The Perinatal Domoate Rat Model: Alterations in Behaviors Mediated by the Mesocorticolimbic Pathway.

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Melissa A. Burt

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Abstract

Glutamate is the primary excitatory amino acid neurotransmitter in the mammalian central nervous system. Glutamate is also critically important in the development of the central nervous system, and in order for normal development to occur, an optimal level of glutamate signalling is needed. Previous studies in our lab have demonstrated that an alteration in the glutamatergic system, through low non-convulsive doses of domoic acid (DOM) and kainic acid (KA) (selective for kainate receptors, a distinct class of glutamate receptors), administered chronically (s.c.) to rats during a critical period of brain development, caused permanent neuroanatomical, neurochemical and behavioural alterations. Such changes include an altered response to a novel, spatial environment, and alterations in hippocampal morphology. The altered response in a novel environment suggests that the rats treated with DOM in these studies had an alteration in systems modulating response to novelty. The dopaminergic system is important in modulating response to both novelty and reward. Glutamate signalling, through kainate receptors, has been shown to modulate dopamine release in the mesocorticolimbic dopamine pathway, and if expression of these receptors is altered by administering DOM, then the result could be alterations in response to novelty and reward. Therefore, the objective of this thesis was to examine the effects of similar low doses of DOM (20μg/kg), administered during a critical period of brain development when the system is undergoing rapid change (postnatal day 8-14), on behaviours specifically designed to assess responses to reward and novelty. It is known that systems undergoing rapid change are the most susceptible to insult, therefore such treatment could result in permanent change to the functional integrity of the nervous system, rather than just producing transient effects.

The first set of experiments involved the administration of 20μg/kg of DOM to rat pups, over PND 8-14. The rats were then assessed as juveniles and adolescents in the open field. During adulthood, rats were tested in an open field, a sucrose consumption task, the playground maze and in a nicotine-induced conditioned place preference (CPP) paradigm. Activity levels were altered in the DOM-treated rats in open field behavior at each time point assessed. In the playground maze the DOM-treated male rats had an altered response to novelty, as indicated by an increase in time spent exploring objects during the novelty trial of the playground maze. This was not found in DOM-treated female or control rats. The DOM-treated female rats had an increased sensitivity to nicotine in the nicotine-induced CPP paradigm, with a conditioning to nicotine as indicated by a preference for the nicotine-paired compartment of the test arena. This preference was maintained for at least a month following the final drug-compartment pairing.

In the second set of experiments, 20 μg/kg of DOM was administered to rat pups over PND 8-14. The rats were assessed in the nicotine-induced CPP paradign, during late adolescence. Peri-adolescence is a period of increased sensitivity to the rewarding properties of nicotine, and a period of maturation for the mesocorticolimbic pathway. In the DOM-treated rats, there was a decrease in the sensitivity to nicotine in the nicotine-induced CPP, compared to the saline control rats. The DOM-treated rats failed to manifest the age-appropriate preference for the nicotine-

paired compartment.

The results of these studies demonstrate the effects of early DOM exposure on the emergence of behaviors mediated by the mesocorticolimbic pathway, specifically activity levels, response to the rewarding properties of nicotine and response to novelty. These differences are also gender related, and manifested differently at differing stages of development. These results are discussed in light of the putative mechanism of initial KA receptor mediated activation and the link to the DA mesocorticolimbic system.

Table of Contents

List of Figures

List of Tables

Abbreviations

Abbreviation	Term
7-OH-DPAT	7-hydroxy-N,N-di-n-propyl-2-aminotetralin
ACh	Acetylcholine
ADHD	Attention deficit hyperactivity disorder
AMPA	Alpha-amino-3-hydroxy-5methylisoxazole-4-propionte
BDNF	Brain derived neurotrophic factor
cAMP	Cyclic-Adenosine monophosphate
CDP	cytidine diphosphate
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CNS	Central Nervous System
COMT	Catechol-o-methyltransferase gene
CPP	Conditioned place preference
CR	Conditioned response
CS	Conditioned stimulus
DA	Dopamine
DAT	Dopamine transporter
DISC1	Disrupted in schizophrenia-1 gene
DOM	Domoate or Domoic Acid
DTNBP1	Dysbindin-1 gene

Abbreviation	Term
FAS	Fetal alcohol syndrome
GABA	Gamma-aminobutyric acid
Glu	Glutamate
HR	High-risk
IL-8	Interleukin-8
i.p.	Interperitoneal
KA	Kainate or Kainic Acid
LI	Latent inhibition
LPS	Lipopolysaccharide
mGluR	Metabotropic glutamate receptors
mPFC	Medial prefrontal cortex
MWM	Morris water maze
NAc	Nucleus accumbens
nAChR	Nicotinic acetylcholine receptors
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f) quinoxaline
NIS-L	Novelty induced seizure-like
NMDA	N-methyl-D-aspartate
NRG1	Neuroregulin-1 gene
nVH	Neonatal ventral hippocampal lesioned
PCP	Phencyclidine

Abbreviation	Term
PFC	Prefrontal cortex
PND	Postnatal day
Poly I:C	polyriboinosinic polyribocytidylic acid
PPI	Pre-pulse inhibition
QA	Quisqualate
RGS4	Regulator of G-protein signaling-4 gene
SAL	Saline
s.c.	Subcutaneous
SD	Sprague-Dawley
US	Unconditioned stimulus
VTA	Ventral tegmental area

Preface

The excitatory amino acid neurotransmitter glutamate, is critically important in the development of the central nervous system, and in order for normal development to occur, an optimal level of glutamate signalling is needed. Previous studies in our lab have revealed that early exposure to low doses of domoic acid (DOM) a naturally occurring glutamate analogue, produces changes in behavioural and neuroanatomical measures of maturation in the rat. Based on known sites of action for DOM, these long term changes are speculated to be mediated initially through activation of kainate (KA) receptors, a glutamate receptor (GluR) subtype, These studies have demonstrated that an alteration in the glutamatergic system, through low non-convulsive doses of DOM or kainic acid (KA) (selective for kainate receptors), administered chronically to rats during a critical period of brain development, caused permanent neuroanatomical, neurochemical and behavioural alterations. Such effects included behavioural changes suggestive of an altered response to novelty and /or a novel spatial environment, and alterations in hippocampal morphology. The altered response in a novel environment suggests that, the rats treated with DOM, had an alteration in systems modulating response to novelty. The dopaminergic system is important in modulating response to both novelty and reward . Glutamate signalling, through kainate receptors, has been shown to modulate dopamine (DA) release in the mesocorticolimbic DA pathway, and if expression of these receptors is altered by administering DOM, then the result could be alterations in response to novelty and reward. Interestingly, neurodevelopmental disorders such as schizophrenia have been linked to both DA and Glu dysfunction. Understanding a link between early modification of glutamate signalling (putatively via KA

receptor activation)and long term behavioural changes in novelty and reward behaviours (mediated via DA signalling) will aid in furthering our understanding of behavioural and neurological changes that may contribute to the development of schizophrenia.

Based on these past observations, the primary hypothesis of this thesis was that DOM exposure would produce long lasting behavioural changes in specific responses to novelty and reward. These behaviours served as behavioural assays to evaluate the functional integrity of the mesocorticolimbic DA pathway. The secondary hypothesis of this thesis was to determine, based on the outcome of these behavioural assays, whether the peri-natal DOM rat model has potential as an animal model of schizophrenia. Therefore, a series of experiments were designed to test the hypotheses.

Chapter 2 "Determining the length of retention for object memory using the playground maze: A pilot study" was a pilot study conducted to determine whether the playground maze methodology could be replicated in our laboratory, producing data similar to that reported in the literature. The playground maze is a relatively new behavioural test used to assess response to novelty in the rat, and it was important to determine the reliability of the maze, as the novelty assessment was central to the thesis. Using this maze, we also tested different retention intervals as a potential variable to be manipulated in the subsequent study. This also allowed us to assess the relative value of the objects used in future testing, verifying that the novel object was not intrinsically more interesting than the familiar objects. Therefore, we were able to replicate the procedure for the maze, and determine the appropriate objects for later use in the maze (Chapter 3).

Chapter 3 "Drug-seeking and altered responses to novelty in adult rats treated neonatally with domoic acid" was designed to directly assess the primary hypotheses. Low doses of DOM were administered, from post natal day (PND) 8-14, and assessment of both developmental measures, and behaviours during adulthood, were conducted. The primary behavioural tests used in the study were; 1) the sucrose consumption test (to assess response to reward), 2) the playground maze (to assess response to novelty), and 3) the nicotine-induced conditioned place preference (CPP) paradigm (to assess response to pharmacological reward). The tests were conducted during adulthood, to determine if there were any permanent alterations in response to novelty and reward.

