin et al., 2007). The $5HT_1$ receptor is coupled to G_i proteins; $5HT_4$, $5HT_6$, and $5HT_7$ receptors are coupled to G_s proteins; and $5HT_{2A}$, $5HT_{2B}$, and $5HT_{2C}$ receptors are coupled to $G_{q/11}$ proteins (Hoyer, Clarke, Fozard, Hartig, Mylecharane, Saxena, & Humphrey, 1994).

Dopamine

Dopamine is a catecholamine involved in a wide range of behaviors and functions, including cognition, motor movement, reward mechanisms, sexual behavior, neuroendocrine regulation, and selective attention (Calne, Chase, & Barbeau, 1975; Missale, Nash, Robinson, Jaber, & Caron, 1998; Roberts, Woodruff, & Iversen, 1978). Dopamine has been linked to neurological and psychiatric

disorders, such as schizophrenia, attention-deficit/hyperactivity disorder (ADHD), and Parkinson's disease (Bortolato, Chen, & Shih, 2008; Giedd, Keshavan, & Paus, 2009). Dopamine neurons are primarily located in the substantia nigra and the ventral tegmental area (Carlson, 2001). Cell bodies located in the substantia nigra project their axons to the neostriatum. Cell bodies in the ventral tegmental area project to several parts of the prefrontal cortex and limbic system, including the nucleus accumbens, amygdala, and hippocampus (Devoto & Flore, 2006; Wanat, Willuhn, Clark, & Phillips, 2009).

Dopamine is synthesized from the amino acid tyrosine (Carlson, 2001; Elsworth & Roth, 1997). Tyrosine is hydroxylated by tyrosine hydroxylase into L-dihydroxyphenylalanine (L-DOPA) (Elsworth & Roth, 1997). This is the rate-limiting step in the synthesis of dopamine (Carlson, 2001; Elsworth & Roth, 1997). L-DOPA is then converted into dopamine via the enzyme DOPA decarboxylase (Elsworth & Roth, 1997). Once dopamine is formed it is moved into vesicles by VMAT (Elsworth & Roth, 1997). When the presynaptic terminal is depolarized, an influx of calcium causes the vesicles to fuse with the membrane and allows dopamine to be released into the

synaptic cleft via calcium-dependent exocytosis (Elsworth & Roth, 1997; Ford, Gantz, Phillips, & Williams, 2010).

Free floating dopamine in the synaptic cleft is inactivated by reuptake, in which dopamine is pumped back in the presynaptic terminal via dopamine transporters (DAT) (Elsworth & Roth, 1997; Ford et al., 2010). Once transported back into the presynaptic terminal by DAT, dopamine is metabolized by MAO (Elsworth & Roth, 1997).

Intracellular MAO metabolizes dopamine into 3,4-dihydroxyphenylacetaldehyde (DHPA). Via oxidization, DHPA is turned into 3,4-dihydroxyphenyl acetic acid (DOPAC) by aldehyde dehydrogenase. The end product, HVA, is found in humans, whereas DOPAC is the major metabolite found in rodents (Elsworth & Roth, 1997; Ford et al., 2010). Dopamine remaining in the extracellular fluid is metabolized into 3-methoxytyramine (3-MT) by the enzyme catechol-o-methyltransferase (COMT). MAO then converts 3-methoxytyramine (3-MT) into 3-methoxy- 4-hydroxyphenylacetaldehyde (MHPA), followed by the conversion of MHPA into HVA by aldehyde dehydrogenase (Elsworth & Roth, 1997; Ford et al., 2010).

Dopamine Receptors

There are two families of dopamine receptor, D1-like and D2-like, which are categorized into five different

subtypes (D₁-D₅) (Civelli, Bunzow, & Grandy, 1993; Gingrich & Caron, 1993; Jackson & Westlind-Danielsson, 1994; O'Dowd, 1993). The two families differ pharmacologically, structurally, and biochemically. In the D₁-like family are the D₁ and D₅ receptors, while the D₂-like family includes the D₂, D₃, and D₄ receptors. Both families belong to a class of seven transmembrane G protein-coupled receptors (Missale et al., 1998; Sealfon & Olanow, 2000). Dopamine receptors are coupled to various G-proteins (G_s, G_{olf}, G_i) (Binder, Kinkead, Owens, & Nemeroff, 2001). D₁-like receptors are coupled to G_{olf}/G_s proteins, which stimulate adenylyl cyclase and promote the production of cyclic AMP (cAMP); whereas, the D₂-like receptors are coupled to the G_i protein, which inhibits adenylyl cyclase (Missale et al., 1998).

Norepinephrine

Norepinephrine is a catecholamine neurotransmitter involved in functions such as arousal, mood, attention, learning, and stress response (Page, Oropeza, & Van Bockstaele, 2008). Norepinephrine has been linked to many neuropsychiatric disorders, including mood and anxiety disorders, posttraumatic stress disorder, ADHD, and Alzheimer's disease (Ressler & Nemeroff, 1999).

Norepinephrine cell bodies are localized in the lateral tegmental area and the brainstem (i.e., locus ceruleus (LC) (Dent, Smith, & Levine, 2001; Page et al., 2008; Sofuoglu & Sewell, 2008; Tully & Bolshakov, 2010). Like dopamine, norepinephrine functions as a hormone in the blood stream or as a neurotransmitter in the brain (Tully & Bolshakov, 2010).

Norepinephrine shares the same biosynthetic pathway as dopamine; however, norepinephrine-specific neurons contain dopamine- β -hydroxylase within their vesicles, which rapidly transforms dopamine to norepinephrine via hydroxylation. When the cell is activated, the vesicles fuse with the presynaptic membrane allowing norepinephrine to be released into the synaptic cleft via calcium-dependent exocytosis (Elsworth & Roth, 1997; Ford et al., 2010).

The presence of norepinephrine in the synaptic cleft triggers the prompt inactivation of norepinephrine through active reuptake. The reuptake of norepinephrine into the presynaptic terminal is controlled by norepinephrine transporters (NET) (Ressler & Nemeroff, 1999; Sofuoglu & Sewell, 2008). Metabolism of norepinephrine occurs by transformation of norepinephrine into 3,4-dihydroxyphenylglycol (DHPG) or 3,4-dihydroxymandelic

acid (DHMA) by dehydrogenase or reductase enzymes within the cell (Ressler & Nemeroff, 1999). COMT transforms DHPG and DHMA into 3-methoxy-4-hydroxyphenylglycol (MHPG) or 3-methoxy-4-hydroxy-mandelic acid (VMA) (Ressler & Nemeroff, 1999). The breakdown of MHPG is relatively specific for central nervous system norepinephrine metabolism and is a useful plasma/urine marker (Ressler & Nemeroff, 1999; Weiner & Molinoff, 1994)

Norepinephrine Receptors

Adrenergic receptors consist of three subtypes, α -1, α -2, and β which differ in pharmacology, molecular structure, and signal transduction pathways (Murrin et al., 2007). These three receptors can be further divided into subtypes (i.e., α 1-A, α -1B, α -1D; α -2A, α -2B, α -2C; β -1, β -2, β -3) (Murrin et al., 2007). All adrenergic receptors are G protein-coupled, with α -1 receptors coupled to G_q , α -2 receptors coupled to $G_{i/o}$, and β receptors coupled to G_s (Murrin et al., 2007).

CHAPTER THREE
ONTOGENY OF MONOAMINES

Serotonin Ontogeny

Prenatal Development

Serotonergic neurons are apparent around embryonic day 12 (Aitken & Törk, 1988; Murrin et al., 2007). Serotonin fiber projections can be detected by embryonic day 14 and reach the prefrontal neocortical pole by embryonic day 17 (Murrin et al., 2007). Around embryonic day 14 descending serotonin fibers enter the spinal cord and axons innervate preganglionic sympathetic neurons and somatic motor neurons to form synapses by embryonic day 17 (Rubenstein, 1998).

Postnatal Development

A rapid growth of serotonin dendrites is evident until the end of the first postnatal week (Murrin et al., 2007). By postnatal day (PD) 15, serotonin synaptogenesis is at approximately 75% of adult levels in the raphe nucleus (Murrin et al., 2007). In the basal forebrain, the percentage of serotonin varicosities engaged in synaptic junctions increase from birth to adult levels by the end of the second postnatal week (Murrin et al., 2007). Serotonin levels in the CNS are low at birth and generally

peak around PD 21 to 30 and then decline to adult levels (Murrin et al., 2007).

Serotonin Receptor Development

5HT₁ serotonin receptors subtypes (5-HT_{1A}, _{1B}, _{1D}, and _{1F}) are expressed by embryonic day 16 in the thalamus and hippocampus (Murrin et al., 2007). 5HT₂ receptors can be detected by approximately embryonic day 17 throughout the whole brain (Murrin et al., 2007).

At birth, 5HT₁ receptor binding in the brainstem is higher than adult levels and appears to be fully functional (Murrin et al., 2007). In the striatum [³H]5-HT binding is only 12% of adult levels at birth, however, increases rapidly by the third week of age (Murrin et al., 2007). Immunocytochemical techniques reveal that 5-HT_{2C}, 5-HT_{5A}, and 5-HT₇ receptors in four regions of the rat hippocampus are at their highest levels at birth and then decrease to adult levels (Murrin et al., 2007).

