PREPULSE INHIBITION OF THE STARTLE REFLEX IN FOREBRAIN OXYTOCIN RECEPTOR KNOCKOUT MICE

A thesis submitted to the Kent State University Honors College in partial fulfillment of the requirements for General Honors

by

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CHAPTER I

INTRODUCTION

Each year approximately 3% of Americans suffer from the neuropsychiatric disorders schizophrenia and bipolar disorder. However the origins of these diseases are poorly understood (Regier et al., 1993). Studying the neurological factors behind normal as well as disrupted human behavior is important to further our understanding of human behavior as well as develop better interventions and therapies for those plagued by mental illness. While it is unlikely that a single gene or a single neurotransmitter is responsible for these disorders, one neurohormone that has been consistently linked to the neuroregulation of many mammalian behaviors, and is thought to contribute to psychiatric illnesses is oxytocin (Oxt) (Feifel and Reza, 1999, Beckmann et al., 1985, Goldman et al., 2008, Caldwell et al., 2009).

The Oxytocin System

Oxytocin

Oxt is a 9-amino-acid neuropeptide that is primarily made in the magnocellular neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus, and is secreted into the peripheral blood stream from the posterior pituitary (Du Vigneaud et al., 1953). Oxt is also made in the parvocellular neurons of the PVN and SON which project their axons throughout the brain; ultimately affecting behavior (Ludwig and Leng, 2006). Oxt is differentiated from its sister hormone
vasopressin in only two of its amino acids, those in the third and eighth positions, and it is these differences that allow for Oxt to specifically bind to its receptor (Barberis et al., 1998). Oxt is synthesized from the hormone precursor preprooxytocin (Swaab et al., 1975). Preprooxytocin has three components, a signaling peptide, the nonapeptide, and a neurophysin (Holmgren and Jensen, 2001).

**Oxytocin Function**

The most investigated peripheral action of Oxt is its role in uterine contractions at the time of parturition and milk ejection during lactation (Gimpl and Fahrenholz, 2001, Zingg, 2001). Central Oxt is heavily linked to the initiation and maintenance of social behaviors, including pair bonding, parental and infant attachment, stress-related behavior, memory, learning, and sexual behavior (Carter, 1992, Carmichael et al., 1987, Winslow and Insel, 2002, Keverne and Curley, 2004). Central microinjections of Oxt have been shown to increase aggressive and sexual behaviors in animals (Whitman and Albers, 1995, Jenike, 1990, Bosch et al., 2005). Excessive grooming, an obsessive compulsive disorder (OCD)-related behavior, is also observed in mice after central microinjection of Oxt (Marroni et al., 2007, Van Wimersma Greidanus et al., 1990). Further, in humans Oxt has also been determined to contribute to aspects of neuropsychiatric diseases (Ozsoy et al., 2009, Tom, 2010, Feifel et al., 2010, Frasch et al., 1995). Within the category of neuropsychiatric disorders Oxt has been shown to play a role in OCD, eating disorders, addiction, post-traumatic stress disorder, anxiety and depression, schizophrenia, autism, and Prader-Willy syndrome (Swerdlow et al., 2006, Schall et al., 1996, Marazziti and
Catena Dell'osso, 2008). Oxt levels in the cerebrospinal fluid (CSF) of adults with OCD are increased compared to controls (Leckman et al., 1994); however, research to the contrary has also been published (Altemus et al., 1999). There are also reports of elevated Oxt concentrations in the CSF of patients diagnosed with schizophrenia. (Beckmann et al., 1985, Leckman et al., 1994). These studies, along with others, suggest a relationship between Oxt and neuropsychiatric disorders. Specifically within neuropsychiatric disorders characterized by psychosis, such as schizophrenia, there is evidence that Oxt may act as a natural antipsychotic (Bakharev et al., 1986, Caldwell et al., 2009, Feifel and Reza, 1999, Bujanow, 1972, Feifel et al., 2010). In animal models, there is mounting evidence that Oxt contributes to some of the symptoms associated with neuropsychiatric disorders. For instance, in rats, treatment with antipsychotics increases Oxt secretion and this elevation in endogenous Oxt may mirror its importance in the effectiveness of antipsychotic drugs (Uvnas-Moberg et al., 1992).

The Oxytocin Receptor

To date only one subtype of Oxt receptor (the Oxtr) has been identified. It is expressed in tissues such as, heart, brain, vascular endothelium, osteoclasts, and myoblasts. The Oxtr is a Class I G protein-coupled receptor whose activation generates 1,2-diacyl-glycerol and inositol triphosphate from the primarily coupled phospholipase C-beta (Gimpl and Fahrenholz, 2001). This activation sequence leads to the promotion of intracellular Ca$^{2+}$ release and activation of protein kinase type C. The end result is an increase in intracellular Ca$^{2+}$ which can lead to a variety of physiological responses such
as smooth cell contraction, modifications of gene transcription and protein synthesis, as well as changes in cellular excitability due to changes in ion gradients (Zingg and Laporte, 2003).