Chapter 4 "Low dose domoic acid in neonatal rats abolishes nicotine induced conditioned place preference during late adolescence" was designed to further investigate the nicotine induced CPP. This study was a specific follow-up based on the finding reported in Chapter 3, of an altered nicotine CPP in the adult DOM females. Low doses of DOM were administered during PND 8-14, and developmental measures were assessed. The nicotine-induced CPP was conducted during late adolescence, to determine whether there was a developmental difference in the DOM rats, with respect to the rewarding properties of nicotine. This was important, as the there is a developmental shift in the normal response to this maze and the DOM-treated rats had demonstrated differences in behaviours during different stages of development in previous studies.

The series of experiments outlined above allow for a thorough assessment of the primary hypotheses of this thesis, that DOM exposure would produce long lasting behavioural changes in specific responses to novelty and reward. These results are then evaluated with respect to the relevance of this DOM rat model to existing animal models and clinical symptoms of schizophrenia.

Chapter 1: General Introduction

1. Introduction

1.1. The Glutamate System

Glutamate (Glu) is an amino acid and is the most abundant excitatory neurotransmitter in the mammalian central nervous system (CNS) (Ozawa et al., 1998). Glutamate signaling underlies many of the normal neurological processes within the brain, and a majority of the neurons are excited by Glu (McDonald & Johnston, 1990). Glutamate is also important in shaping the neuronal circuitry involved in learning and memory throughout an organisms life span (Ozawa et al., 1998). However, excessive levels of Glu, and related compounds, are also implicated in neuronal pathogenesis and disease such as Huntington's disease, Parkinson's disease, Alzhiemer's disease, epilepsy and ischemia (Bettler & Mulle, 1995). Optimal levels of Glu signaling are also needed for normal CNS development, as it plays an important role in many physiological processes that underlie CNS maturation, including neuronal survival, dendritic and axonal structure, synaptogenesis and synaptic plasticity (McDonald & Johnston, 1990). However, while an optimal level of Glu is needed for normal CNS development, excessive Glu can have excitotoxic effects that result in abnormal development, neuronal injury and even cell death, while too little Glu can impede development and also have detrimental effects (McDonald & Johnston, 1990).

1.1.1. Glutamate Receptor Classification

Glutamate functioning is mediated by a family of receptors which include both ionotropic and metabotropic receptors (Bleakman & Lodge, 1998; Ozawa et al., 1998). The ionotropic receptors are further subdivided into N-methyl-D-aspartate (NMDA) receptors and non-NMDA recptors. The non-NMDA receptors are alpha-amino-3-hydroxy-5methylisoxazole-4-propionate (AMPA) and kainate (KA) receptors (Bettler & Mulle, 1995; Bleakman & Lodge, 1998) (see Figure 1.1). The terms ionotropic and metabotropic originated in an article by Eccles and McGeer (1979) and described the two major forms of neurotransmission. Ionotropic transmission allows ions to cross the membrane of the neuron, through the ion channel and produces rapid and relatively large conductance changes in the post-synaptic membrane. In contrast, metabotropic transmission stimulates cyclic nucleotide production or activates/inhibits other second messenger cascades (Ozawa et al., 1998) which, in turn, cause metabolic changes in the post-synaptic neuron; these effects are slow mediating (Eccles and McGeer, 1979).

1.1.2. Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluR) are coupled to G-proteins which in turn, are linked to various effector systems such as phospholipase C, adenylate cyclase and ion channels (Schoepp et al., 1999). The mGluR subtypes are divided into three groups based on their degree of shared sequence homology and the G-proteins to which they are coupled. Group 1 includes mGluR1 and mGluR5. Group 2 includes mGluR2 and mGluR3. Group 3 includes mGluR4, mGluR6,

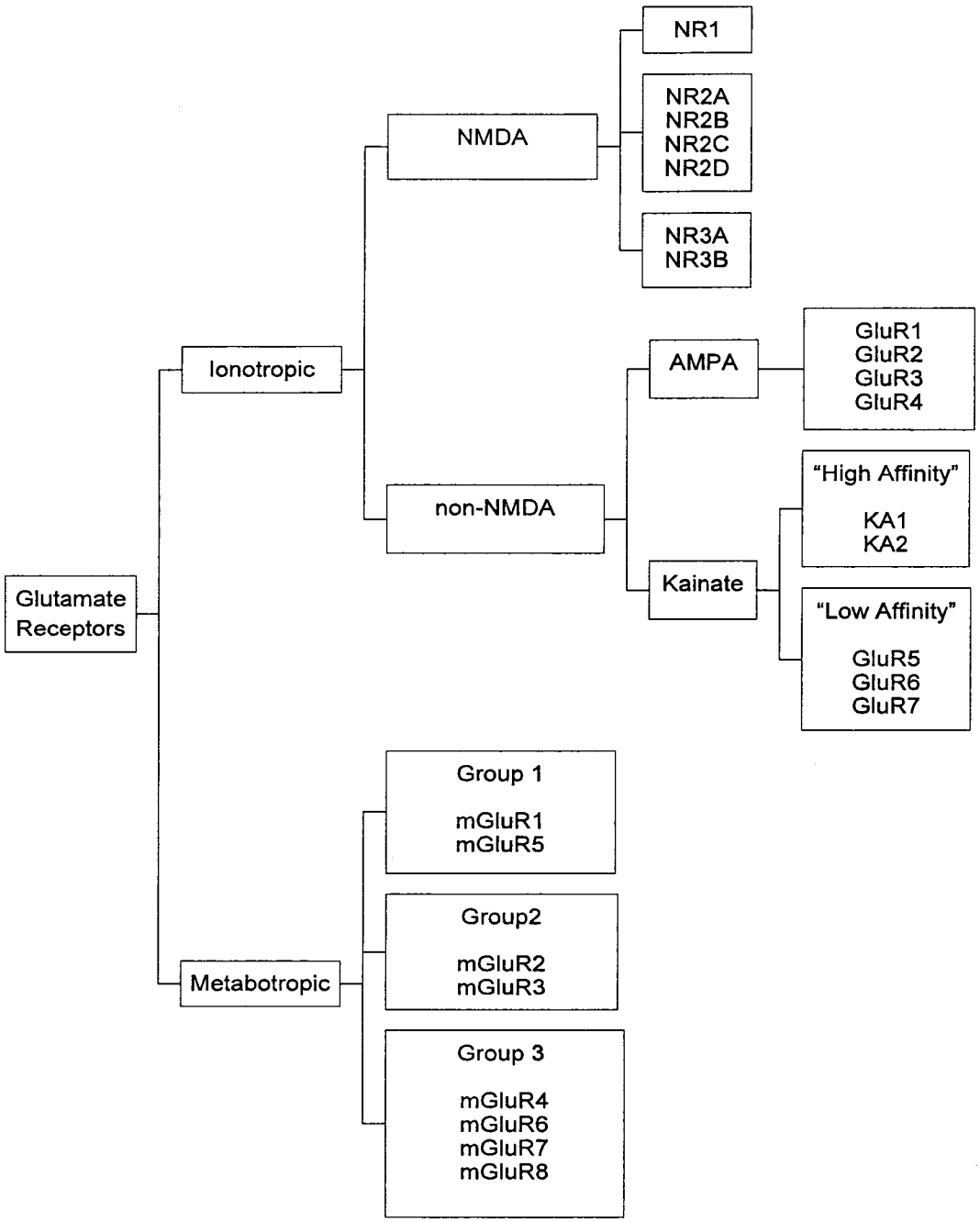


Figure 1.1. Glutamate receptor classification. Adapted from Bettler & Mulle, 1995; Bleakman & Lodge, 1998; Kew & Kemp, 2005; Lerma et al., 2001; Ozawa et al., 1998; Schoepp et al., 1999.

mGluR7, and mGluR8 (Schoepp et al., 1999) (see Figure 1.1). The mGluR family of receptors generally exert a modulatory role, regulating neuronal excitability and synaptic transmission. The group 1 mGluRs are predominately located post-synaptically in somatodentric domains, whereas the group 2 and 3 mGluRs are located predominately pre-synaptically on axon terminals and in axon domains (Kew & Kemp, 2005).

1.1.3. NMDA Receptors

NMDA receptors are both voltage- and ligand-gated cation channels. They are considered voltage-gated because the channel pore is blocked by a magnesium ion (Mg^{2+}) at resting potential of the cell, and ligand-gated because they require the activation of the glycine and glutamate binding sites on the outside of the NMDA receptor protein complex (Ozawa et al., 1998). The Mg^{2+} is released when the cell membrane is depolarized by the influx of monovalent cations, typically achieved via ion-flux through non-NMDA channels (Ritter et al., 2002). The NMDA receptors are permeable to calcium ions (Ca^{2+}) and are located primarily post-synaptically (McDonald & Johnston, 1990). To date total of seven NMDA subunits have been cloned (NR1, NR2A-NR2D, NR3A, and NR3B) (see Figure 1.1). A functional NMDA receptor requires a tetramer composed of two NR1 subunits and two NR2 subunits, usually composed of a dimer (i.e. pair), of dimers (Kew & Kemp, 2005). The NR1 subunit is considered obligatory, while NR2 functions as a modulatory subunit (e.g. as the properties of the NMDA receptor are dependent on which NR2 subunits are present). For example incorporation of the NR2A and NR2B subunits generally result in higher conductance through the NMDA receptor than do the

NR2C and NR2D subunits (Ozawa et al., 1998). However, it is likely that if a NR3 subunit is present, it will substitute for one of the NR2 subunits in an NMDA receptor complex, because they cannot form a functional receptor when expressed with NR1 or NR2 alone (i.e. both NR1 and NR2 subunits must be present) (Kew & Kemp, 2005). Also, the presence of a NR3 subunit results in a decrease in Ca^{2+} permeability through the NMDA receptor (Kew & Kemp, 2005).