Dopamine Ontogeny

Prenatal Development

Dopamine neurons in the striatum appear between embryonic day 10 and embryonic day 14, (Maciag, Altschuler, Slack, Krogan, Emili, Greenblatt, Maniatis & Wu, 2006). On embryonic day 13, dopamine axons from the

substantia nigra and the ventral tegmental area start to extend dorsally to the ventral striatum, but are not fully mature until birth (embryonic day 21) (Hu, Cooper, Crockett, & Zhou, 2004). By embryonic day 14 extensive development of dopamine activity is evident in the striatum (Specht, Pickel, John, & Reis, 1981; Maciag et al., 2006). HPLC studies measuring catecholamines and their metabolites show a 9 fold increase in dopamine concentrations in the midbrain from embryonic day 17 to PD 0 (Parès-Herbutè, Tapia-Arancibia, & Astier, 1989).

Postnatal Development

At PD 7, dopamine synapses in the dorsal striatum peak and then gradually decline to adult levels around PD 21 (Antonopoulos, Dori, Dinopoulos, Chiotelli, Parnavelas, 2002). Dopamine distribution in the prefrontal cortex develops slower than in the striatum (Kalsbeek, Voorn, Buijs, Pool, & Uylings, 1988).

Dopamine Receptor Development

By embryonic day 14, D1-like and D2-like receptor mRNA is detectable in the caudate-putamen, olfactory tubercle, frontal cortex, and cingulate cortex (Schambra, Duncan, Breese, Fornaretto, Caron, & Fremeau, 1994). D1-like receptor mRNA levels at birth are 75% of adults, however, D-like receptor binding is very low (Schambra et

al., 1994). The density of D1-like binding sites increases between PD 14 to 21, where it remains relatively stable throughout adulthood (Schambra et al., 1994). D1-like receptors reach adult levels faster and have greater densities than D2-like receptors (Broaddus & Bennett, 1989; Schambra et al., 1994). Some studies, however, show that D2-like receptors are expressed by PD 3 and reach adult levels by PD 21 (Rao, Molinoff, & Joyce, 1991; Sales, Martes, Bouthenet, & Schwartz, 1989).

Norepinephrine Ontogeny

Prenatal Development

Noradrenergic neurons appear between embryonic day 10 and 13 (Murrin et al., 2007). From this point there is a linear development of markers for noradrenergic neurons in the central nervous system, approximately increasing 100- to 1000-fold by adulthood (Murrin et al., 2007). Axial projections reach their terminal areas a week before birth and start to form varicosities (Murrin et al., 2007).

Postnatal Development

The development of norepinephrine concentrations to adult levels in rat brain is relatively slow compared to other monoamines (Murrin et al., 2007). Norepinephrine levels increase steadily from birth onward, however, adult

concentrations are not reached until PD 30 to 40 (Murrin et al., 2007).

Adrenergic Receptor Development

α -1 Adrenergic receptors are found in low levels in rat brain at birth (Murrin et al., 2007). Density of α -1 adrenergic receptors increases rapidly between PD 15 and 20 (Murrin et al., 2007). By PD 20, levels of α -1 adrenergic receptors are greater than in adults, followed by a decrease to adult levels in the following weeks (Murrin et al., 2007). α -2 Adrenergic receptors (i.e., α -2A, α -2B, and α -2C), exhibit a distinct transcriptional developmental profile throughout the central nervous system (Murrin et al., 2007). An increase in α -2 adrenergic receptors density occurs in most brain regions after birth and reaches peak levels at about PD 15 (Murrin et al., 2007). β -Adrenergic receptors in the rat brain exist in very low levels shortly after birth (Murrin et al., 2007). By the second week of postnatal development, however, β -adrenergic receptors levels are greater than in adults (Murrin et al., 2007).

CHAPTER FOUR
RODENT SOCIAL COMMUNICATION THROUGH PLAY
BEHAVIOR AND VOCALIZATION

The development of social communication and interactions in children is evident early in life and increases in complexity as a child matures (Brown, Odom, & Holcombe, 1996). It has been proposed that appropriate and effective social behavior acquisition with peers is important for early social development (Brown et al., 1996). The formation of strong social bonds is also important for the well being of individuals and is a critical criterion for mental health (Young, Liu, & Wang, 2004). Thus, the inability to form social bonds has been used as a diagnostic component of various psychological disorders, including autism, social anxiety, and schizophrenia (Young et al., 2004).

Social Communication through Play Behavior

In rodents, social play is one of the earliest forms of non-mother-directed social behavior (Vanderschuren, Niesink, & Van Ree, 1997; Young et al., 2004). Play behavior is also necessary for normal social development in rodents and is a common model used to assess impairments in social interactions (Vanderschuren et al.,

1997). Social play in young rats consists of various behavioral patterns, including pouncing, chasing, social grooming, crawling over/under, charging, boxing, wrestling, pinning, social sniffing, and lateral display (Baenninger, 1967; Bolles & Woods, 1964; Meany & Stewart, 1981; Panksepp & Beatty, 1980; Poole & Fish, 1975). Social development (i.e., social, sexual, and aggressive behaviors) in rodents is facilitated by social play (Vanderschuren et al., 1997). Moreover, play deprivation causes abnormal patterns of social behaviors (Vanderschuren et al., 1997). Within a litter, rats show preferences for specific play partners and rats that play less have weaker ties with the group in later life (Bekoff, 1974; Carlsen & Heimer, 1986). Social play in infant rats is also important for learning to express and understand intraspecies communicative signals, which may serve to inhibit aggression and increase group stability (Bekoff, 1972; Lore & Planelly, 1977; Meany & Stewart, 1981; Meany, Stewart, & Beatty 1985). Thus, social play behavior facilitates different aspects of social development, which may contribute to the acquisition of adequate social functioning (Vanderschuren et al., 1997).

An important disadvantage to using play behavior as a model of social communication, is that play behavior is

typically assessed in rats after weaning and peaks after PD 30 (Auger & Olesen, 2009; Siviy & Panksepp, 2011). This play behavior model corresponds to late childhood to early adolescence in humans, and therefore, cannot be used as a model of early onset social communication disorders like autism.

Social Communication through Vocalization

Ultrasonic vocalization is another form of social communication in rodents. Unlike social play, however, ultrasonic vocalizations are evident just hours after birth (Dastur, McGregor, & Brown, 1999; Hofer, 1996). The emission of ultrasonic vocalizations also plays an important role in parent-infant interactions (Yu, Wang, Tai, Broders, An, Zhang, He, An, & Wu, 2011).

Ultrasonic vocalizations in infant rats have been studied under a variety of conditions; however, one of the most effective methods for inducing vocalization is through isolation stress (Shair, Masmela, & Hofer, 1999). Emission of ultrasonic vocalizations in young rodents is a distress response produced when under cold stress or when separated from their dam and littermates (Allin & Banks, 1970; Hofer & Shair, 1978; but see Blumberg, Sokoloff, & Kent, 1999). Importantly, isolation-induced ultrasonic

vocalizations have been interpreted as a communicative behavior, which can be used to model several neuropsychiatric and neurodevelopmental disorders associated with communicative/social deficits (Scattoni, Crawley, & Ricceri, 2009).

Neurobiology of Ultrasonic Vocalization and Social Play

Multiple neurochemical systems modulate ultrasonic vocalization emission, including serotonin, norepinephrine, and dopamine (Dastur et al., 1999). Serotonin's effect on ultrasonic vocalization emission varies and depends on which receptor subtype is activated (Dastur et al., 1999). For example, in 10-day-old rats serotonin 5HT_{1A} receptor agonists reduce ultrasonic vocalizations (Kehne, McCloskey, Baron, Chi, Harrison, Whitten, & Oalfreyman, 1991; Winslow & Insel, 1990), while 5HT_{1B} receptor agonists increase ultrasonic vocalizations (Winslow & Insel, 1991). Antagonists at 5HT_{2A} receptors and serotonin reuptake inhibitors suppress ultrasonic vocalizations (Winslow & Insel, 1990). Norepinephrine also modulates ultrasonic vocalizations, because the β-adrenoceptor antagonist, propranolol, the α-2-adrenoceptor antagonist, yohimbine, and the α-1-adrenoceptor antagonist, prazosin, all decreases

ultrasonic vocalizations in 8- to 12-day-old rat pups, while the α -2-adrenoceptor agonist, clonidine, increases ultrasonic vocalizations in 4- to 16-day-old rat pups (Kehoe, 1988; Winslow & Insel, 1991). Lastly, studies looking at dopamine modulation of ultrasonic vocalizations have shown that dopamine D1 and D2 receptors may modulate ultrasonic vocalizations emission differently (Dastur et al., 1999). In 10-day-old rat pups, the D1 receptor agonist SKF 81297 suppressed ultrasonic vocalizations, whereas the selective D2 receptor antagonist sulpiride decreased ultrasonic vocalizations in 12- to 14-day-old rat pups (Cuomo, Cagiano, Renna, DeSalvia, & Racagni, 1987; Dastur et al., 1999).