Oxt binding sites in the human central nervous system are primarily located in the pars compacta of the substantia nigra and the globus pallidus, along with the anterior cingulate and medial insula (Loup et al., 1991). In rodents Oxtr is often prominent in the olfactory bulb and tubercle, neocortex, endopiriform cortex, hippocampal formation, central and lateral amygdala, bed nucleus of the stria terminalis, nucleus accumbens, and ventromedial hypothalamus (Insel et al., 1991, Veinante and Freund-Mercier, 1997). In general, Oxt binding sites vary greatly between species, and it is hypothesized that this variation contributes to the species-specific effects of Oxt on behavior (Marazziti and Catena Dell'osso, 2008).

Schizophrenia

Emil Kraepelin is credited with the first comprehensive definition of schizophrenia, although recorded cases had been documented much earlier. He defined the disease as an early-onset “dementia” with cognitive deficits and a poor prognosis. The term “schizophrenia” was coined by Eugen Bleuler, giving the distinct emphasis of the “splitting of the mind” believed to be a defining characteristic of the illness. The symptoms of schizophrenia occur heterogeneously and are consistently present after onset of the disease. Unlike other disorders, the typical definition of remission is a 20% decrease in symptoms (Moscarelli et al., 1996).
The symptoms of schizophrenia are divided into three categories, negative symptoms, positive symptoms, and cognitive dysfunction. Negative symptoms include affective blunting, speech lacking normal unprompted responses (aloria), a lack of desire to achieve meaningful goals (avolition), inability to make close bonds with others (asociality), anhedonia, and attention impairment. These symptoms are described as negative because they are the result of the loss of normal function (Meador-Woodruff and Healy, 2000, Thaker and Carpenter, 2001, Morris et al., 2005, Andreasen, 2000). Positive symptoms, however, are the distortion or excess of normal function including; hallucinations, delusions, thought broadcasting, thought insertion, and dysfunction of logical thought patterns (Morris et al., 2005). Two types of cognitive dysfunction largely present in schizophrenic patients are sensory flooding and cognitive fragmentation. Sensory flooding can be described as too much data being collected with no organization or filtering, while cognitive fragmentation is viewing data in pieces instead of as a whole (Arieti, 1966); both symptoms are linked to the loss of sensory motor gating (McGhie and Chapman, 1961).

**Sensorimotor Gating**

Sensorimotor gating is a type of information processing also known as the ability to “filter” or “gate” information (Swerdlow and Perry, 1996). Gating acts as an attentional mechanism, filtering potentially distracting stimuli so that attention can be focused on relevant sensory information. Patients diagnosed with schizophrenia are known to have disrupted sensorimotor gating (Braff et al., 1992). This disruption is
thought to be the cause of sensory flooding and cognitive fragmentation present in schizophrenia (McGhie and Chapman, 1961). Sensorimotor gating can be measured experimentally by prepulse inhibition of the startle reflex (PPI).

**Prepulse Inhibition of the Startle Reflex**

PPI is a measurement of sensorimotor gating defined by the normal subduing of the startle reflex when an intense startle stimulus “pulse” is immediately given after a less intense stimulus “prepulse” as shown in Figure 1 (Graham, 1975). Acoustic startle response (ASR) is the term used when the stimulus is auditory and is defined by the rapid contraction of the facial and cranial muscles after an intense, unexpected auditory stimulus (Koch and Schnitzler, 1997). Startle, including ASR, is known to have a non-zero baseline, meaning that it can be enhanced and inhibited as is presented with PPI (Koch, 1999). PPI is unlearned and has been used in both humans and animal models as a mode of measuring information processing (Feifel and Reza, 1999, Koch and Schnitzler, 1997, Morris et al., 2005, Braff et al., 2001, Koch, 1999). Modulation of PPI is controlled by the forebrain cortic-striato-pallido-pontine (CSPP) circuitry, which includes multiple circuits and neurotransmitters. This wide-spread modulation leads to the link of PPI, and thus the CSPP pathway, with multiple neuropsychiatric disorders including schizophrenia (Braff et al., 2001).
Figure 1: Comparison of two acoustic startle response (ASR) trials. Trial #1 contains a pulse alone, and Trial #2 contains a prepulse + pulse. The weak prepulse in Trial #2 precedes the pulse alone by 30-500 ms, significantly reducing the startle amplitude. The degree to which the motor response is inhibited by the prepulse provides an operational measure of sensorimotor gating. (Modified from Swerdlow et al., 2000)
The CSPP Circuitry

Studies in rats suggest that there are sequential and parallel neural connections between the limbic cortex, the ventral striatum, the ventral pallidum, and the pontine tegmentum, which regulate PPI (Koch and Schnitzler, 1997, Swerdlow et al., 2001). The primary startle circuitry and the limbic CSPP circuitry converge at the level of the nucleus reticularis pontis caudalis (Davis et al., 1982, Davis and Gendelman, 1977). The CSPP circuit regulates the degree of inhibition of the response to stimuli when preceded by a prepulse, although the main inhibitory effect of prepulse on the on the startle is exerted at the level of the pons. Reports show that many neurotransmitters are active within the CSPP circuitry (Swerdlow et al., 2001).