NMDA receptors are located throughout the mammalian CNS, with particularly high densities expressed in the hippocampus and the forebrain (Ozawa et al., 1998). This receptor complex also plays a pivotal role in learning (Bliss & Collingridge, 1993) and in the many neuronal processes that occur during development (McDonald & Johnston, 1990). However, the NMDA receptor has also been implicated in a wide variety of neuropathological states (e.g. cell death accompanying both acute and chronic neurodegenerative diseases) (Bettler & Mulle, 1995).

1.1.4. AMPA Receptors

AMPA receptors are ligand-gated, are responsible for mediating fast, desensitizing responses and are located post-synaptically (Bleakman & Lodge, 1998). To date a total of four AMPA subunits have been cloned (GluR1-GluR4) (see Figure 1.1) (Bettler & Mulle, 1995). AMPA receptor subunits assemble as functional heteromeric tetramers, forming ion channels which are usually only permeable to sodium ions (Na^+) (Kew & Kemp, 2005). The GluR2 subunit is important in the permeability of Ca^{2+} through the AMPA receptor and if the GluR2 subunit is not present, the receptor is also permeable to Ca^{2+} (Kew & Kemp, 2005). During development there are a high

concentration of AMPA receptors which are permeable to Ca^{2+}, however, in the mature brain there are few AMPA receptors which flux Ca^{2+}, except in disease states (e.g. epilepsy). This is believed to play a major role in the plasticity of the developing brain (Simeone et al., 2004). The AMPA receptor complexes are located throughout the CNS, however there are high concentration located in the hippocampus (Ozawa et al., 1998).

1.1.5. Kainate Receptors

The KA receptor complex is ligand-gated and elicits a fast onset and a desensitizing response and can be located both pre- and post-synaptically (Chittajallu et al., 1999; Lerma, 2003). To date five KA receptor subunits, including GluR5, GluR6, GluR7, KA-1 and KA-2, have been identified through molecular cloning (see Figure 1.1) (Bettler & Mulle, 1995; Ozawa et al., 1998). As with other ionotropic Glu receptors, the pharmacological properties and channel kinetics of KA receptors depend on the subunit composition (Chittajallu et al., 1999; Lerma, 2003). The KA receptor is permeable to Na^{+}, however, when the GluR6 subunit is incorporated into the complex, the channel may display permeability to Ca^{2+} (Ozawa et al., 1998).

The KA subunits are classified as either "high affinity" (KA1, KA2) or "low affinity" (GluR5-GluR7) based on their affinity to the naturally occurring toxin KA. At relatively low concentrations, KA will bind selectively to high affinity subunits, and the naturally occurring toxin, domoic acid (DOM), will selectively bind to the low affinity subunits (Lerma et al., 2001), and at very low concentrations DOM shows the greatest affinity for GluR6 (Verdoorn et al.,

1994). Both KA and DOM are agonists for kainate receptors, however as concentrations increase, selectivity is lost (Lerma et al., 2001).

1.2. Glutamate Involvement in CNS Development

Ionotropic and metabotropic Glu receptor subunit mRNA is present in the rat embryonic brain, however Glu receptor complexes are not functional until later in development (Luján et al., 2005). Metabotropic Glu receptors are differentially regulated throughout development, with some receptor subtypes (mGlu1 and mGlu5) detected, although at low levels, as early as embryonic development (Luján et al., 2005). While the NMDA receptor subunit NR2B can be detected throughout the embryonic brain, other subunits such as NR2A, are not expressed until postnatal development. The NR2A subunit is also expressed only in the forebrain (Luján et al., 2005, Takai et al., 2003). This demonstrates differential expression of NMDA subtypes, however, all subunits are present by postnatal day (PND) 7 (Takai et al., 2003). Similar patterns of differential receptor subtype expression is apparent for AMPA and KA receptor subtypes (Luján et al., 2005, Ritter et al., 2002) and both have receptor subtypes present as early as embryonic development (Luján et al., 2005). There are periods of transient increases for certain AMPA receptor subtypes, such as an increase in the expression of GluR 2 and GluR4, around PND 7, and PND 28 for GluR3 (Ritter et al., 2002). There is also a complex pattern of transient increase in expression of KA receptor subtypes during early postnatal development (e.g. in the first and second week of postnatal life), each with a specific period of increased expression (Ritter et al., 2002). Such differential expression of Glu subunits and subunit combinations

throughout development may play a functional role in neuronal development. These changes are likely to occur during critical periods of known plasticity and may be important in development, with different properties associated with different subunit composition in Glu receptors (Ritter et al., 2002).

Evidence in support of the importance of Glu in normal neuronal development is apparent in a study conducted by Mattson et al., (1988) which was designed to examine the effects of Glu on the outgrowth of dendrites and axons in isolated hippcampal pyramidal-like neurons in cell culture. Hippocampal cells were obtained from Sprague-Dawley (SD) rat fetus (i.e. embryonic day 18), and placed in cell culture. The isolated hippocampal pyramidal-like neurons were exposed to varying doses of Glu and the Glu agonists, quisqualate (QA), KA and NMDA (the largest dose 1 mM). Results indicate that while the highest dose resulted in cell death, lower doses caused dose-dependent alterations in neuronal structure (e.g. reduction in dendritic length and with increasing doses, a reduction in axonal length) (Mattson et al., 1988).

In a similar study by Wilson & Keith (1998), the effects of Glu on the growth of hippocampal pyramidal cells was also examined. Tissue was obtained from SD rats (embryonic day 18-20) and placed into cell culture. Data from this study demonstrated that 50μM of Glu (which does not cause cell death) initially caused dendritic outgrowth after 4 hours. However, after 8 hours, Glu caused dendritic retraction (i.e. from prolonged exposure) (Wilson & Keith, 1998).

Data from these studies, and others, underscore the importance of optimal Glu on normal

neuronal development, illustrating that Glu can alter axonal and dendritic growth during development, altering neuronal form, and can even result in cell death.

Often, studies exploring the effects of Glu signaling in development, focus on the excitotoxic effects of Glu agonists on the developing rodent brain. This has been a focus in research, since Glu excitotoxicity will result in convulsions and has been used as a model of epilepsy. However, it has been suggested that the adverse effects of excitoxicity are less pronounced in the immature brain then the mature brain (Sarkisian et al., 1997). For instance, in one study, multiple administrations (4 administrations, i.p.) of a convulsant dose of KA was administered to rats over two day intervals early in development (PND 20, 22, 24, 26). These animals were then compared to other rats subjected to the same procedure during adulthood (PND 60, 62, 64, 66). The results demonstrated that the developing rats had less severe seizures, and after a one month period demonstrated no impairment in spatial memory, and no gross cell loss in the hippocampus. In contrast, these effects were apparent in the adult rats treated with KA (Sarkisian et al., 1997).

However, this does not mean that the developing brain is entirely protected against the excitoxic effects of Glu. This is demonstrated by Sayin et al., (2004) whose work demonstrated that a single administration of KA, which induced seizures early in development (ranging from PND 1-24) resulted in permanent alterations in cognition and anxiety in these same rats during adulthood (Sayin et al., 2004). It has also been demonstrated that relatively low doses of KA (2 mg/kg, i.p.), administered early in development (PND 12), will lead to non-convulsive seizures and produce alterations in exploratory behavior on PND 18 and PND 25, with an increase in

locomotor activity noted, even in the absence of gross morphological changes (Kubova et al., 2001). These studies demonstrate that early exposure to KA-induced seizures can result in long-term behavioral consequences.