Like ultrasonic vocalizations, play behavior in rats is modulated by monoamine activity. For example, social play is reduced when blockers of dopaminergic transmission, such as chlorpromazine and haloperidol, are administered. Moreover, the serotonin agonist, quipazine, and the partial agonist, methysergide, both reduce social play (Beatty, Costello, & Berry, 1984; Normansell & Panksepp, 1985). In addition, blockade of presynaptic α -2-adrenoreceptors decreases social play, presumably by increasing noradrenergic activity (Siviy, Fleischhauer, Kuhlman, & Atrens, 1994).

CHAPTER FIVE
THESIS STATEMENT AND HYPOTHESES

Impairments in social interactions are one of the earliest signs of developmental delay in children and are an important diagnostic criteria in many pediatric mental disorders. Autism, in particular, can be distinguished from other developmental disorders by the presence of social communication deficits. Unfortunately, these deficits in social communication are difficult to assess in animal models and have hampered progress in understanding the neurobiological bases of disorders, like autism, and in the development of effective treatments.

Play behavior in young rats is an established model used to study complex social interactions (Vanderschuren et al., 1997). Supporting this idea is research showing that: (a) play behavior is disrupted in a number of development disorders, including autism, Asperger's syndrome, and attention deficit hyperactivity disorder (Buitelaar, Swinkels, De Vries, van derGaag, & Van Hooff, 1994; Buitelaar, Van England, de Kogel, De Vries, & Van Hooff, 1991; Kirkpatrick, 1994); and (b) play behavior is necessary for normal social development (Bekoff, 1972; Meany et al., 1985; Meany & Stewart, 1981; Vanderschuren,

Niesink, Spruijt, & Van Ree, 1995). A major advantage to the rat play behavior model is that there are known differences in playfulness between rat strains that can be used to find genetic and neurobiological substrates for this behavior. For example, F344 are less playful when compared to other strains of rats, including SD rats from which they were initially derived. F344 rats are less likely to solicit play and are less likely to respond playfully when approached or contacted by another rat (Siviy, Love, DeCicco, Giordano, & Seifert, 2003). Consistent with decreased play behavior, F344 rats show differences in monoamine transmission when compared to SD rats (Varty & Geyer, 1998).

A disadvantage to the play behavior model, however, is that play behavior is usually assessed in rats after weaning and peaks after PD 30 (e.g., a period corresponding to late childhood to early adolescence in humans). Thus, this model cannot be used to assess deficits in social interaction in ages analogous to human children. The purpose of the present thesis, therefore, was to determine if F344 rats showed decreased emission of social communication at a developmental period analogous to early childhood (e.g., 2-3 years). For this reason, separation-induced ultrasonic vocalizations were measured

in F344 and SD preweanling rats on PD 15. In addition, the ability of specific monoaminergic compounds to modulate ultrasonic vocalizations was assessed in both rat strains. These data were then analyzed to determine whether there was an involvement of serotonin, dopamine, and norepinephrine systems in separation-induced ultrasonic vocalizations. Additionally, striatal serotonin, dopamine, and norepinephrine levels, as well as their major metabolites, were measured in vehicle-treated rats on PD 17. Monoamines were assessed in the striatum because the striatum is important for the mediation of social behavior (Siviy & Panksepp, 2011; Kirsten et al., 2012) and abnormalities in this brain area are frequently seen in autistic individuals (Di Martino et al. 2011; De monte et al., 2012).

Based on past social play and ultrasonic vocalization studies, as well as investigations using F344 and SD rats, a number of hypotheses were made concerning the current thesis. First, we predicted that vehicle-treated F344 rats would emit fewer isolation-induced ultrasonic vocalizations than SD rats, because F344 have a natural tendency to engage in less social play (Siviy, Love, DeCicco, Giordano, & Seifert, 2003). We also predicted that administration of GBR-12909 [a dopamine reuptake

inhibitor (DRI)] would result in less ultrasonic vocalization production for both SD and F344 rats, our rational was that dopamine agonists decrease ultrasonic vocalizations, while dopamine antagonists decrease social play (Knutson, Burgdorf, & Panksepp, 1998; Nelson & Panksepp, 1998). We predicted, however, that GBR-12909 would have a smaller effect on F344 rats, as compared to SD rats, because F344 rats appear to be less sensitive to activation of the dopamine system (Siviy, Crawford, Akopian, & Walsh, 2011).

In contrast to the predicted effects of GBR-12909, we hypothesized that administering atomoxetine [a selective norepinephrine reuptake inhibitor (NRI)] would increase ultrasonic vocalization-emissions in both strains, because norepinephrine agonists increase ultrasonic vocalizations and decrease social play (Siviy et al., 1994; Winslow & Insel, 1991). We also hypothesized that atomoxetine administration would have an enhanced effect in F344 rats, as compared to SD rats, because norepinephrine agonists, in general, produce greater behavioral effects in F344 rats (Skolnick & Daly, 1977).

Interestingly, serotonin transmission has mixed effects on social communication. For example, serotonin reuptake inhibitors decrease ultrasonic vocalization

production, while serotonin agonists decrease social play (Beatty et al., 1984; Normansell & Panksepp, 1985). Based on prior studies using serotonin reuptake inhibitors, we predicted that fluoxetine [a serotonin reuptake inhibitor (SRI)] would decrease ultrasonic vocalization emission in all rats (Winslow & Insel, 1990). Additionally, we predicted that fluoxetine would have a smaller effect on F344 rats, as compared to SD rats, because F344 rats appear to be less sensitive to activation of the serotonin system (Uphouse, Maswood, Jackson, Brown, Prullage, Myers, & Shaheen, 2002).

Finally, we predicted that monoamine turnover (defined as metabolite level/neurotransmitter level) would be differentially affected by strain. Specifically, we hypothesized that dopamine and serotonin turnover would be lower in F344 rats, when compared to SD rats, while norepinephrine turnover would be greater in F344 rats. These monoamine turnover predictions were based primarily on agonist sensitivity studies (Beatty et al., 1984; Nelson & Panksepp, 1998; Normansell & Panksepp, 1985; Winslow & Insel, 1991) and turnover studies in adult and adolescent rats (Uphouse et al., 2002; Varty & Geyer, 1998).

CHAPTER SIX

METHODS

Subjects

Subjects consisted of 142 F344 rat pups (67 male and 75 female) and 145 SD rat pups (77 male and 68 female). Untimed pregnant rats were purchased from Charles River Laboratories (Wilmington, MA) and their pups, after receiving different drug treatments, were tested on PD 15. Table 1 summarizes the number of male and female pups from each strain (F344 or SD) tested under each treatment condition.

The colony room was maintained at 22-24°C and kept under a 12-hr light/dark cycle, with behavioral testing occurring during the light phase of the cycle. Food and water were freely available. Subjects were treated according to the "Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council, 2010) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

Table 1. Subject Assignment

Treatment Conditions	F344		SD	
	Male Pups	Female Pups	Male Pups	Female Pups
Vehicle-treated (saline or 50% DMSO)	21	24	21	15
Rat pups treated with dopamine reuptake inhibitor (DRI: GBR-12909)	1.5 mg/kg	4	6	5
	5 mg/kg	5	6	7
	15 mg/kg	6	5	6
Rat pups treated with selective norepinephrine reuptake inhibitor (NRI: Atomoxetine)	0.3 mg/kg	5	6	7
	1 mg/kg	6	5	6
	3 mg/kg	5	6	7
Rat pups treated with serotonin reuptake inhibitor (SRI: Fluoxetine)	3 mg/kg	4	6	5
	10 mg/kg	5	6	7
	30 mg/kg	6	5	6
Total		67	75	77
		N = 142		N = 145

Design of the Experiments

Three experiments were designed to test the proposed hypotheses: The first experiment involved the administration of the dopamine reuptake inhibitor (DRI: GBR-12909), the second experiment involved the administration of the selective norepinephrine reuptake inhibitor (NRI: atomoxetine), and the third experiment involved the administration of the serotonin reuptake inhibitor (SRI: fluoxetine).

In the first experiment, a $2 \times 4 \times 4$ mixed factorial design was adopted. The first independent variable "rat strain" was a between-subjects variable with two levels (the F344 rats and the SD rats). The second independent variable "drug condition" is a between-subjects variable with one vehicle-treated control condition and three levels (1.5 mg/kg, 5 mg/kg, and 15 mg/kg) of GBR-12909. The third independent variable "time block" is a within-subjects variable with four 5-minute blocks. The dependent variable is the frequency of ultrasonic vocalizations produced by each rat during each 5-minute testing block. The design (not including the "time block" variable) is illustrated in Table 2.