Neurotransmitter Systems Regulating PPI

The dopamine (DA) system has been the most frequently studied model for PPI disruption. When PPI is measured following injection of apomorphine (APO), a direct DA agonist, a similar decrease in PPI is seen as of those suffering from schizophrenia (Mansbach et al., 1988). This effect is only partially rescued by the typical neuroleptic haloperidol, a DA agonist (Koch, 1999). Amphetamine (AMP) is also used to induce PPI dysfunction similar to that of schizophrenic patients. However, AMP acts as a nonspecific indirect DA agonist (Mansbach et al., 1988). The agonist effects of APO and AMP support the hypothesis of DA hyperactivity in PPI disruption.

Phencyclidine (PCP), a non-competitive N-Methyl-D-aspartate (NMDA) receptor antagonist, has also been shown to disrupt PPI (Mansbach and Geyer, 1989). NMDA
receptors are glutamate receptors, implicating the glutamate system in the regulation of
PPI. In normal humans, PCP administration has been shown to induce psychosis and
amplify psychosis in schizophrenic patients (Pearlson, 1981).

Oxytocin and Schizophrenia

When used clinically as an antipsychotic, Oxt has been reported to have curative
properties; specifically in the areas of mood stabilization, anxiety and depression, apathy,
and sleep. Given these properties, Oxt has been studied as a therapeutic agent in patients
suffering from schizophrenia (Bakharev et al., 1986, Bujanow, 1974). As an atypical
neuropeptide, Oxt has a blocking effect on the 5-HT2-receptor, which has been
hypothesized to be the cause of its successful ability to normalize social behaviors in
schizophrenic patients. Two antipsychotics, clozapine and amperozide, increase levels of
central and peripheral Oxt in those being treated for schizophrenia (Uvnas-Moberg et al.,

Oxt has also been located, along with its receptors, in the nucleus accumbens and
the hippocampus, which are areas of the brain that are hypothesized to be involved in
schizophrenia (van Leeuwen et al., 1985). Patients suffering from the disorder have also
shown disturbed Oxt function (Linkowski et al., 1984, Beckmann et al., 1985, Mai et al.,
1993). Specifically, in a study by Beckmann et. al. (1985) schizophrenic patients showed
higher levels of Oxt in CSF samples than the normal control group. Levels of Oxt in CSF
also increase after neuroleptic treatment (Beckmann et al., 1985). Oxt neurophysins, part
of the preprooxytocin, were shown to be at abnormal levels in the CSF of schizophrenic
patients when compared to controls (Linkowski et al., 1984). Increasing knowledge about the relationship of Oxt and neuropsychiatric disorders has lead to research implicating Oxt as a therapeutic agent. In a recent study, Oxt has been shown to have a greater effect on ameliorating positive symptoms when compared to negative symptoms as demonstrated when intranasal Oxt was used therapeutically for schizophrenic subjects (Feifel et al., 2010).

In rats, subcutaneous injections of Oxt restored PPI disrupted by dizocilpine and AMP. Dizocilpine acts as a non-competitive NMDA agonist, thus allowing for a pharmacological examination of the contributions of glutamatergic pathways in schizophrenia. In contrast, AMP and APO treatment allows for a pharmacological examination of dopaminergic dysfunction in schizophrenic patients. Oxt’s restoration of PPI-deficits induced by dizocilpine and AMP suggest a role of Oxt in both the glutamatergic and dopaminergic pathways involvement in PPI, and its possible role as an antipsychotic (Feifel and Reza, 1999, Swerdlow et al., 1994).

Research by Caldwell and colleagues found that inducing a “psychotic-like” state in Oxt knockout (Oxt −/−) mice with PCP prior to PPI testing could be used to test the potential antipsychotic effects of Oxt. Their results were in agreement with their hypothesis as the Oxt −/− mice were more susceptible to the PPI-disrupting effects of PCP than the Oxt wildtype (Oxt +/+ ) mice (Caldwell et al., 2009). There has also been progress in creating a conditional knockout of the Oxtr in the forebrain of mice (Oxtr FB/FB). These mice avoid developmental effects of Oxtr inactivation due to post partum knockout of the receptor. Knockout is accomplished through the expression of
Ca\(^{2+}\)/calmodulin-dependent protein kinase II\(\alpha\) (Camk2a) promoter-driven Cre recombinase. Camk2a is only located in the forebrain, and thus only receptors in this area are subject to knockout. The difference in Oxtr presence in Oxtr wildtype (Oxtr +/+), Oxtr full body knockout (Oxtr −/−) and Oxtr FB/FB is shown in figure 2. This knockout model will allow studies to focus on the role of Oxt in the central nervous system, specifically in the forebrain (Lee et al., 2008). Using the Oxtr FB/FB mice, the work described in this thesis set out to determine if Oxt interacts with PPI circuitry in the forebrain.