Few studies, however, have looked at the effects of Glu receptor activation on development without looking at the excitotoxic effects. A few studies, however, have looked at the effects of very low equi-efficacious non-convulsive doses of DOM (5 or 20 μg/kg, s.c.) and KA (25 or 100 μg/kg, s.c.) administered during a critical period of development (Doucette et al., 2004, Doucette et al., 2003). Such studies are important, since they aid in furthering our understanding of the role of KA receptors in CNS development. In this series of studies, DOM and KA were administered between PND 8-14 in the rat (i.e. a period known as the brain growth spurt) (see section 1.6), and the rats were then assessed, during development, and as adults, for behavioural, neuroanatomical and neurochemical alterations. Developmental changes that were observed in these animals included early eye opening in the rats treated with 20 μg/kg of DOM and an increase in conditioning in the 20 μg/kg of DOM animals in an odor-conditioning place preference (CPP) paradigm. In the KA treated female rats, but not in the KA treated males, there was a decrease in activity found in the CPP with fewer center crosses, however, no conditioning was present in either the KA treated or saline control rats. No drug-induced alterations were found for weight gain or maternal retrieval, which suggests that neither DOM or KA produce any sign of overt toxicity at these doses (Doucette et al., 2003). During adulthood, drug-induced differences were apparent in a number of assessments. Firstly, alterations were seen in response to a novel spatial environment. When rats were placed in the Morris water maze (MWM), the

treated rats displayed behaviors on the platform that were characterized by hunched posture, mastication with tongue protrusion, facial clonus, repetitive eye blinking and squinting, head bobbing, ear twitching and forelimb clonus. These behaviors are similar to partial seizure behaviors and have been termed NIS-L (novelty induced seizure-like) behaviors. Additionally, along with such behavioral changes, alterations were noted in hippocampal structure and chemistry, evidenced as an increase in mossy fibre sprouting, an elevation in hippocampal brain derived neurotrophic factor (BDNF) and a reduction in hippocampal cell counts; all found in toxin-treated rats, relative to saline controls (Doucette et al., 2004). Therefore, although the doses used in these studies were low non-convulsive doses, there were long lasting alterations in behavior and in hippocampal morphology throughout development and into adulthood (Doucette et al., 2004, Doucette et al., 2003).

Some of the differences found in treated animals (reported above) suggest that the dopaminergic system may also be altered as a consequence of early DOM or KA exposure (e.g. such as altered responses to a novel spatial environment and morphological and neurochemical changes in the hippocampus) (Doucette et al., 2004). The dopaminergic system, especially the mesocorticolimbic pathway, is important in the modulation of response to novelty (Bevins et al., 2002, Legault & Wise, 2001) and has connections with the hippocampus (Pennertz & Kitai, 1991) (see sections 1.3 and 1.5).

1.3. The Dopamine System

Dopamine (DA) is a catecholamine neurotransmitter and is distributed with specific projection pathways in the brain. The DA system is important in many functions which include, but are not limited too; locomotor activity, endocrine regulation, cognition, emotion, regulation of food intake, and positive reinforcement (Missale et al., 1998).

The midbrain dopaminergic neurons are organized into three major pathways: the tuberoinfundibular/ tuberohypophysial, nigrostriatal and mesocorticolimbic (sometimes referred to as mesocortico and mesolimbic pathways separately) pathways (Prakash & Wurst, 2006; Hess & Creese, 1987) (see Figure 1.2). The tuberoinfundibular/ tuberohypophysial pathway originates in the para- and peri-ventricular hypothalamic nucleus, innervates the median eminence of the hypothalamus and the pituitary gland, and is important in neuroendocrine control (Prakash & Wurst, 2006). The nigrostriatal pathway originates in the substantia nigra and innervates the caudate nucleus, putamen, and globus pallidus (which, together, are referred to as the basal ganglia) (Hess & Creese, 1987). This pathway is important in functions such as voluntary muscle movements and body posture (Prakash & Wurst, 2006). The mesocorticolimbic pathway originates in the ventral tegmental area (VTA) and innervates the ventral striatum, nucleus accumbens (NAc), amygdala and olfactory tubercle (mesolimbic pathway), and also projects to the prefrontal cortex (PFC) (mesocortico pathway) (Prakash & Wurst, 2006). This pathway appears important in cognition and in the regulation of reward behaviors (Prakash & Wurst, 2006).

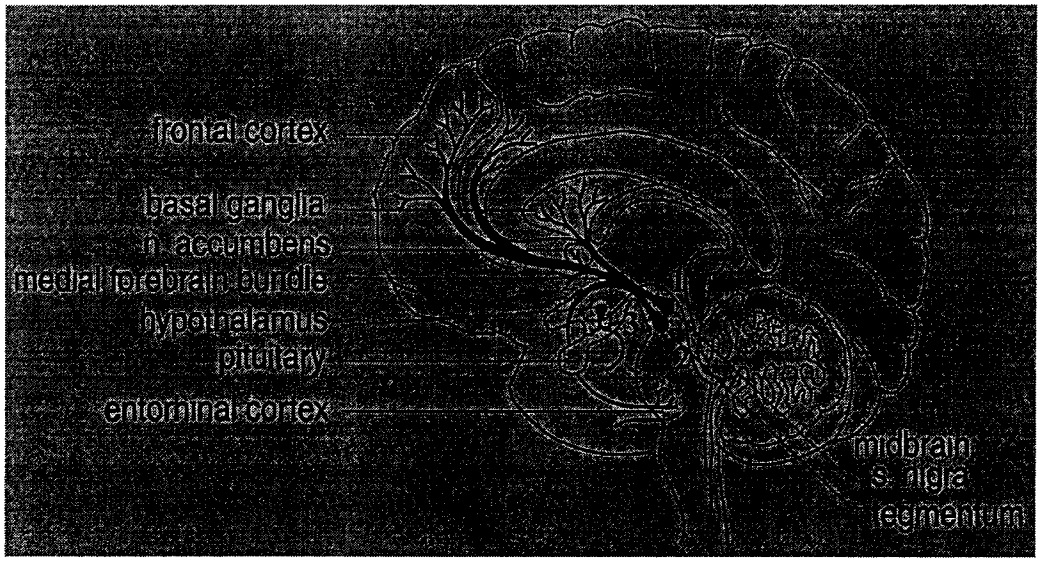


Figure 1.2. The Dopamine Pathways. 1) Nigrostriatal pathway, 2) Mesocorticolimbic pathway, 3) Tuberoinfundibular pathway. Adapted from McKim (2003).

Dysfunctions in DA pathways are associated with the development of various disorders. For instance, dysfunction in the tuberoinfundibular system is associated with hyperprolactinemia (e.g. a condition with elevated levels of prolactin); dysfunction in the nigrostriatal pathway is associated with Parkinson's disease; and dysfunction in the mesocorticolimbic pathway is associated with addictions and schizophrenia (Prakash & Wurst, 2006). The mesocorticolimbic pathway is of particular interest to this thesis and, as mentioned earlier, is important in modulating responses to novelty (Bevins et al., 2002, Legault & Wise, 2001) and to reward (Wise, 2005). Other neurotransmitter systems are involved in modulating the activity of this pathway including the gamma-aminobutyric acid (GABA) and the Glu systems (see section 1.5).

All known DA receptors are metabotropic (i.e. coupled to G-proteins) (Missale et al., 1998). It was first proposed in1979 that two classes of DA receptors existed, and were classified based on the effects of receptor activation on adenylyl cyclase activity (Kebabain & Calne, 1979). A total of five DA receptors have been cloned to date and are divided into two major classes; the D1-like receptors and the D2-like receptors (Missale et al., 1998). The D1-like receptors include the D1 and D5 receptors and the D2-like receptors include D2, D3 and D4 receptors.

1.3.1. D1-Like Receptors

The D1-like receptors were first described based on their ability to activate adenylyl cyclase and promote cyclic-AMP (cAMP) accumulation in cells, and are therefore stimulatory (Kebabain & Calne, 1979). Historically the D1-like receptors, D1 and D5, have not been easily differentiated

using pharmacological techniques. Recently, however there has been remarkable progress in identifying compounds which demonstrate selectivity for the D5 receptor (Mohr, et al., 2006, Wittig et al., 2004). D1 and D5 receptors are primarily located post-synaptically, although some expression has been observed pre-synaptically (Missale et al., 1998). In the brain, the D1 receptor is widely distributed throughout the mammalian CNS, however, the D5 receptor is poorly expressed in the rat brain in comparison, with distribution restricted to the hippocampus, the lateral mammillary nucleus and the parafascicular nucleus of the thalamus (Missale et al, 1998).

1.3.2. D2-Like Receptors

The D2-like receptors were first described based on the ability to cause a decrease in cAMP in cells (Kebabain & Calne, 1979). The D2-like receptors are negatively coupled to adenylyl cyclase and inhibit cAMP, and are therefore inhibitory (Missale et al., 1998). There has been better progress in the pharmacological differentiation of D2-like receptors, and although the pharmacological profile is similar, there are compounds which show selectivity to D2, D3 and D4 receptors, illustrating that they can be readily differentiated (Missale, et al., 1998). The D2 receptors are predominately localized within in the striatum, the NAc core, and the olfactory tubercle. The D3 receptors have more specific distribution throughout the limbic system, they are found in the shell of the NAc, but have poor expression in the striatum and are found in low concentrations within the hippocampus. Finally the D4 receptor is highly expressed in the hippocampus, hypothalamus, mesencephalon, amygdala and frontal cortex (Missale et al., 1998).