Table 2. Experiment 1: GBR-12909 Administration

		Rat Strain	
		F344 rats	SD rats
Drug condition	Vehicle-treated (saline or 50% DMSO)		
	1.5 mg/kg		
	5 mg/kg		
	15 mg/kg		

In the second experiment, a $2 \times 4 \times 4$ mixed factorial design was adopted. The first independent variable "rat strain" was a between-subjects variable with two levels

(the F344 rats and the SD rats). The second independent variable "drug condition" is a between-subjects variable with one vehicle-treated control condition and three levels (0.3 mg/kg, 1 mg/kg, and 3 mg/kg) of atomoxetine. The third independent variable "time block" is a within-subjects variable with four 5-minute blocks. The dependent variable is the frequency of ultrasonic vocalizations (USVs) produced by each rat during each 5-minute test block. The design (not including the "time block" variable) is illustrated in Table 3.

Table 3. Experiment 2: Atomoxetine Administration

		Rat Strain	
		F344 rats	SD rats
Drug condition Atomoxetine	Vehicle-treated (saline or 50% DMSO)		
	0.3 mg/kg		
	1 mg/kg		
	3 mg/kg		

In the third experiment, a $2 \times 4 \times 4$ mixed factorial design was adopted. The first independent variable "rat strain" was a between-subjects variable with two levels (the F344 rats and the SD rats). The second independent variable "drug condition" is a between-subjects variable with one vehicle-treated control condition and three levels (3 mg/kg, 10 mg/kg, and 30 mg/kg) of fluoxetine.

The third independent variable "time block" is a within-subjects variable with four 5-minute blocks. The dependent variable is the frequency of ultrasonic vocalizations (USVs) produced by each rat during each 5-minute test block. The design (not including the "time block" variable) is illustrated in Table 4.

Table 4. Experiment 3: Fluoxetine Administration

		Rat Strain	
		F344 rats	SD rats
Drug condition Fluoxetine	Vehicle-treated (saline or 50% DMSO)		
	3 mg/kg		
	10 mg/kg		
	30 mg/kg		

Note that, in this thesis, the same vehicle-treated (saline or 50% DMSO) condition was used as the control condition of the "drug condition" variable for all three experiments.

Drugs

Atomoxetine, GBR-12909, and pentobarbital were mixed in saline, while fluoxetine was mixed in 50% DMSO. All drugs were injected intraperitoneally (IP) at a volume of 5 ml/kg. GBR-12909 and pentobarbital were purchased from Sigma-Aldrich (St. Louis, MO). Atomoxetine and fluoxetine

were purchased from Toronto Research Chemicals (Toronto, Ontario).

Apparatus

The testing apparatus was a Plexiglas testing chamber ($20 \times 20 \times 20$ cm) housed inside a white wooden chamber ($24 \times 24 \times 22$ cm) housed inside a heated terrarium (31°C , $\pm 1^\circ\text{C}$). A Mini-3 ultrasonic detector (Ultrasound Advice, London, UK) was suspended above the floor of the testing apparatus. The ultrasonic detector was tuned to 40 kHz (± 1 kHz), because a setting of 40–45 kHz provides the highest rate of USV detection (Hofer & Shair, 1978). Ultrasonic vocalizations were recorded using the Ultravox program and by observers blind to drug treatment conditions. Body temperatures were recorded using a BAT-12 microprobe thermometer (Physitemp Instruments, Piscataway, NJ).

Isolation-Induced Ultrasonic Vocalizations Procedure

On PD 15, rats were weighed and injected with either GBR-12909 (DRI; 1.5, 5, or 15 mg/kg), atomoxetine (NRI; 0.3, 1, or 3 mg/kg), fluoxetine (SRI; 3, 10, or 30 mg/kg), or vehicle 30 min before testing. Half of the vehicle-treated subjects were injected with saline and the other half with 50% DMSO. (see Tables 2-4.) Thirty minutes after

injection, each rat was removed individually from the home cage, placed in a travel container, and moved to the testing room. The rat was then placed in the heated chamber (31°C , $\pm 1^{\circ}\text{C}$) where ultrasonic vocalizations were measured for 20 min. Rectal temperatures were measured immediately after rats were removed from the test chamber. Afterwards, the rat were anesthetized with pentobarbital (20 mg/kg) and returned to the home cage. Rats were anesthetized to prevent them from emitting ultrasonic vocalizations while in the home cage. Rat pups were monitored until fully responsive.

Tissue Monoamine and Metabolite Assay

On PD 17, rats in the vehicle condition from each strain were decapitated and their striatum rapidly dissected. Tissue samples were then frozen until day of assay. On assay day, frozen brain sections were sonicated in 10 volumes of 0.1 N HClO_4 and centrifuged at $20,000 \times g$ for 20 min at 4°C . The supernatant was then filtered through a $0.22 \mu\text{m}$ centrifugation unit at $2,000 \times g$ for 5 min at 4°C . Twenty microliters of the resulting extracts were then assayed for dopamine, norepinephrine, and serotonin using high performance liquid chromatography (1525 binary pump, 717plus autosampler; Waters, Milford,

MA, USA, and an MD-150 column; ESA, Chelmsford, MA) with electrochemical detection (Coulochem II; ESA). The major monoamine metabolites (i.e., DOPAC, HVA, 5-HIAA, and MHPG) were also measured. The mobile phase consisted of 75 mM NaH₂PO₄, 1.4 mM 1-octane sulfonic acid, 10 mM EDTA, and 7.5% acetonitrile [(pH 3.0) MD-TM Mobile Phase; ESA] and was pumped at a rate of 0.5 ml/min.

Statistical Analysis

Litter effects were controlled by the subject assignment procedure with no more than one subject per litter placed in any group. An approximately equal number of male and female rats were assigned to each group. Although "sex" of the rat pups was not treated as a major independent variable in the above-mentioned three experiments, preliminary data analyses indicated some interesting and potentially important differences between male and female pups' differential responses to different drug treatments. Thus, in some statistical analyses, the variable "sex" was included.

In this thesis, several ANOVAs for mixed designs were conducted to test the proposed hypotheses. To test the first hypothesis regarding the difference between the vehicle-treated F344 rat pups and the SD rat pups in their

production of separation-induced ultrasonic vocalizations, a 3-way [2 (rat strain) \times 2 (sex) \times 4 (5-minute time block)] ANOVA was conducted. For each of the three experiments, to test the hypothesis concerning the effect of drug treatment on production of ultrasonic vocalizations, if the variable "sex" was included, then a 4-way [2 (rat strain) \times 2 (sex) \times 4 (drug condition) \times 4 (5-minute time block)] ANOVA was conducted. If the variable "sex" was not included, then a 3-way [2 (rat strain) \times 4 (drug condition) \times 4 (5-minute time block)] ANOVA was conducted. The corresponding SPSS program used was labeled "Repeated measures..." for "General Linear Model" because, in each experiment, besides the related between-subjects variables, a within-subjects (or repeated-measures) variable (namely, time block) was included. For between-subjects variables where three or more treatment conditions were included, the post hoc test ("Tukey's Honestly Significant Difference (HSD)") was further performed to identify significant differences between any two conditions.

For each experiment, data regarding body temperature were analyzed using a 3-way [2 (rat strain) \times 2 (sex) \times 4 (drug condition)] ANOVA for a between-subjects design.

Moreover, to analyze dopamine, norepinephrine, serotonin, their metabolites, and turnover rates in the striatum, 2-way [2 (rat strain) × 2 (sex)] ANOVAs for between-subjects designs were conducted. Turnover was defined as (metabolite/neurotransmitter level). The corresponding SPSS program used for between-subjects designs was labeled "Univariate..." for "General Linear Model."

CHAPTER SEVEN

RESULTS

Ultrasonic Vocalizations in Vehicle-treated Rats

The main effect of rat strain on ultrasonic vocalization production was significant, $F(1, 23) = 6.473$, $p < .05$. However, contrary to our prediction, vehicle-treated F344 rats ($M = 107.93$; SEM = ± 23.64) emitted more (rather than fewer) ultrasonic vocalizations than vehicle-treated SD rats ($M = 40.92$; SEM = ± 14.76). The main effect of time block on ultrasonic vocalizations was not significant; ultrasonic vocalizations did not vary in frequency over the four five-minute time blocks for vehicle-treated rats. The main effect of sex and the interaction effect between rat strain and sex on ultrasonic vocalizations were also not significant. Both the F344 female rats and the F344 male rats produced more ultrasonic vocalizations than their SD counterparts.

Ultrasonic Vocalizations after GBR-12909 Administration

The results regarding the administration of the dopamine reuptake inhibitor (GBR-12909) are illustrated in Figures 1 and 2. As can be seen from Figure 1, administration of GBR-12909 [dopamine reuptake inhibitor

(DRI)] did not result in less ultrasonic vocalization production for either the SD or the F344 rats. For the SD rats, no significant difference in ultrasonic vocalization production was observed between any two of the three GBR conditions (1.5, 5, and 15 mg/kg GBR-12909) or between any one of the three GBR-12909 conditions and the vehicle-treated condition (0 mg/kg GBR-12909). This result was consistently observed across all four 5-minute time blocks. For the F344 rats, ultrasonic vocalization production was higher for those treated with 1.5 mg/kg GBR-12909 as compared to those treated with the vehicle or those treated with either 5 or 15 mg/kg GBR-12909, especially for the last two time blocks. [This set of results was suggested by the significant rat strain \times drug condition \times time block interaction effect on the ultrasonic vocalization production, $F(9,216) = 2.721$, $p < .005$, and the outcome of the Tukey tests.] The results illustrated in Figure 1, also did not suggest that GBR-12909 had a smaller effect on F344 rats as compared to SD rats.