**Objective**

The central hypothesis of this experiment was that Oxt signaling is important to the severity of the disruption of sensorimotor gating. Oxtr FB/FB mice provided the opportunity to narrow in on possible neural substrates where Oxt may be interacting with the PPI neural circuitry since the disruption of Oxt signaling is confined mostly to the forebrain (Lee et al., 2008). Thus, in this study we administered the psychotomimetics, PCP, APO, and AMP to Oxtr FB/FB mice, as well as control Oxtr +/+ mice and measured their effects on PPI. Using PPI to measure sensorimotor gating is a valuable tool in research for neuropsychiatric disorders as its neural circuitry is conserved across species. The work described in this thesis will hopefully add to our understanding of the role of Oxt in neuropsychiatric disorders that are known to exhibit PPI deficits, including schizophrenia.
Figure 2: Oxtr binding. Sagittal slices of three different mouse brains. Absence of black coloration indicates absence of Oxtr. Presence of black coloration indicates Oxtr location within the brain. (a.) Oxtr +/+ mouse (b.) Oxtr −/− mouse (c.) Oxtr FB/FB mouse
CHAPTER II

MATERIALS AND METHODS

Animals

In this experiment, a total of N=41 three to five month old Oxtr FB/FB and Oxtr +/+ mice were tested. Of those mice 17 were Oxtr +/+ mice (8 males and 9 females) and 18 were Oxtr FB/FB mice (12 males and 9 females). Six mice tested (3 males and 3 females) were determined not to be homozygous after being re-genotyped at the end of the study so only 35 mice (Oxtr FB/FB or Oxtr +/+ ) were included in the statistical analysis. The mice were tested in two separate cohorts, starting in the Fall 2010 semester and ending in the Spring 2011 semester. Mice were generated as described previously (Lee et al., 2008) with the original breeding pairs imported from the National Institutes of Health to the Kent State University Vivarium in Summer 2007.

After weaning, and through the duration of the experiment, mice were housed with same-sex littermates in groups of at least two and no more than five animals per cage. A 12:12 light/dark cycle (2am-2pm “lights on”) was observed, with food and water available ad libitum. On any given day, testing began during the end of the light phase, at approximately 12:30pm, and progressed into the dark cycle, until as late as 8pm. During testing sessions, the testing room lights were kept on. Thus, on days of testing, subjects were exposed to a prolonged light cycle. All procedures were approved by the Kent State University Institutional Animal Care and Use Committee.
**Drugs**

The drugs, used as well as their concentrations, were based on previous work (Caldwell et al., 2009). Drugs used were APO (10mg/kg), AMP (12mg/kg), and PCP (8mg/kg), and are summarized in Table 1. The control injection was a sterile saline solution of 0.9% NaCl. This saline solution was given in the same volume as APO and AMP as a control and also used to dilute the drugs. APO and AMP were diluted to 1.5mg/kg and PCP to 1.2mg/ml. All drugs, including the control saline solution, were kept on ice and injected intraperitoneally (i.p.) into the lower abdomen of the mouse.

**Prepulse Inhibition of the Startle Reflex**

SR-LAB startle chambers (San Diego Instruments, San Diego, CA) were used to measure the whole-body startle reflex of the mice in response to an acoustic stimulus. For the first cohort only one chamber was used. For the second cohort two chambers were used. When two chambers were used they were calibrated to one another, so for all intensive purposes they were identical. For each session the house light within the chamber was kept on.

Each chamber is composed of a clear plexiglas tube attached to a larger plexiglass stage piece as seen in Figure 3. This apparatus is known as the “animal enclosure.” A piezoelectric unit is attached directly to the bottom of the animal enclosure. Acoustic stimulus is emitted from a loudspeaker within the chamber. The vibrations (startle) sensed and transduced by the piezoelectric unit were converted into arbitrary units by the potentiometer and stored on a computer.
Table 1: Drug table. All drugs were administered intraperitoneally (i.p.). Doses were selected based on previous work (Abbreviations: PCP, phencyclidine; APO, apomorphine; AMP, amphetamine)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>N/A</td>
<td>i.p.</td>
</tr>
<tr>
<td>PCP</td>
<td>8mg/kg</td>
<td>i.p.</td>
</tr>
<tr>
<td>APO</td>
<td>10mg/kg</td>
<td>i.p.</td>
</tr>
<tr>
<td>AMP</td>
<td>12mg/kg</td>
<td>i.p.</td>
</tr>
</tbody>
</table>
Figure 3: Acoustic startle chamber (SR-LAB; San Diego). The animal enclosure, piezoelectric unit, and loudspeaker (source of acoustic startle) have been labeled. Two identical chambers were used.
Each PPI test consisted of sixty trials and lasted approximately 20 minutes. Five trial types were presented: no acoustic stimulus, which is when only background noise was presented, 120dB pulse, a 40-ms 120 dB pulse, and a 77dB PREPULSE, a 80 dB PREPULSE, and a 86dB PREPULSE, all of which are a 20-ms prepulse of the appropriate dB level followed 100-ms later by a 40-ms 120dB pulse. The block of five trials were presented ten times. The sessions always started and ended with a block of five PULSE alone trials. A background noise was present throughout the test at 68dB. Prior to the start of each testing session there was a 5-minute acclimation time with the animal in the chamber.

Procedure

Each cohort was tested over the course of five weeks as summarized in Figure 4. In week 1, the baseline was determined for the animals with no drug treatment administered. Mice of each cohort were then separated into one of two groups. Mice in the first group were only tested on Mondays and those in the second group only tested on Wednesdays. Thus, there was always a one week wash-out between each test for all animals during the five-week test period. Prior to testing mice were taken to the testing room and allowed to acclimate for one hour.