1.4. Dopamine in CNS Development

Dopamine receptors are evident during embryonic development, with fully differentiated DA neurons present by approximately embryonic day 12 in the rat (Prakash & Wurst, 2006). The DA neuron projections reach adult morphology by the third postnatal week in the rat (Broening & Slikker, 1998; Prakash & Wurst, 2006). In the second postnatal week of the rat, D1 receptor densities are over expressed by approximately 200% in the PFC. The normal DA content does not reach adult concentrations in the striatum until approximately PND 50-60 in the rat (Broening & Slikker, 1998)

Data obtained from studies using 6-hydroxydopamine in developing rats have revealed the importance of DA in normal neurodevelopment. For instance, in one study, where rats were given a intracisternal administration of 6-hydroxydopamine at 5 days of age (which results in the depletion of brain DA), a decrease in habituation to spontaneous locomotor activity between 15 and 19 days of age (a period of development when there is already a heightened arousal) was found (Shaywitz et al., 1977). It is suggested that habituation is mediated by catecholamines, especially DA, and habituation of activity is dependent on a normal functioning DA system (Shaywitz et al., 1977). In another study, effects of 6-hydroxydopamine was shown to produce alterations in measures of activity, resulting in stereotyped behaviors, a decrease in anxiety on the elevated plus maze, and impaired self stimulation of the lateral hypothalamus during adulthood. However, the effects were demonstrated to a greater degree when exposure occurred postnatally rather than prenatally. This demonstrates not only the importance of normal development of the

DA system, but also an increased sensitivity to manipulations of the system during early postnatal development, when the system is undergoing rapid change (Shabenov et al., 2005).

1.5. Interactions between the Glutamate and Dopamine Systems in the Mesocorticolimbic Pathway.

There is substantial evidence of glutamatergic interactions with the mesocorticolimbic DA pathway. Glutamate receptors have been demonstrated to be located on DA neurons within the mesocorticolimbic pathway, and can modulate the release of DA (Crowder & Weiner, 2002, Legault & Wise, 2001, Mathé et al., 1998). Functional KA receptors, in particular, have been located both pre- and post-synaptically in the rat NAc, and it is believed that KA receptors inhibit excitatory transmission through presynaptic activation (Crowder & Weiner, 2002). The exact mechanism for this inhibition is not fully understood, but could be mediated through KA receptors on GABA neurons (e.g. by potentiation of GABAergic synaptic transmission in the NAc via presynaptic KA receptors located on these GABA neurons) (Crowder et al., 2006).

Dopamine release in the NAc and the caudate nucleus is also modulated by KA and AMPA receptors in freely moving rats (Imperato et al., 1990). The modulation of DA through Glu receptors, was demonstrated with enhanced release of DA in the caudate nucleus and NAc, after the perfusion of QA and KA into these structures. In this study the effect was antagonized with an AMPA/KA antagonist but not with an NMDA antagonist (Imperato et al., 1990). Therefore, KA receptors are not only located in the NAc, but also appear to play a functional role in the

modulation of DA release.

There is also a role for AMPA and KA receptor modulation of DA release within the VTA as demonstrated using AMPA/KA antagonist (LY293558) applied to the VTA. In a study by Takahata & Moghaddam (2000), microdialysis probes were used to perfuse 100 μM of LY293558 into the VTA, and DA content in the NAc and the medial prefrontal cortex (mPFC) were measured. An increase in DA levels in the NAc and a decrease in DA levels in the PFC were found (Takahata & Moghaddam, 2000). Further evidence to support the role of Glu receptors on DA release comes from an electrophysiological study by Wang & French, (1995). Their work provides support for the idea that non-DA neurons in the VTA, perhaps GABAergic interneurons and projection neurons, contain NMDA, AMPA and KA receptors. In non-dopamine neurons, obtained from the VTA of male SD rats, AMPA, KA, and NMDA resulted in a depolarization of the neurons with a rank order of potency (AMPA>KA>NMDA) (Wang & French, 1995). These studies collectively demonstrate the importance of Glu receptors, particularly non-NMDA receptors, in the modulation of DA release within the mesocorticolimbic pathway, through the VTA and the NAc.

There is also Glu interaction from the mPFC to the mesocorticolimbic pathway. The primary output of the mPFC occurs through Glu pyramidal cells, and have inputs in the VTA and NAc. Activation of the DA VTA projections to the mPFC causes a decrease in Glu activity, creating a feedback loop to the mesocorticolimbic pathway (Steketee, 2003). The VTA projections provide an inhibitory influence in the mPFC, either by activating inhibitory GABA interneurons in the

mPFC, or by directly inhibiting Glu pyramidal neurons in the mPFC (Steketee, 2003) (see Figure 1.3).

Glutamate receptors also appear to play a role in many of the behaviors that are modulated by the mesocorticolimbic pathway. Alvarez & Ruarte (2001) demonstrated a glutamatergic modulation, through the NAc, of the response to novelty, in the rat. Administrations of antagonists for mGlu receptors (AP3), NMDA receptors (AP7) and AMPA/KA receptors (CNQX), and glutamic acid were injected into the NAc and the different groups were compared on exploratory behaviors in a conflictive (elevated plus maze) and a non-conflictive (hole-board) environment. Results indicated that administration of glutamic acid resulted in an inhibition of some exploratory behaviors in both the conflictive and non-conflictive environments. In the conflictive environment, the effects of glutamic acid were blocked by the Glu receptor antagonists. However, only AP7 and CNQX (NMDA and AMPA/KA antagonists, respectively) were effective in blocking some of the effects of glutamic acid in the non-conflictive environment (Alvarez & Roarte, 2001). This demonstrates the importance of Glu receptors (metabotropic and ionotropic) in the modulation of exploration in environments producing anxiety (elevated-plus maze). However, only the ionotropic receptors appear to be important in the modulation of exploration in a non-conflictive environment (hole-board). This demonstrates a differential role for metabotropic and ionotropic Glu receptors in exploration, depending on the level of anxiety

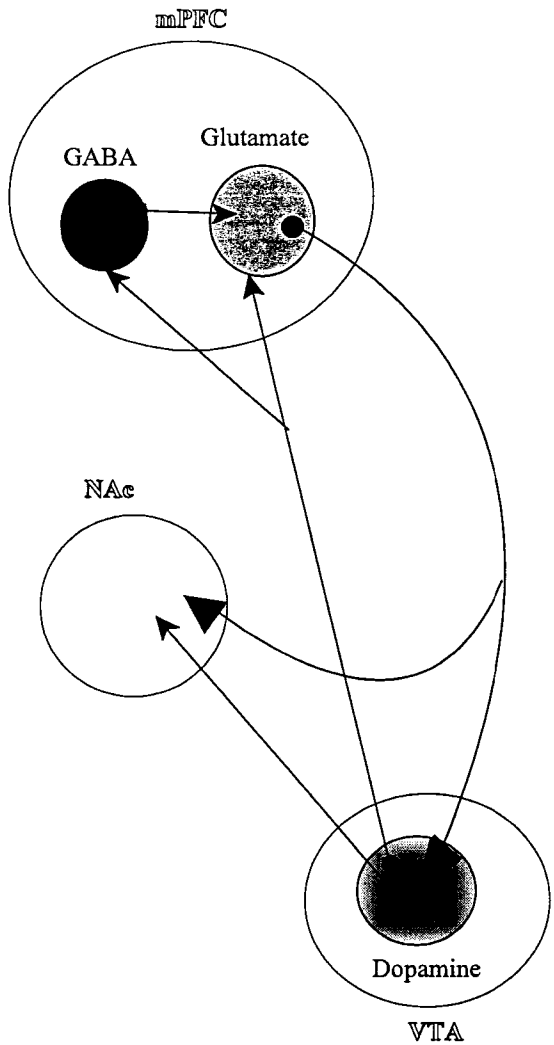


Figure 1.3. Connections from the Prefrontal Cortex to the Mesocorticolimbic Pathway. (mPFC= medial prefrontal cortex, NAc= nucleus accumbens, VTA= ventral tegental area). Adapted from Steketee (2003).

produced by the environment (Alvarez & Ruarte, 2001).

Also AMPA/KA receptors play a role in the "rewarding properties" attributed to activity with the mesocorticolimbic pathway. As mentioned earlier, the mesocorticolimbic pathway is important in the modulation of positive reinforcement (Missale et al., 1998). Evidence to support AMPA/KA involvement comes from a study by Choi et al. (2005) where AMPA/KA receptor antagonists (CNQX and NBQX) were shown to be effective in blocking brain self stimulation of the VTA, when administered with a DA D2/3 receptor agonist (7-OH-DPAT) in the NAc of the rat. However, brain self stimulation could not be effectively blocked when CNQX, NBQX or 7-OH-DPAT was administered alone. Therefore, AMPA/KA and D2/3 receptors may act synergistically within the NAc in mediating brain self stimulation, however the mechanism(s) of action are unclear. This demonstrates the importance of the interaction between non-NMDA receptors and DA receptors on the behavioral modulation of response to reward (Choi et al., 2005). Further evidence has been provided with receptor blockade of NMDA and AMPA/KA receptors which resulted in prevention of the acquisition of D2/3 dopamine receptor stimulation conditioned place preference (CPP) (Biondo et al., 2005).