Figure 2 illustrates how rat strain, sex, and drug condition interact to affect ultrasonic vocalization production. Over the 20 minute testing period, ultrasonic

vocalization production was higher for F344 rats as compared to SD rats. [This is suggested by the significant "rat strain" main effect, $F(1, 72) = 8.037, p < .05$]. Compared to their female counterparts, F344 male rats appeared to be more sensitive to GBR-12909 administration. As can be seen from Figure 2, male F344 rats (but not female F344 rats) treated with the 1.5 mg/kg dose produced more ultrasonic vocalizations than those treated with the 5 or 15 mg/kg doses [Tukey test, $p < .05$]. As can be seen from Figure 1, for some yet to be determined reason, both the vehicle-treated F344 rats and SD rats tended to produce considerably low amounts of ultrasonic vocalizations. Due to the very low levels of baseline vocalization production, a potential floor effect might have occurred, thus making it difficult for any potential drug-induced decline in vocalizations to occur.

Body Temperature

The administration of different amounts of GBR-12909 did not differentially affect the body temperatures of rats from either strain. However, F344 preweanling rats had lower rectal temperatures ($M = 36.89^{\circ}\text{C}, SEM = \pm 0.15$) than preweanling SD rats ($M = 37.45^{\circ}\text{C}, SEM = \pm 0.79$) [strain main effect, $F(1, 80) = 9.733, p < .05$].

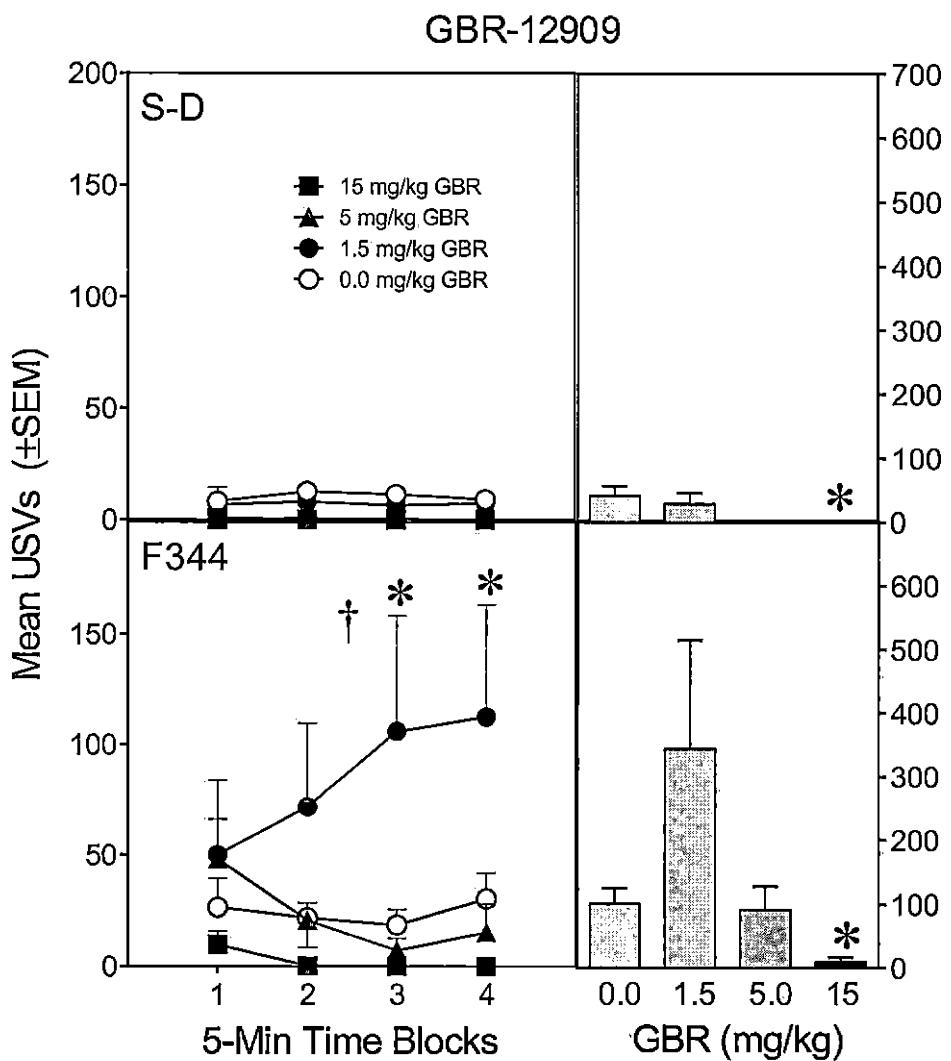


Figure 1. Mean Ultrasonic Vocalizations (USVs) for F344 Rats and SD Rats on PD 15. Rats were Treated with Vehicle or GBR-12909 (1.5, 5, or 15 mg/kg, IP) 30 Minutes Prior to Testing. † Indicates a Significant Difference from SD rats (Main Effect of Strain). *Indicates Significant Difference from Rats Treated with GBR-12909 (5 or 15 mg/kg) or vehicle (Main Effect of Drug).

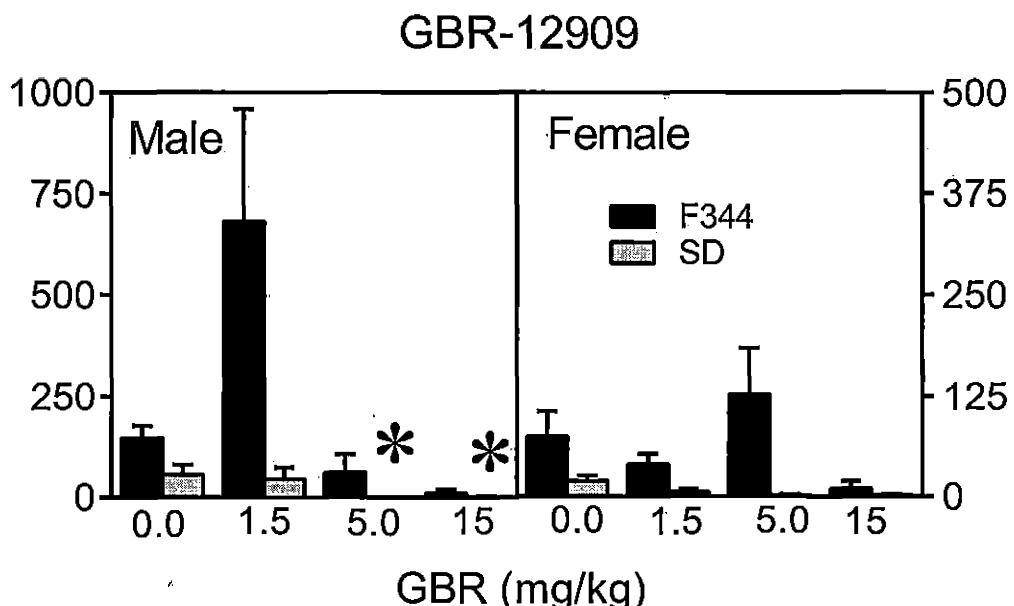


Figure 2. Mean Ultrasonic Vocalizations (USVs) for Male and Female F344 Rats and SD Rats on PD 15. Rats were Treated with Vehicle or GBR-12909 (1.5, 5, or 15 mg/kg, IP) 30 Minutes Prior to Testing. *Indicates a Significant Difference from Male Rats Treated with 1.5 mg/kg (Sex by Drug Interaction).

Ultrasonic Vocalization after Atomoxetine Administration

Preliminary analyses revealed that the frequency of ultrasonic vocalizations did not differ across the four five minute time blocks, thus subsequent analyses were performed with the data collapsed over time blocks.

The results regarding the administration of the norepinephrine reuptake inhibitor (atomoxetine) are

illustrated in Figure 3. Consistent with our prediction, administration of atomoxetine did increase ultrasonic vocalization emissions in both the SD and the F344 rats. The amount of ultrasonic vocalizations differed according to atomoxetine dose; rats treated with the two highest doses of atomoxetine (1 or 3 mg/kg) exhibited a significantly greater amount of vocalizations than the vehicle-treated rats. [This is supported by the significant main effect of drug condition, $F(3, 79) = 4.937$, $p < .05$, and the outcome of the Tukey tests]. Also consistent with our prediction, administration of atomoxetine did appear to have an enhanced effect in F344 rats as compared to SD rats. As can be seen from Figure 3, the difference in ultrasonic vocalization production between those rats treated with the two highest doses of atomoxetine (1 or 3 mg/kg) and those treated with vehicle was larger for the F344 rats than for the SD rats. However, F344 rats had more vocalizations than SD rats regardless of drug dose. [The strain main effect is significant, $F(1, 79) = 25.250$, $p < .05$]. The effect of atomoxetine administration on ultrasonic vocalization production did not differ according to sex.