In weeks 2-5 animals were injected with either: 1) Saline, 2) PCP, 3) APO, or 4) AMP in a counterbalanced order, meaning that the order of the injections differed across animals. The amount injected for each mouse was based on their weight. After the syringe was prepared the mouse was scruffed and the injection delivered. Following
Figure 4: Experiment timeline. (a.) Timeline of testing session beginning with a one-hour room acclimation period. This was followed by the drug injection, except for baseline, and a five-minute wait until the mouse was moved to the chamber. Once in the chamber there was a five-minute acclimation to chamber preceding the 60-trial prepulse session. A one-week washout occurred between all testing sessions. (b.) Timeline of experiment beginning with baseline week. There was one week between each injection/test session. If necessary retests followed the washout after week 4.
injection the mouse was temporarily housed in a new cage for three minutes. During this

time identifying data was entered into the computer to set-up for the session. The mouse

was then placed into the chamber by holding the tail and orienting towards the animal

enclosure. Thus, approximately five minutes after the injection the testing session was

initiated.

After testing the mouse was returned to a clean cage with the same littermates it

was housed with before the session. Between sessions the chamber was cleaned with

germicidal disposable cloths (PDI Sani-Cloth Plus, Professional Disposable International

Inc., Orangeburg, NY). Fumes and moisture from the wipes made a five-minute waiting

period necessary before the next animal could be placed in the chamber. If re-tests were

needed they were made at least one week after the five-week experiment period on

Monday/Wednesday depending on what day the mouse was previously tested on.


Data Analysis

Due to differences in responses to startle the sexes were analyzed separately.

Habituation of startle was calculated using the average responses to the first block (P1-
Pulse 1) and last block (P2-Pulse 2) of five pulse alone (120dB) trials. Percent prepulse

inhibition for each level (77dB, 80dB, 86dB) was calculated using the formula %PPI

(level)=(1-(PPI (level)/PPI120dB)) x100. Average PPI was calculated by taking the

average of the percent PPI of the three levels. Startle was calculated using the average

response to the 120db pulse alone trials within the 10 trial blocks, excluding the first and
last blocks of five. These calculations are represented in Figure 5. A repeated measure analysis of variance (ANOVA) was used to make comparisons within the data. When the within drug effects on the startle response were analyzed, the $\alpha$ was adjusted to 0.013. For all other comparisons the $\alpha$ was 0.05.
**Figure 5: Experimental design.** Used to examine startle amplitude, habituation of startle, and PPI percentage in mice.
CHAPTER III
RESULTS

Females

Habituation of Startle

Within each drug treatment, there was no main effect for saline, PCP, or APO. However, there was a main effect of AMP \( (F_{1,15}=8.343, p<0.013) \). There were no main effects of genotype or any other interactions on habituation of startle (Figure 6).

Startle Amplitude

There was a main effect of the drug treatment on the startle amplitude \( (F_{3,45}=25.612, p<0.05) \). Specifically, PCP increased the startle amplitude while APO/AMP decreased the startle, all relative to saline. There was no main effect of genotype, or any interactions (Table 2).

PPI Percentage

Baseline

There was no main effect of genotype on the baseline PPI percentage. As expected there was a main effect of prepulse intensity \( (F_{2,30}=24.855, p<0.05) \), with the percent PPI increasing with increased prepulse tone (Figure 7).
Figure 6: Habituation to startle in female Oxtr +/+ and Oxtr FB/FB mice. There was habituation of startle to AMP (12mg/kg) in Oxtr +/+ and Oxtr FB/FB female mice. There was no habituation of startle to the other drugs; Saline (0.9%), APO (10mg/kg), and PCP (8mg/kg). Data are expressed as mean + standard error of the mean. (Abbreviations: PCP, phencyclidine; APO, apomorphine; AMP, amphetamine)
Table 2: Startle amplitude in female Oxtr +/+ and Oxtr FB/FB mice. There was an effect of drug treatment (PCP, APO, AMP) on startle amplitudes as compared to saline. PCP increased the startle amplitude while APO and AMP decreased the amplitude. (Abbreviations: PCP, phencyclidine; APO, apomorphine; AMP, amphetamine)

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Oxtr +/+</th>
<th>Oxtr FB/FB</th>
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<tbody>
<tr>
<td>Saline</td>
<td>39.52 ± 4.53</td>
<td>39.94 ± 4.48</td>
</tr>
<tr>
<td>PCP</td>
<td>58.03 ± 9.55</td>
<td>55.28 ± 8.79</td>
</tr>
<tr>
<td>APO</td>
<td>19.98 ± 4.23</td>
<td>15.08 ± 4.08</td>
</tr>
<tr>
<td>AMP</td>
<td>25.06 ± 3.23</td>
<td>33.09 ± 6.36</td>
</tr>
</tbody>
</table>
Figure 7: Baseline PPI percentage in female Oxtr +/- and Oxtr FB/FB mice. There was no genotypic difference in baseline PPI percentage but there was a main effect of prepulse intensity with the higher decibel prepulse tones resulting in greater PPI percentage. Data are expressed as mean + standard error of the mean. (* indicates significance of p<0.05)
**Drug Treatment**

There was a main effect of the drug treatment ($F_{3,45}=12.153, p<0.05$) with PCP, APO and AMP all resulting in disruption of PPI compared to saline. There was also a main effect of prepulse intensity ($F_{2,30}=27.881, p<0.05$). However, there was no main effect of genotype in the females on PPI percentage or any other interactions (Figure 8) (Table 3).