Therefore, Glu receptors are important in the modulation of DA release and in behaviors, such as response to novelty and reward, which are modulated by the mesocorticolimbic pathway. Also there appears to be an interplay between DA receptors and Glu receptors in the functional integrity of the mesocorticolimbic pathway.

1.6. Neurodevelopment in the Rat

Development is a continuous process which lasts throughout an organisms lifespan (Vorhees, 1986a). However, there are a number of developmental stages in the CNS during which there is an increased susceptibility to toxins (Vorhees, 1986b). Understanding the fundamental principles of neurodevelopment and the time course for critical periods of vulnerability in the rat is important in order to more clearly interpret the practical significance of research findings, and to better understand the relevance of animal models to the human condition.

The development of the rodent brain is similar, in many respects, to human brain development. However, unlike humans, the regional development of the rodent brain occurs on a timeline of days, rather than weeks or even months (Rice & Barone, 2001). Stages of development where there is increased susceptibility to disruption include pre-implantation, organogenesis, histogenesis and functional organization. Each of these stages (in the human) occur prenatally, with the exception of functional organization, which extends throughout the postnatal period and into adolescence (Vorhees, 1986b; Rice & Barone, 2001).

If a developing organism is exposed to a toxin during pre-implantation, the result is an "all or nothing" effect, either the toxin kills the organism (e.g. by preventing implantation into the uterine wall) or the organism survives and there is no apparent secondary consequence (Vorhees, 1986b). Interference during organogenesis, a period of cellular organization, will usually result in a physical malformation in the organism. Organogenesis occurs predominately during the

embryonic stage of development in the rodent (Vorhees, 1986b). The stages of increased susceptibility for the maturing brain is during finer levels of CNS development (e.g. histogenesis and functional organization). With respect to time lines, histogenesis overlaps with organogenesis, and occurs during the fetal period of development in the rodent. It is a period of finer cellular organization, and disruptions due to toxins can result in cellular abnormalities, changes in growth, and even functional impairments (Vorhees, 1986b). The last stage of development is known as functional organization, with its commencement overlapping with histogenesis and extending into adolescence (humans and rodents). This stage of CNS development involves even finer levels of organization, such as establishment of receptor and secretory sites and the activation of biochemical pathways, resulting in the final function of each system. Interference due to toxins during this period could result in functional impairments, such as changes in behavior or cognition which manifest in absence of gross physical malformations (Vorhees, 1986b).

Events occurring in the brain during the stages of development discussed above include neurogenesis, neuronal migration, differentiation, synaptogenesis, gliogenesis, myelination and apoptosis (Rice & Barone, 2001). Neurogenesis is the growth and development of neurons and is a highly regulated process with different periods of development for various brain regions (e.g. rostral to caudal) (Rice & Barone, 2001). These neuronal cells then migrate to the appropriate locations within the brain and if neurogenesis is disrupted, migration will also be disrupted (Rice & Barone, 2001). Also integral to neurogenesis and migration is the differentiation of neuronal cells and the expression of the terminal phenotype (Rice & Barone, 2001). Synaptogenesis is the

formation of functional synapses, is important for cell-to-cell communication, and occurs during the first three weeks of postnatal development in the rat. Each of these events of neuronal development can be susceptible to interference due to drugs, toxins and other environmental agents. Synaptogenesis, in particular, is a period of increased susceptibility (e.g. especially during the first two weeks of postnatal life in the rat) (Rice & Barone, 2001) and occurs during functional organization. The development and maturation of supporting cells, such as neuroglial cells in the brain is also crucial for normal brain development (e.g. gliogenesis and myelination) Gliogenesis and myelination is an extended developmental process (i.e. myelination occurs into adolescence in both rodents and humans), and interference with either process will result in functional impairments in the brain (Rice & Barone, 2001). Finally, normal brain development also requires an appropriate degree of apoptosis (programmed cell death), which systematically removes neurons during development (Rice & Barone, 2001).

A particularly vulnerable period of development, is known as the brain growth spurt. This is a period of development when the brain is undergoing rapid growth, where the brain has many of the complexities of the adult brain, but also additional complexities due to the rapid development (Dobbing & Smart, 1974). This period of development occurs during the first two weeks of postnatal life in the rat, and is approximately equivalent to the third trimester in humans (Dobbing & Smart, 1974). The brain growth spurt occurs during functional organization and includes many of the developmental processes mentioned above, such as synaptogenesis, and is of particular interest to this thesis.

1.7. Neurodevelopmental Disorders

The term neurodevelopmental disorder, is a relatively new term used in the medical community. It is used for disorders in which there are developmental delays or variations in the brain, believed to be caused by environmental insults, genetic variations, unspecified reasons which are not known yet, or some combination therein. These variations or delays in neurodevelopmental process may result in problems in learning and behavior with varying degrees of dysfunction. To date, there are many neurodevelopmental disorders that have been characterized, such as epilepsy, attention deficit hyperactivity disorder (ADHD), fetal alcohol syndrome (FAS), autism spectrum disorder, and potentially schizophrenia, as just a few examples.

Many of the neurodevelopmental disorders mentioned above are believed to be a result of a combination of genetic variations and environmental insults, however the exact etiology is not presently known (Little, 2000). However, even for those neurodevelopmental disorders in which the etiology is known, such as is the case for FAS (e.g. due to alcohol consumption during pregnancy), there are still many questions that presently remain unanswered (e.g. How much alcohol must be consumed to result in functional alterations in the child? During which stage of development is the child the most susceptible? How is the child functionally impaired?) (Riley & McGee, 2005), demonstrating the complexity of, and the need for, further research on neurodevelopmental disorders.

1.8. Schizophrenia as a Neurodevelopmental Disorder

According to the American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders (4th ed.) text revision (2000), schizophrenia is a serious mental disorder which is characterized by symptoms such as delusions, hallucinations, disorganized speech, disorganized or catatonic behavior and negative symptoms (e.g. such as affective flattening or alogia). In order for a diagnosis of schizophrenia to be made, two or more of these symptoms must be present for significant portions of time for a duration of a month (or less, if treatment is successful) (DSM-IV-TR, 2000). Schizophrenia also results in social and occupational dysfunction, and the symptoms and dysfunctions are not due to a general medical condition or due to substance use (DSM-IV-TR, 2000). Substance use can, however, precipitate or aggravate psychosis in schizophrenia (Carlson, 2007), and substance abuse is often co-morbid with schizophrenia (see section 1.8.5.).

Schizophrenia is a devastating disorder which results in a great emotional and financial costs to the individual and those directly affected. This disorder also results in great financial costs to the Canadian economy. The total cost of this disorder in Canada in 2004 was estimated at 6.85 billion dollars. For instance the greatest cost to the economy was the loss of productivity due to the decrease of functioning within the work force and mortality (predominately due to suicide) of the afflicted individuals, accounting for over two thirds of the total cost (Goeree, et al., 2005).

Schizophrenia afflicts approximately 0.9% of the Canadian population, resulting in

hospitalization, unemployment, the need for assisted services and, unfortunately, even suicide (Goeree et al., 2005). Therefore the need for researchers to better understand the etiology of this disorder is great, as this will aid in the development of better treatment, which in turn, will improve functioning in the afflicted individuals and perhaps ultimately may result in ways by which schizophrenia may be prevented.

Symptoms of schizophrenia are classically defined as positive or negative symptoms. A patient may have other symptoms such as cognitive deficits, and may be described as having predominately positive or negative symptoms, or may present with a mixture of positive and negative symptoms (Andreasen & Olsen, 1982). Positive symptoms include hallucinations, delusions, positive formal thought disorder, and bizarre or disorganized behavior (Andreasen & Olsen, 1982). Hallucinations are perceptions of stimuli that are not present (Carlson, 2007). They can include auditory, olfactory, visual or tactile sensory hallucinations. Delusions are false beliefs which may be prosecutory, jealous, religious or grandiose in content. Marked formal thought disorder includes marked incoherence, illogical thought, or derailment of thought (Andreasen & Olsen, 1982). In turn, negative symptoms include alogia, affective flattening, anhedonia, social withdrawal, avolition, and attentional impairment. Alogia is a marked poverty of speech, with a very limited content. Affective flatting is a decrease in a range of expressing emotions. Anhedonia is a decrease in the ability to experience pleasure, and often is interrelated with social withdrawal, as it often results in the inability to feel intimacy. Avolition is the inability to finish something, such as a project, once it is started and is often related to a decrease in occupational or school functioning (Andreasen & Olsen, 1982).