Body Temperature

The administration of different amounts of atomoxetine did not differentially affect the rectal temperature of rats in either rat strain. However, preweanling F344 rats ($M = 36.94^{\circ}\text{C}$, SEM = ± 0.14) did have a lower rectal temperature than preweanling SD rats ($M = 37.29^{\circ}\text{C}$, SEM = ± 0.13) [strain main effect, $F (1, 79) = 10.186$, $p < .05$].

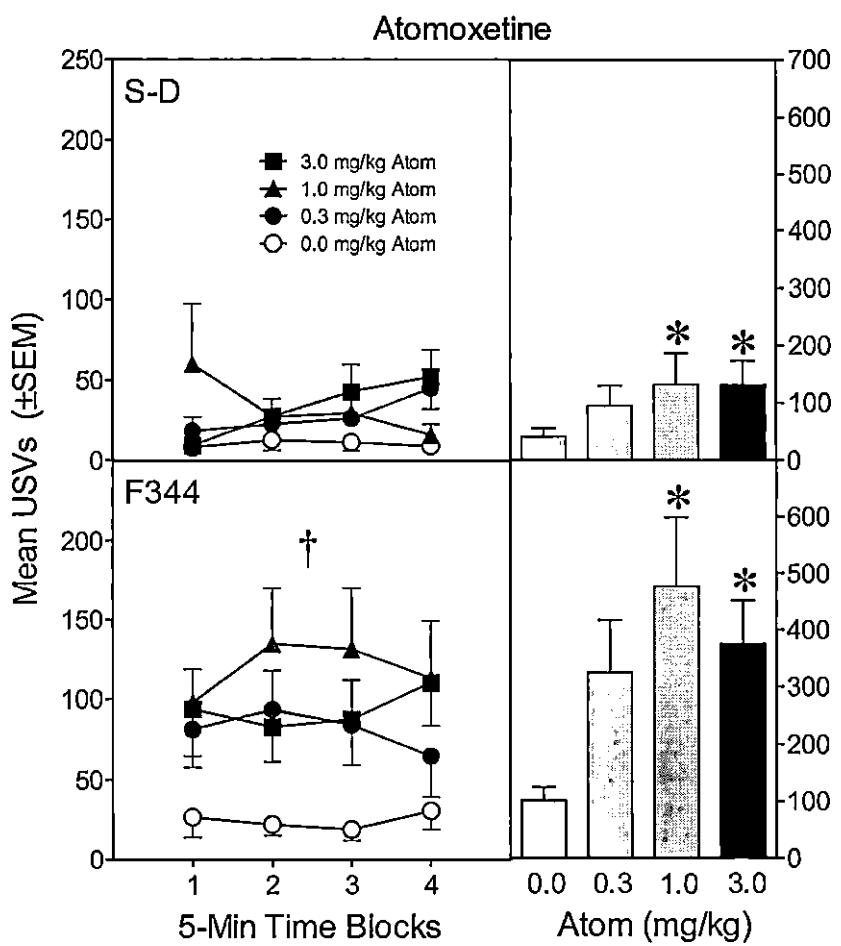


Figure 3. Mean Ultrasonic Vocalizations (USVs) for F344

Rats and SD Rats on PD 15. Rats were Treated with Vehicle or Atomoxetine (0.3, 1, or 3 mg/kg, IP) 30 Minutes Prior to Testing. †Indicates a Significant Difference from SD Rats (Main Effect of Strain). *Indicates a Significant Difference from Vehicle-Treated Rats (Main Effect of Drug).

Ultrasonic Vocalizations after Fluoxetine Administration

similar to the effects of atomoxetine, vocalizations after fluoxetine administration did not significantly differ across the four time blocks. Therefore, data were collapsed over the time blocks in the analyses for this experiment.

The results regarding the administration of the serotonin reuptake inhibitor (fluoxetine) are illustrated in Figure 4. As can be seen from Figure 4, ultrasonic vocalizations were dose-dependently affected by fluoxetine. Partially consistent with our hypothesis, although both the F344 and the SD rats treated with a low or medium amount of fluoxetine (3 or 10 mg/kg) did not produce fewer ultrasonic vocalizations as compared to the vehicle-treated rats, both the F344 and the SD rats treated with a high amount of fluoxetine (30 mg/kg) did produce fewer ultrasonic vocalizations as compared to the vehicle-treated rats. [This is supported by the significant main effect of drug condition, $F(3, 80) = 2.878, p < .05$; and the outcome of the Tukey tests]. However, inconsistent with our prediction, fluoxetine did not seem to have a smaller effect on F344 rats as compared to SD rats. In general, F344 rats emitted

more vocalizations than SD rats regardless of drug dose. [The strain main effect is significant, $F(1, 80) = 4.424$, $p < .05$]. Ultrasonic vocalizations after fluoxetine administration did not differ in accordance with sex. As can be seen from Figure 1, for some yet to be determined reason, both the vehicle-treated F344 rats and SD rats tended to produce considerably low amounts of ultrasonic vocalizations. Due to the very low levels of baseline vocalization production, a potential floor effect might have occurred, thus making it difficult for any potential drug-induced decline in vocalizations to occur.

Body Temperature

Considering body temperature, rats were differentially affected by the administration of different amounts of fluoxetine [the main effect of drug condition was significant, $F(3, 88) = 12.009$, $p < .05$]. Moreover, F344 rats were affected by the administration of different amounts of fluoxetine, while SD rats were not. Specifically, F344 rats treated with 3 mg/kg of fluoxetine ($M = 35.80^{\circ}\text{C}$, $\text{SEM} = \pm 0.19$) had lower body temperatures than F344 rats treated with 10 mg/kg ($M = 37.03^{\circ}\text{C}$, $\text{SEM} = \pm 0.18$) or 30 mg/kg of fluoxetine ($M = 36.94^{\circ}\text{C}$, $\text{SEM} = \pm 0.18$). The 3mg/kg group also had lower body temperatures than the vehicle-treated F344 rats.

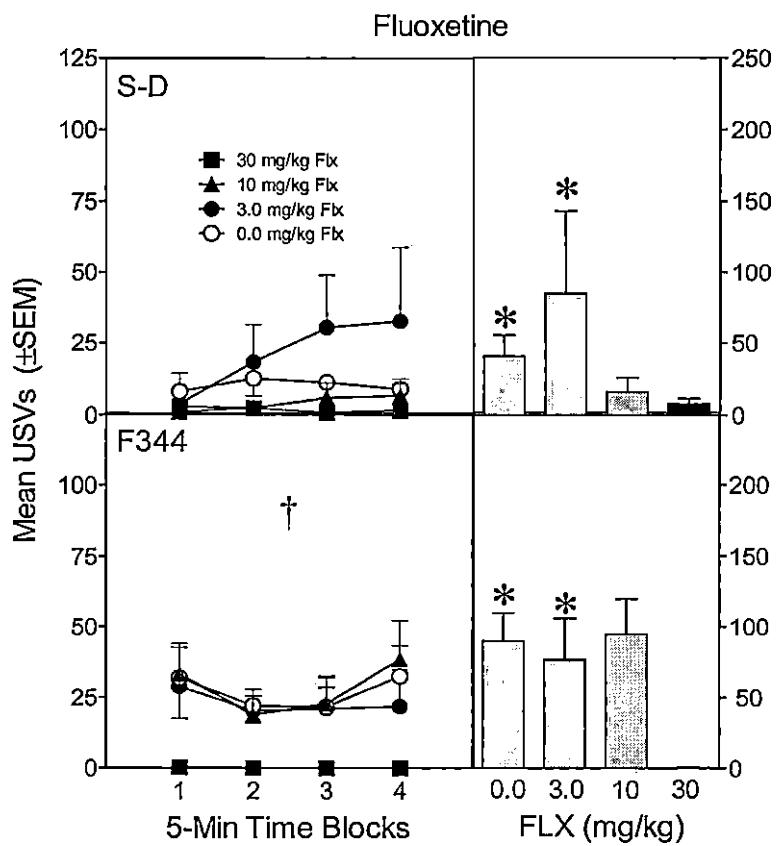


Figure 4. Mean Ultrasonic Vocalizations (USVs) for F344 Rats and SD Rats on PD 15. Rats were Treated with Vehicle or Fluoxetine (1, 10 or 30 mg/kg, IP) 30 Minutes Prior to Testing. †Indicates a Significant Difference from SD Rats (Main Effect of Strain). *Indicates a Significant Difference from rats treated with 30 mg/kg Fluoxetine (Main Effect of Drug).

Striatal Monoamine Content and Turnover

Norepinephrine content varied according to rat strain and sex (strain × sex interaction, $F(1, 22) = 10.924$,

$p < .01$) (see Table 5). Specifically, male F344 rats had higher levels of norepinephrine than male SD rats; while female 344 rats had lower levels of norepinephrine than female SD rats. [Tukey tests, $p < .05$]. Dopamine and serotonin content did not differ by rat strain or sex (see Table 5).