**Males**

*Habituation of startle*

Within each drug treatment, there was a main effect for saline ($F_{1,15}=12.490, p<0.013$), APO ($F_{1,15}=12.618, p<0.013$), and AMP ($F_{1,15}=21.758, p<0.013$). There was no main effect of PCP, genotype, or any other interactions on habituation of startle (Figure 9).

*Startle amplitude*

There was a main effect of drug treatment ($F_{3,45}=21.510, p<0.05$) on startle amplitude. Specifically, PCP increased the startle amplitude while APO/AMP decreased the startle, all relative to saline. There were no main effects of genotype or any other interactions (Table 4).
Figure 8: PPI percentage in female Oxtr +/+ and Oxtr FB/FB mice following drug treatment. Treatment with all three drugs (PCP, APO, AMP) disrupted PPI as compared to saline. Prepulse intensity also had a main effect, increasing the startle amplitude as the decibel of the prepulse increased. There was no genotypic difference between the female Oxtr +/+ and Oxtr FB/FB mice. Data are expressed as mean + standard error of the mean. (Abbreviations: PCP, phencyclidine; APO, apomorphine; AMP, amphetamine)
### Table 3: PPI Percentage in female Oxtr +/+ and Oxtr FB/FB mice. PPI percentage (means ± standard error of the mean) for drug treatments in female Oxtr +/+ and Oxtr FB/FB mice. (Abbreviations: PCP, phencyclidine; APO, apomorphine; AMP, amphetamine)

<table>
<thead>
<tr>
<th>Drugs &amp; Prepulse Level</th>
<th>Oxtr +/+</th>
<th>Oxtr FB/FB</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline 77</td>
<td>76.69 ± 5.27</td>
<td>78.16 ± 3.25</td>
<td></td>
</tr>
<tr>
<td>Saline 80</td>
<td>78.57 ± 3.11</td>
<td>81.48 ± 2.59</td>
<td></td>
</tr>
<tr>
<td>Saline 86</td>
<td>84.92 ± 2.16</td>
<td>84.22 ± 1.85</td>
<td></td>
</tr>
<tr>
<td>PCP 77</td>
<td>67.15 ± 6.61</td>
<td>57.60 ± 7.37</td>
<td></td>
</tr>
<tr>
<td>PCP 80</td>
<td>71.41 ± 8.89</td>
<td>71.34 ± 6.08</td>
<td></td>
</tr>
<tr>
<td>PCP 86</td>
<td>80.96 ± 5.46</td>
<td>77.29 ± 4.78</td>
<td></td>
</tr>
<tr>
<td>APO 77</td>
<td>54.91 ± 5.96</td>
<td>36.07 ± 11.62</td>
<td></td>
</tr>
<tr>
<td>APO 80</td>
<td>54.85 ± 7.14</td>
<td>37.15 ± 11.58</td>
<td></td>
</tr>
<tr>
<td>APO 86</td>
<td>58.17 ± 6.35</td>
<td>53.86 ± 10.70</td>
<td></td>
</tr>
<tr>
<td>AMP 77</td>
<td>55.60 ± 1.86</td>
<td>46.21 ± 7.60</td>
<td></td>
</tr>
<tr>
<td>AMP 80</td>
<td>62.57 ± 4.08</td>
<td>51.28 ± 9.51</td>
<td></td>
</tr>
<tr>
<td>AMP 86</td>
<td>66.60 ± 4.72</td>
<td>60.37 ± 7.46</td>
<td></td>
</tr>
</tbody>
</table>
Figure 9: Habituation of startle in male Oxtr +/+ and Oxtr FB/FB mice. There were main effects of three of the drugs; Saline (0.9%), APO (10mg/kg), and AMP (12mg/kg) on habituation of startle on Oxtr +/+ and Oxtr FB/FB. There was no main effect of PCP (8mg/kg). Data are expressed as mean + standard error of the mean. (Abbreviations: PCP, phencyclidine; APO, apomorphine; AMP, amphetamine)
<table>
<thead>
<tr>
<th>Drugs</th>
<th>Oxtr +/+</th>
<th>Oxtr FB/FB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>68.80 ± 11.01</td>
<td>68.67 ± 12.02</td>
</tr>
<tr>
<td>PCP</td>
<td>104.96 ± 16.81</td>
<td>69.76 ± 10.23</td>
</tr>
<tr>
<td>APO</td>
<td>33.91 ± 6.52</td>
<td>22.54 ± 4.89</td>
</tr>
<tr>
<td>AMP</td>
<td>38.49 ± 9.85</td>
<td>32.27 ± 5.60</td>
</tr>
</tbody>
</table>

**Table 4: Startle amplitude in male Oxtr +/+ and Oxtr FB/FB mice.** There was an effect of drug treatment (PCP, APO, AMP) on startle amplitudes as compared to saline. PCP increased the startle amplitude while APO and AMP decreased the amplitude. (Abbreviations: PCP, phencyclidine; APO, apomorphine; AMP, amphetamine)
**PPI Percentage**

**Baseline**

There was a main effect of prepulse intensity ($F_{2,30}=11.765$, $p<0.05$) with increasing prepulse tone resulting in increased PPI percentage. There was no effect of genotype on the baseline PPI percentage (Figure 10).