Schizophrenia appears to have a fairly specific time course with respect to the manifestation of clinical and pathological stages (e.g. during development there appear to be different stages of the disorder) (see Figure 1.4). First to manifest is the premorbid stage, appearing at childhood - adolescence with symptoms such as mild cognitive and social impairments and mild impairments in motor coordination. The following stage is the prodromal stage, starting approximately during adolescence - early adulthood with many symptoms such as cognitive and attentional impairments, anxiety, social withdrawal, substance abuse and even mild psychotic symptoms. Both stages appear before the onset and diagnosis of schizophrenia, which in turn, is known as the onset stage, with active psychosis warranting a diagnosis (described previously in this section) with onset most often occurring during early adulthood. This stage is often brought on by a life stressor which triggers a psychotic episode. Finally the chronic or residual stage occurs after the onset of schizophrenia and lasts from adulthood into senescence, with symptoms for chronic stages similar to onset, and residual stages consisting of primarily negative and cognitive symptoms. During the chronic or residual stage symptoms are attempted to be managed with antipsychotic treatment (Lieberman et al., 2001). Although this the typical time-course pattern for schizophrenia, there are also cases of child-onset schizophrenia, and in such cases the symptoms are often more severe (Rapoport et al., 2005).

The predominate theory to date of the etiology of schizophrenia is that it is a neurodevelopmental

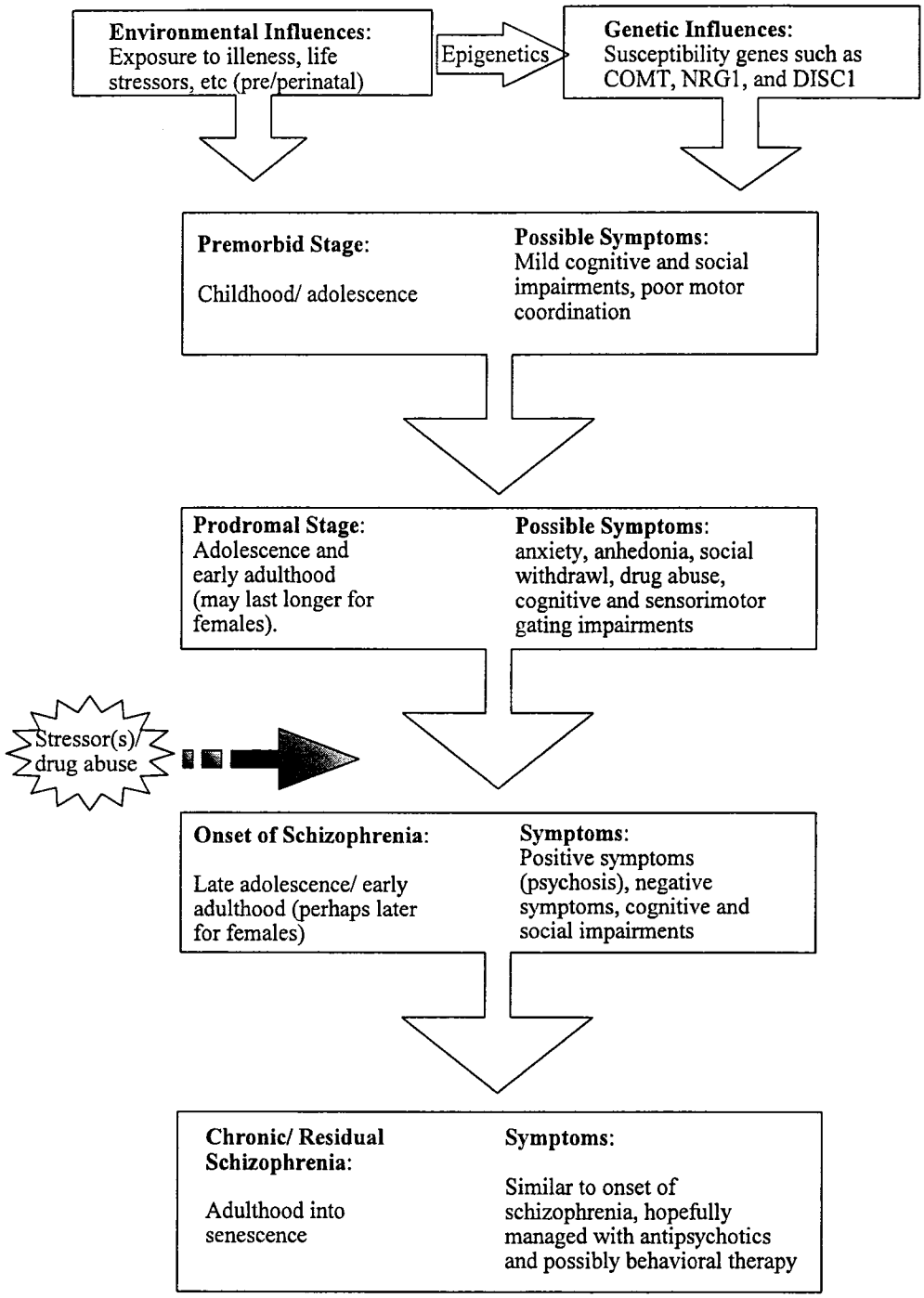


Figure 1.4. Developmental sequence of schizophrenia. Adapted from Lieberman et al., (2001) and Rapoport et al., (2005). COMT = catechol-O-methyltransferase, NRG1= neuregulin 1 DISC1=Disrupted-In-Schizophrenia 1.

disorder with an interplay between genes and environment. The theory of schizophrenia as a neurodevelopmental disorder proposes that schizophrenia is a result of an aberration in neurodevelopmental processes which occurs before the onset of schizophrenia symptoms. However alterations in normal behavioral and social functioning may be present throughout development (e.g. such as attentional deficits). This is considered to be due to environmental and genetic interactions with CNS development, such as exposure to stress early in development (reviews Duncan et al., 1999; Keshavan, 1999; Lieberman, et al., 2001; Murray, et al., 2004; and Rapoport et al., 2005). There is an abundance of evidence in support of this theory, however, it is broad and increased specificity for the potential environmental and genetic risk factors and the resulting neurostructural and neurochemical alteration, is needed to strengthen and modify the theory.

1.8.1. Evidence of Prenatal and Perinatal Environmental Factors

There are a number of environmental factors that have been investigated as potential risk factors for the later development of schizophrenia. Much of the investigation has recently focused on prenatal and perinatal environmental factors, which provides support for schizophrenia as a neurodevelopmental disorder. Such environmental factors have included obstetric complications (Clarke et al., 2006), nutrition (Hoek et al., 1998; McNamara & Carlson, 2006), prenatal (Brown et al., 2004) and early postnatal illness (Abrahao et al., 2005), prenatal exposure to radiation (Imamura et al., 1999) and trauma/stress (e.g. child abuse and neglect) (van Os et al., 2005).

One environmental factor for which there is strong evidence and focus within the literature, is the link between early exposure to infection and the later development of schizophrenia. Studies have shown there to be a strong relation between prenatal immune infections (e.g. such as polio) and schizophrenia (Suvisaari et al., 1999). Also, a relation between a general elevation of pro-inflammatory cytokines (IL-8) in the second trimester of pregnancy and adult onset schizophrenia has also been reported (Brown et al., 2004). It has also been demonstrated that there is an increased risk of schizophrenia in the offspring of mothers diagnosed with maternal genital and reproductive infections (Babulas et al., 2006) and an increased risk has also been reported for offspring of mothers infected with the parasite Toxoplasma gondii (Mortensen et al., 2006).

The relation between infection and later development of schizophrenia is not completely restricted to prenatal infections. For instance Abrahao et al., (2005) demonstrated a relation between early childhood meningitis and an increased risk for psychosis and schizophrenia in adulthood. In this study, the authors report that children who were diagnosed with meningitis during the first four years of life had an increased rate for the diagnosis of schizophrenia and the occurrence of psychotic symptoms (without being diagnosed with schizophrenia) later in life than did sibling controls (i.e. who did not have meningitis as a child). Such research offers strong support for the involvement of early exposure to infection as an environmental risk factor for schizophrenia.

1.8.2. Evidence of Genetic Factors

Schizophrenia appears to be a heritable disorder, with an incidence among first degree relatives,

such as parents and siblings, of approximately 15% (Kandel et al., 2000). The involvement of genes in schizophrenia is most readily apparent in studies of identical twins and fraternal twins. When comparing monzygotic twins to dizygotic twins the heritability of schizophrenia is evident, as monozygotic twins have a much higher concordance 41-65 % than dizygotic twins 0-28% (Cardno & Gottesman, 2000). The concordance of monozygotic twins demonstrates that genetic factors are just part of the etiology of schizophrenia (Cardno & Gottesman, 2000).