Similar to serotonin, the content of the serotonin metabolite 5-HIAA, did not vary by sex or rat strain (see Table 6). In contrast, the dopamine metabolite, DOPAC, did differ by strain and sex. For male rats, F344 rats had higher levels of DOPAC than SD rats. However, for female rats, no significant difference was observed between the F344 and SD rats. [strain \times sex interaction, $F(1,22) = 6.607$, $p < 0.05$, and Tukey tests]. Data for the norepinephrine metabolite, MHPG are unavailable because MHPG levels fell below the detection range of our HPLC.

Turnover or usage rates for both dopamine and serotonin varied by rat strain and sex (see Table 7). For male rats, dopamine turnover was lower for the F344 rats as compared to the SD rats ($F344 = 0.436$, $SD = 0.536$). For female rats, no significant difference in dopamine turnover was observed between the F344 and SD rats ($F344 = 0.437$, $SD = 0.440$). [This is supported by the

significant strain \times sex interaction, $F(1, 22) = 5.877$, $p < .05$, and the outcome of the Tukey tests.] For male rats, serotonin turnover was lower for the F344 rats as compared to the SD rats ($F_{344} = 0.759$, $SD = 0.824$). However, for female rats, serotonin turnover was greater for F344 rats as compared to the SD rats ($F_{344} = 0.864$; $SD = 0.747$). [This is supported by the significant strain \times sex interaction, $F(1, 22) = 4.835$, $p < .05$, and by the outcome of the Tukey tests.] Norepinephrine turnover could not be calculated because MHPG levels were not obtained.

Table 5. Monoamine Content in F344 and SD Rats on PD 17

Norepinephrine (ng/mg wet weight tissue)			
Rat Strain	Male rats	Female rats	X (M, F)
SD	0.205 (\pm 0.023)	0.284 (\pm 0.040)	0.241 (\pm 0.024)
F344	0.280 (\pm 0.026)**	0.180 (\pm 0.063)*	0.227 (\pm 0.021)
Dopamine (ng/mg wet weight tissue)			
Rat Strain	Male rats	Female rats	X (M, F)
SD	4.327 (\pm 0.048)	4.305 (\pm 0.029)	4.317 (\pm 0.285)
F344	4.270 (\pm 0.054)	4.427 (\pm 0.056)	4.354 (\pm 0.043)
Serotonin (ng/mg wet weight tissue)			
Rat Strain	Male rats	Female rats	X (M, F)
SD	0.718 (\pm 0.015)	0.749 (\pm 0.041)	0.732 (\pm 0.020)
F344	0.737 (\pm 0.024)	0.723 (\pm 0.016)	0.729 (\pm 0.017)

F344 and SD male and female rats (n = 6-8 per group) were injected with vehicle on PD 15 and ultrasonic vocalizations were assessed for 20 min. On PD 17, monoamine content was measured.

* Indicates significant difference from female SD rats.

** Indicates significant difference from male SD rats.

Table 6. Monoamine Metabolite Content in F344 and SD Rats
on PD 17

5-HIAA (ng/mg wet weight tissue) in the dorsal striatum			
Rat Strain	Male rats	Female rats	\bar{x} (M, F)
SD	0.591 (\pm 0.043)	0.569 (\pm 0.048)	0.581 (\pm 0.038)
F344	0.562 (\pm 0.040)	0.626 (\pm 0.038)	0.596 (\pm 0.022)
DOPAC (ng/mg wet weight tissue) in the dorsal striatum			
Rat Strain	Male rats	Female rats	\bar{x} (M, F)
SD	2.320 (\pm 0.098)	1.894 (\pm 0.107)	2.126 (\pm 0.099)
F344	1.867 (\pm 0.090)*	1.931 (\pm 0.084)	1.902 (\pm 0.057)

F344 and SD male and female rats (n = 6-8 per group) were injected with vehicle on PD 15 and ultrasonic vocalizations were assessed for 20 min. On PD 17, monoamine metabolite content was measured.

* Indicates significantly different from SD male rats.

Table 7. Monoamine Turnover Content in F344 and SD Rats on PD 17

DOPAC/Dopamine in the dorsal striatum			
Rat Strain	Male rats	Female rats	\bar{x} (M, F)
SD	0.536 (\pm 0.020)	0.440 (\pm 0.018)	0.492 (\pm 0.022)
F344	0.436 (\pm 0.019)**	0.437 (\pm 0.018)	0.436 (\pm 0.012)
5-HIAA/Serotonin in the dorsal striatum			
Rat Strain	Male rats	Female rats	\bar{x} (M, F)
SD	0.824 (\pm 0.042)	0.747 (\pm 0.046)	0.789 (\pm 0.039)
F344	0.759 (\pm 0.039)	0.864 (\pm 0.037)*	0.815 (\pm 0.025)

F344 and SD male and female rats (n = 6-8 per group) were injected with vehicle on PD 15 and ultrasonic vocalizations assessed for 20 min. On PD 17, monoamine content and monoamine metabolite content were measure and monoamine turnover rates were calculated.

* Indicates significant difference from female SD rats.

** Indicates a significance difference from male SD rats.

CHAPTER EIGHT

DISCUSSION

Introduction

Delays in social communication are a defining feature of many developmental disorders such as autism (Constantino & Todd, 2003; Flynn & Healy, 2012). Unfortunately, few effective treatments exist for alleviating deficits in social communication and the development of new therapies has been hampered by a lack of appropriate animal models (Klauck & Poustka, 2006). Recently, F344 rats have emerged as a possible model of social communication deficits as they naturally display less play behavior, an important type of developmental social interaction, than other rat strains such as SD and Lewis rats (Siviy et al., 2011). However, a disadvantage to this model is that play behavior has a relatively late onset, while social communication delays appear early in human children. Thus, the goal of the present thesis was to determine if F344 rats would also show fewer emissions of isolation-induced ultrasonic vocalizations (an early onset form of social interaction) as compared to SD rats. We also investigated the role of monoamines in social communication by using selective monoaminergic compounds

(i.e., atomoxetine, GBR-12909, and fluoxetine) to modulate monoamine activity and by determining monoamine activity in the striatum.

Ultrasonic Vocalizations

Effects of Rat Strain

We predicted that vehicle-treated F344 rats would emit fewer isolation-induced ultrasonic vocalizations than SD rats. However, F344 rats treated with vehicle emitted more, not fewer, ultrasonic vocalizations. Our findings, although contrary to our prediction, are supportive of studies showing that F344 rats differ in social interaction (i.e., social play) when compared to other rat strains. The higher number of ultrasonic vocalizations exhibited by F344 rats may also be consistent with a previous result showing robust behavioral differences in the F344 rat phenotype, including hyper-responsiveness to stress (Kosten & Ambrosio, 2002). Specifically, the greater number of ultrasonic vocalizations emitted by F344 rats may be a result of their hyper-responsiveness when put in stressful situations, such as isolation. Because both the SD and the F344 rats were tested in the same environment, it is unlikely that environmental stimuli were responsible for the differences in vocalization emission rates.

The difference in the number of vocalizations between F344 and SD rats could also be related to variations in body temperature, because body temperature is known to modulate ultrasonic vocalization production in rat pups. Specifically, lowering body temperatures by placing rat pups in a cool testing environment (10-15 °C) induces greater amounts of ultrasonic vocalizations than when rats are placed in a thermoneutral (35 °C) testing environment (Blumberg, Efimova, & Alberts, 1992). Thus, the increased number of ultrasonic vocalizations emitted by F344 rats could be a consequence of lower body temperatures as compared to SD rats. Differences between F344 and SD rats in body temperature and thermoregulation have been previously observed in adult male rats, with F344 rats having a greater loss of body heat in cool environments (Brown & Pham-Le, 2012). In addition, adult male F344 rats have elevated body temperature when placed in a warm chamber (37.1 °C), whereas body temperatures of SD rats are unaffected by placement in a heated chamber (Brown & Pham-Le, 2012). These data suggest that F344 rats are less able to maintain a homeostatic body temperature than SD rats and this deficit may be partially responsible for their greater production of ultrasonic vocalizations.

Effect of Monoamine Modulation

We predicted that GBR-12909 administration would result in less ultrasonic vocalization production for F344 and SD rats. GBR-12909 is a highly selective dopamine re-uptake blocker that increases dopamine in the synapse (Loupe, Bredemeier, Schroder, & Tessel, 2002). Thus it was hypothesized that increased dopamine activity would decrease vocalizations. Additionally, we predicted that GBR-12909 administration would have a smaller effect on F344 rats than on SD rats.

The hypothesis regarding the administration of GBR-12909 was not supported; however, although not statistically significant, the high dose of GBR-12909 (15 mg/kg) did decrease ultrasonic vocalizations in male F344 rats. Contrary to our prediction, F344 rats treated with 1.5 mg/kg GBR-12909 emitted more ultrasonic vocalization than similarly treated SD rats. The increased ultrasonic emissions of F344 rats may be a result of lower levels of synaptic dopamine, because F344 rats release significantly less dopamine than SD rats when electrically stimulated or challenged with amphetamine (Cadoni & Di Chiara, 2007; Siviy et al., 2011).