**Drug Treatment**

There was a main effect of drug treatment ($F_{3,45}=14.964$, $p<0.05$) with PCP, APO, and AMP all resulting in disruption of PPI when compared to saline. There was also an effect of prepulse intensity ($F_{2,30}=11.791$, $p<0.05$). There was no main effect of genotype in the males on PPI percentage or any other interactions (Figure 11) (Table 5).
Figure 10: Baseline PPI percentage in male Oxtr +/+ and Oxtr FB/FB mice. There was no genotypic difference in baseline PPI percentage but there was a main effect of prepulse intensity. Greater decibel tones resulted in greater disruption of PPI. Data are expressed as mean + the standard error of the mean. (* indicates significance of p<0.05)
Figure 11: PPI percentage of male Oxtr +/+ and Oxtr FB/FB mice following drug treatment. Treatment with PCP, APO, and AMP resulted in disruption of PPI as compared to saline. A main effect of prepulse intensity showed that increasing decibel of prepulse resulted in an increase of PPI percentage. There was no main effect of genotype or any other interactions. Data are expressed as mean + the standard error of the mean. (Abbreviations: PCP, phencyclidine; APO, apomorphine; AMP, amphetamine)
<table>
<thead>
<tr>
<th>Drugs</th>
<th>Oxtr +/-</th>
<th>Oxtr FB/FB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline 77</td>
<td>84.43 ± 3.52</td>
<td>83.31 ± 3.01</td>
</tr>
<tr>
<td>Saline 80</td>
<td>87.20 ± 2.40</td>
<td>86.86 ± 2.17</td>
</tr>
<tr>
<td>Saline 80</td>
<td>86.59 ± 2.66</td>
<td>88.85 ± 2.21</td>
</tr>
<tr>
<td>PCP 77</td>
<td>64.69 ± 7.16</td>
<td>67.80 ± 5.79</td>
</tr>
<tr>
<td>PCP 80</td>
<td>72.20 ± 8.14</td>
<td>74.49 ± 4.52</td>
</tr>
<tr>
<td>PCP 86</td>
<td>76.61 ± 9.24</td>
<td>82.62 ± 3.42</td>
</tr>
<tr>
<td>APO 77</td>
<td>63.03 ± 5.06</td>
<td>59.70 ± 4.03</td>
</tr>
<tr>
<td>APO 80</td>
<td>65.78 ± 8.24</td>
<td>63.76 ± 2.80</td>
</tr>
<tr>
<td>APO 86</td>
<td>66.39 ± 5.71</td>
<td>63.79 ± 5.49</td>
</tr>
<tr>
<td>AMP 77</td>
<td>46.35 ± 5.60</td>
<td>55.15 ± 6.77</td>
</tr>
<tr>
<td>AMP 80</td>
<td>51.92 ± 6.27</td>
<td>68.42 ± 5.49</td>
</tr>
<tr>
<td>AMP 86</td>
<td>64.66 ± 4.47</td>
<td>67.67 ± 8.16</td>
</tr>
</tbody>
</table>

Table 5: PPI Percentage in male Oxtr +/- and Oxtr FB/FB mice. PPI percentage (mean ± standard error of the mean) for drug treatments: PCP (8mg/kg), APO (10mg/kg), AMP (12mg/kg) on male Oxtr +/- and Oxtr FB/FB mice. (Abbreviations: PCP, phencyclidine; APO, apomorphine; AMP, amphetamine)
CHAPTER IV

DISCUSSION

The experiment described in this thesis has added to our knowledge of the role of Oxt in sensorimotor gating deficits. It has already been established that Oxt is important to the neural circuits that regulate PPI (Caldwell et al., 2009, Feifel and Reza, 1999). To narrow in on where in the brain this might be occurring, we used Oxtr FB/FB mice. Interestingly, we found that there were no genotypic differences in the disruption of PPI between Oxtr +/- and Oxtr FB/FB mice after administration of psychotomimetic drugs as compared to saline; these results were consistent for male and female mice.

Baseline for male and female mice both showed a significant (p<0.05) main effect of prepulse intensity. The three prepulse tones (77dB, 80dB, 86dB) gave rise to different levels of PPI percentage, i.e., the greater the dB of the tone the greater the % PPI. There was however no main effect of genotype. The presence of normal increases in %PPI with increasing prepulse tone amplitude suggests that the parameters of the test itself were working.

Drug treatment affected the startle amplitude, with PCP increasing the startle amplitude, and APO and AMP decreasing the startle amplitude. These effects of the drugs on startle amplitude are consistent with previous work (Breier et al., 2010, Yee et al., 2004, McFadden et al., 2010). Given the pharmacology of PCP as a non-competitive NMDA receptor antagonist, the increase in startle caused by the PCP was expected (Yee
et al., 2004). Prior studies also show that APO and AMP have decreasing effects on startle amplitude (Breier et al., 2010, McFadden et al., 2010).