In our second experiment, we predicted that stimulating norepinephrine transmission with atomoxetine

would result in an increase of ultrasonic vocalization emissions in F344 and SD rats. Additionally, we predicted that atomoxetine would have a potentiated effect on F344 rat vocalizations when compared to SD rats. As expected, F344 rats emitted more ultrasonic vocalizations than SD rats regardless of atomoxetine dose and atomoxetine increased ultrasonic vocalizations in both strains in a dose dependent manner. As compared to F344 rats, treatment with atomoxetine (1 and 3 mg/kg) caused similar (but smaller) increases in the ultrasonic vocalizations production of SD rats.

Increased ultrasonic emissions after atomoxetine may be attributed to the effects of atomoxetine on presynaptic NET. Atomoxetine has a high affinity for presynaptic NETs, while leads to significant increases in extracellular norepinephrine (Bymaster et al., 2002). In regard to the current experiment, the greater amount of ultrasonic vocalizations emitted after atomoxetine is probably the result of higher level of synaptic norepinephrine. Adrenergic receptors are known to modulate ultrasonic vocalizations, because administration of the adrenergic agonists clonidine or yohimbine increase ultrasonic vocalizations (Blumberg et al., 1999; Krall, Andicochea, &

McDougall, 2005; Nazarian, Krall, Osburn, & McDougall, 2001).

As predicted, F344 rats, when compared to SD rats exhibited a potentiated response to atomoxetine. It is possible that the increased norepinephrine content observed in F344 rats is indicative of greater adrenergic system sensitivity. Moreover, the elevated levels of striatal norepinephrine, may explain why F344 rats have more basal ultrasonic vocalizations than SD rats.

In our third experiment, we increased serotonin neurotransmission with fluoxetine. Fluoxetine is a selective serotonin re-uptake inhibitor (SSRI) used in the treatment of major depression and other disorders such as autism (DeLong, Teague, & McSwain Kamran, 1998; Taylor, Fricker, Devi, & Gomes, 2005). We hypothesized fluoxetine would decrease ultrasonic vocalization emissions in both rat strains and that the fluoxetine induced enhancement of serotonin transmission would have a smaller effect on F344 rats than SD rats. In partial support of our hypothesis, rats treated with the highest dose of fluoxetine (30 mg/kg) exhibited fewer ultrasonic vocalizations than rats treated with the lowest dose of fluoxetine (3 mg/kg) or vehicle-treated rats. We did not find support for the second part of our hypothesis because there were no

strain-dependent differences in the effects of fluoxetine on ultrasonic vocalizations.

The role of serotonin in the regulation of ultrasonic vocalizations of infant rats is determined by which serotonin receptor subtypes are stimulated (Olivier, Molewijk, van der Heyden, van Oorschot, Ronken, Mos, & Miczek, 1998). For example, agonists acting on 5HT_{1A} receptor reduce ultrasonic vocalizations, whereas 5HT_{1B} receptors agonists and 5HT_{2A/2C} receptor antagonists stimulate ultrasonic vocalization production (Olivier et al., 1998). Because fluoxetine (30 mg/kg) decreased ultrasonic vocalizations it can be inferred that this reduction in vocalizations was the result of 5HT_{1A} activation. This hypothesis is supported by a prior investigation showing fluoxetine-induced 5HT_{1A} activation (De Vry, 1996).

Basal Monoamine Activity

Many previous investigations have demonstrated that ultrasonic vocalizations are modulated by monoamine activity (Brunelli & Hofer, 2007; Brunelli & Kehoe, 2005; Dastur et al., 1999). Furthermore, F344 rats differ from other strains in monoaminergic functioning, including differences in dopaminergic, serotonergic, and norepinephrine functions (Varty & Geyer, 1998). Thus, we

hypothesized that differences in basal monoamine activity could be responsible for differences in the ultrasonic vocalization emissions of F344 and SD rats. We predicted that monoamine and monoamine metabolite levels and monoamine turnover (defined as metabolite/neurotransmitter level) would differ between F344 and SD rats.

Specifically, we hypothesized that dopamine and serotonin turnover would be lower in F344 rats, when compared to SD rats, while norepinephrine turnover would be greater in F344 rats.

Considering monoamine content, we found that levels of norepinephrine varied according to rat strain and sex.

Specifically, consistent with our prediction, male F344 rats had higher levels of norepinephrine than male SD rats; however, different from their male counterparts, female F344 rats had lower levels of norepinephrine than female SD rats. Dopamine and serotonin did not differ between the two rat strains.

Considering monoamine metabolite content, we found that the serotonin metabolite 5-HIAA did not vary by sex or rat strain. In contrast, the dopamine metabolite (DOPAC) differed by strain and sex. Contrary to our prediction, male F344 rats had higher levels of DOPAC than male SD rats. This increase of striatal DOPAC levels in

F344 rats, however, was not observed in female rats. Levels of MHPG were not analyzed because the levels of MHPG in most of the samples fell below the detection range of our HPLC.

Considering monoamine turnover, we found that serotonin and dopamine turnover did differ by strain, but the strain effect was moderated by sex. For male rats, serotonin turnover was lower for the F344 rats as compared to the SD rats; however, for female rats, serotonin turnover was greater for F344 rats as compared to the SD rats. Overall F344 rats had less dopamine turnover than SD rats, but the difference was only significant in male rats. Norepinephrine turnover could not be calculated because MHPG levels were not obtained.

Noradrenergic neurons in the locus coeruleus have been implicated in the response of the CNS to environmental stressors (Nisenbaum, Zigmond, Sved, & Abercrombie, 1991). Specifically, increases in norepinephrine levels have been linked to stress-related events such as isolation (Harvey & Hennessy, 1995; Nisenbaum et al., 1991). The results of our study may indicate that the greater amount of norepinephrine found in the striatum of male F344 rats may be due to an enhanced responsiveness to stress during testing.

The increase in DOPAC levels in male F344 rats is also consistent with this hypothesis because high levels of DOPAC are indicative of increased conversion of dopamine to norepinephrine (Abercrombie & Zigmond, 1989; Nisenbaum et al., 1991). Lastly, the increase in serotonin turnover in female F344 rats seen in this study may be attributed effects of stress on serotonin turnover (Papaioannou, Dafni, Alikaridis, Bolaris, & Stylianopoulou, 2002). Previously, the increase of serotonin turnover was suggested to occur in response to adaptation of environmental stress during isolation rearing (Fone & Porkess, 2008). Thus, high levels of serotonin turnover may suggest that female F344 rats in this study may also be sensitive to stressful situations.

It should be pointed out that, in our experiments, the predicted strain effect (i.e., F344 < SD) for dopamine and serotonin turnover was moderated by sex. As reported earlier, for male rats, dopamine turnover was lower for the F344 rats as compared to the SD rats, whereas, for female rats, no significant difference in dopamine turnover was observed between the F344 and SD rats. For male rats, serotonin turnover was lower for the F344 rats as compared to the SD rats. However, for female rats, serotonin turnover was greater for F344 rats as compared

to the SD rats. The reason why male but not female F344 rats showed the expected differences in monoamine systems is still an open question. However, if the observed sex difference can be validated, it could be important for understanding the differential prevalence of social communication disorders like autism in male and female children (Fombonne, 2005; Matson & Kozlowski, 2011).

Conclusions

Overall our study revealed that atypical social communication can be detected in F344 rats at a young age. When compared to SD rats, F344 rats had increased ultrasonic vocalizations emission. This finding of ultrasonic vocalization production did not support our hypotheses, but our data do support the existing literature indicating that F344 rats when compared to other strains of albino rats display different patterns of social communication. Thus, I believe that isolation-induced ultrasonic vocalizations in F344 rats could be a useful model for social communication disorders, if the potential confounding effect associated with differences in body temperature regulation evident in F344 rats compared to SD rats can be properly controlled.

Although our results revealed differences in monoaminergic systems between F344 and SD rats, the activation of dopaminergic and serotonergic systems produced similar effects on ultrasonic vocalization production in both strains. In contrast the activation of adrenergic systems by atomoxetine produced greater ultrasonic vocalization production in F344 rats than SD rats. This strain dependent difference in ultrasonic vocalization production suggests that enhanced norepinephrine activity may be important for understanding the differences in social communication between F344 and SD rats. However, it is likely that other neurochemical messengers such as hormones (i.e., oxytocin and vasopressin), and opioids may play an important role in the modulation of social deficits. Vasopressin and oxytocin are known to be important in the regulation of social bonding (Klopfer, 1971) and both hormones reduce the frequency of isolation-induced ultrasonic vocalizations in infant rats (Insel & Winslow, 1991; Winslow & Insel, 1993). Endogenous opioids have also been associated with social behaviors in human children (Panskepp, Nelson, & Bekkdal, 1997) and in the deficits of social behavior seen in psychiatric disorders such as autism (Panskepp, 1979). In summary, we believe our

findings provide a possible direction for future research on the use of ultrasonic vocalizations in F344 rats as a model of social communication deficits. Moreover, we believe studies assessing the modulation of ultrasonic vocalizations by vasopressin, oxytocin, and the endogenous opioid activity are warranted.

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