Habituation of the startle reflex occurs when there is a significant reduction of measured startle from the beginning five blocks of pulse alone trials to the ending five blocks (Yee et al., 2004). The effects of drug treatment on the habituation of startle differed between male and female mice, although again no genotypic effect was observed. For female mice there was habituation after administration of AMP (p<0.013); with the α adjusted to 0.013 since the effects of the drugs were analyzed separately. Male mice experienced habituation with administration of saline, APO, and AMP (p<0.013). While habituation and startle amplitude are measured, they do not directly coincide with PPI, rather they work through different pathways. Therefore any significance in their values does not detract from the significance of the percentage of PPI as they do not act within the same neural circuits (Swerdlow et al., 2001).

For the percent PPI, following treatment with psychotomimetics, there were main effects of prepulse intensity and drug treatment (p<0.05) for both male and female mice. The effects of prepulse intensity were the same as described above for baseline. For the drug treatments, all of the psychotomimetics administered, i.e., PCP, APO, and AMP, disrupted PPI when compared to saline.

The significance of this work is it helps to narrow in on possible neural substrates where Oxt may interact with the PPI neural circuitry. Oxt −/− mice, which have no Oxt peptide throughout the entire body, are more susceptible to the PPI-disrupting effects of PCP (Caldwell et al., 2009). Further, work in Dr. Caldwell’s lab has also shown that Oxtr
−/− mice, that are missing the Oxtr throughout their body, are also more susceptible to the PPI-disrupting effects of PCP (Caldwell, unpublished data). Thus, it appears that following treatment with the psychotomimetic PCP, that Oxt interacts with the PPI neural circuitry. In this study the use of Oxtr FB/FB mice limited the knockout of the Oxtr to the forebrain. Mice without functional Oxtr in the forebrain means that they have Oxt peptide present, but that Oxt cannot act in the forebrain through its receptors. By using these mice the interaction of Oxt in the forebrain in the PPI pathway could be determined. Given that there was no genotypic difference, it can be concluded that Oxt does not impact the PPI circuitry in the forebrain.

Pathways involved in sensorimotor gating, and thus PPI, are still under study due to their complicated nature. Although the forebrain has been named as a central location for the PPI pathway, there are also many other brain areas involved (Koch, 1999, Koch and Schnitzler, 1997, Swerdlow et al., 2001). The results of this study suggest that Oxt is not acting in the forebrain to affect the sensorimotor gating neural circuitry. This is particularly interesting, because Oxt’s regulation of social cognition, such as social memory, is known to be forebrain mediated (Kirsch et al., 2005, Domes et al., 2007). Thus, the findings of this work suggest that the modulation of PPI by Oxt following treatment with PCP may not be the same Oxt signaling that is important to social cognition.

Narrowing in on specific neural substrates where Oxt interacts with the PPI neural circuitry will be important in future studies. With this study’s elimination of forebrain neuroanatomical areas, it will now be appropriate to conduct experiments focusing on
different brain areas. Such experiments could take place by knocking out the Oxtr in other specific brain areas. The Caldwell Lab (Kent State University) is planning to continue research in this direction with the use of Oxtr −/− mice in combination with measures of immediate early gene activation, e.g. c-fos, following treatment with PCP. By identifying brain areas in which immediate early genes are activated after treatment with psychotomimetics it would be possible to hone in on brain areas where Oxt may interact with the PPI neural circuitry. Use of small inhibitory RNA to knockdown Oxt or Oxtr mRNA combined with pharmacological treatments, such as Oxtr antagonists, could further pinpoint the location of Oxt interaction with PPI circuitry.

Several studies have suggested that Oxt may have antipsychotic properties (Feifel and Reza, 1999, Caldwell et al., 2009, Uvnas-Moberg et al., 1992, Feifel et al., 2010, Bakharev et al., 1986, Bujanow, 1974). While mouse knockouts for Oxt are basically indistinguishable from wildtype mice in baseline measures of PPI, the induction of a “psychosis-like” state, following treatment with psychotomimetics, reveals the deficits that are present in the knockout mice. This is consistent with the idea that under conditions of psychosis that Oxt may improve symptom severity. This idea is supported by a study that found that intranasal administration of Oxt decreased overall symptom severity when given in coordination with antipsychotics (Feifel et al., 2010). Though, further testing in humans is needed to ascertain the effects of long term Oxt therapy.

Since Oxt may be helpful to the treatment of schizophrenia it is important to understand how it works to affect the symptoms associated with schizophrenia. This is why identification of the pathways, specifically the PPI neural circuitry, is important to
research involving schizophrenia. The positive and cognitive aspects of the disease have been shown to have a relationship with Oxt, mostly in the severity of these symptoms (Goldman et al., 2008, Feifel et al., 2010, Keri et al., 2009). However, PPI studies, including this one will be important to improve our understanding of the role of Oxt in the neural underpinnings of cognition. So, while results of this experiment may not be specific to schizophrenia, they can certainly be applied to the disease. Work in this area holds the promise to improve our knowledge of one neurohormone that may be important to the severity of schizophrenic symptoms.
REFERENCES


variability and evidence for altered staining patterns in schizophrenia. *J Hirnforsch*, **34**: 133-54.