To date there have been several susceptibility genes identified for schizophrenia, including catechol-O-methyltransferase (COMT), dystrobrevin binding protein 1 (DTNBP1), neuregulin 1 (NRG1), regulator of G-protein signaling 4 (RGS4), Disrupted-In-Schizophrenia 1 (DISC1), and G72. There are also several candidate genes under investigation, leading to a promising future for understanding the genetics which may underlie schizophrenia (Harrison & Weinberger, 2005). Interestingly, it has been demonstrated that intermediate phenotypic measures of impaired neurodevelopment are actually associated with many of the same genes that have been identified as susceptibility genes for schizophrenia (Rapoport et al., 2005). This gives support to the neurodevelopmental model of schizophrenia, as genes associated with schizophrenia also result in phenotypic measures during development that are associated with the later onset of schizophrenia (Rapoport et al., 2005).

Another promising area of study for understanding schizophrenia, is the study of epigenetics, where regulation of gene activity is tissue specific, age-dependent, and susceptible to developmental stage and environmentally induced changes (Rapoport et al., 2005). Such

influences on gene expression may be important in understanding the variations in susceptibility genes in schizophrenia. An interesting study looking at the influences of epigenetics of schizophrenia, investigated the DNA modification status of the DA D2 receptor gene in monozygotic twins. Interestingly, the study demonstrated a greater epigenetic similarity between unrelated individuals with schizophrenia, in the D2 receptor gene, than with their own monozygotic twin who was unaffected by schizophrenia (Petronis et al., 2003) This provides evidence of the influence of epigenetics in the expression of genes related to schizophrenia. Further investigation in this area may provide greater clarity in understanding the interplay between environment and genetics and the later development of schizophrenia.

1.8.3. Summary of the Neurodevelopmental model of Schizophrenia

The etiology of schizophrenia appears to be a complex interaction between genetics, epigenetics, and environmental factors (e.g. such as exposure to illness and life stressors, preceding the onset of the symptoms). This supports the idea that schizophrenia is a neurodevelopmental disorder (Rapoport et al., 2005). While the exact mechanism(s) which underlie the development of schizophrenia may actually be unique for each individual, it is still of utmost importance to understand specific environmental and genetic risk factors and the course of neurological maladaptive development, because this may aid in prevention or at least better treatment(s) and/or earlier interventions for this disorder.

1.8.4. Brain Alterations in Schizophrenia

While the precise etiology of schizophrenia is not known, many neuroanatomical and neurochemical correlates are consistently present. The negative and cognitive symptoms present in schizophrenia is thought to be due to an underactivity in the PFC, predominately in Glu activity. The positive symptoms are thought to be due to an increase in DA activity in the mesocorticolimbic pathway (Carlson, 2007). There have been alterations found in the hippocampus and PFC in the brains of patients with schizophrenia (see section 1.8.4.1.). Such neuroanatomical alterations provide evidence of brain structures that might be altered and responsible for the negative and cognitive symptoms found in schizophrenia. Other disorders, such as depression, often have similar alterations in such structures which could account for an overlap in symptomatology between schizophrenia and other disorders (Carlson, 2007). There has also been strong support for altered DA transmission in the mesocorticolimbic pathway, and in Glu transmission from the PFC into the mesocorticolimbic pathway (see section 1.8.4.2.). These neuroanatomical and neurochemical correlates found in schizophrenia will be discussed further in the following sections.

1.8.4.1. Gross Neuroanatomical Alterations in Schizophrenia

The hippocampus has been extensively investigated in schizophrenia patients, and there appear to be many alterations present within this forebrain structure. Using immunoreactivity, a post mortem study found a decrease in the dendritic expression of GluR 5, 6, and 7 subunits in the hippocampus

of patients who were diagnosed with schizophrenia (Benes et al., 2001), a decrease which was not present in patients diagnosed with manic depression or in controls (Benes et al., 2001). Takahashi et al., (2000) report an increase in BDNF mRNA in the hippocampus and in the anterior cingulate cortex of patients with schizohprenia. A reduction in hippocampal pyramidal cell counts, and a disarray in the organization of hippocampal pyramidal cells has also been reported following post mortem analyses of brain tissue obtained from patients with schizophrenia (Jönsson et al., 1999). Further anatomical anomalies have been reported by Bogerts et al., (1985) who found shrinkage in not only the hippocampus, but also the amygdala, parahippocamapal gyrus, and the pellidum internum. There have also been differences found in the ventricles in patients with schizophrenia, with an increase in ventricle size. Increased ventricle size, in turn, has been associated with poor-outcome in schizophrenia patients (Davis et al., 1998). Other changes that have been reported include reductions in dendritic spine density in the PFC (Kolluri et al., 2005), increases in mGlu receptor expression (mGLuR1 and mGluR2/3), in the PFC (detected using immunohistochemistry) (Gupta et al., 2005), and altered KA receptor mRNA expression in the PFC (i.e. an increase in GluR7 mRNA and a decrease in KA1 mRNA) of patients with schizophrenia (Meador-Woodruff et al., 2001).

1.8.4.2. Dopamine and Glutamate Dysfunction in Schizophrenia

Ample evidence exists to support the idea that Glu and DA dysfunction may be the primary candidates underlying the neuropathology associated with schizophrenia. The role of DA in the pathology and treatment of schizophrenia has been the subject of investigation for approximately

50 years. It was originally hypothesized that DA hyperactivity was responsible for positive symptoms manifested by patients with schizophrenia (Carlsson & Lindqvist, 1963). The focus of DA dysfunction in schizophrenia has, historically, been largely due to two reasons, 1) the efficacy of antipsychotics in treating the symptoms associated with this disorder and 2) the action and effects of psychomotor stimulants (i.e. regarding the manifestation of schizophrenia-like symptoms, in otherwise "normal" individuals).

Antipsychotics were originally developed by a French surgeon, Henri Laborit, who discovered that a drug used as a tranquilizer for surgery was also successful in reducing anxiety (Carlson, 2007). A related compound, chlorpromazine, was later developed and found to be effective in treating positive symptoms in patients with schizophrenia (Carlson, 2007). This drug is a typical antipsychotic and acts primarily as a DA receptor blocker. The typical antipsychotics are primarily blockers of the D2 receptor, and those with the greatest efficacy in treating the symptoms of schizophrenia also have the greatest affinity for the D2 receptor. Typical antipsychotics appear to be primarily effective against positive symptoms (McKim, 2003). Atypical antipsychotics were developed later, and show a preferential affinity for the D3/4 receptors. They also have an advantage, in that some of the Parkinson's- like side effects manifested by patients treated with the typical antipsychotics are largely avoided (McKim, 2003). Differential expression of DA receptors throughout the brain results in different brain areas affected with antipsychotics (e.g. atypical antipsychotics primarily target cortical and limbic structures, and typical antipsychotics target the striatum resulting in Parkinson's-like side effects) (see Figure 1.5) (McKim, 2003).

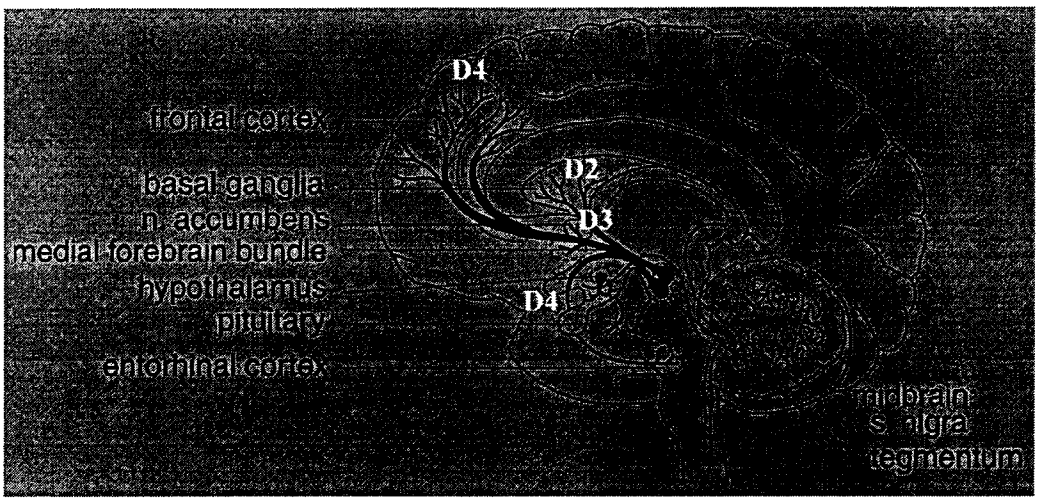


Figure 1.5. Dopamine receptor distribution of targeted receptors of typical and atypical antisychotics. (Typical antipsychotics - D2; Atypical antipsychotics - D3, D4). Adapted from McKim, 2003.

Psychomotor stimulants, such as amphetamine and cocaine, are DA agonists. These drugs increase activity within the mesocorticolimbic pathway, they strongly reinforce behavior, and they can cause positive symptoms of schizophrenia, if taken in large doses (Carlson, 2007). Such drugs act by increasing DA activity in the synapse. For instance cocaine and amphetamine block DA re-uptake, and amphetamine will also cause the spontaneous release of DA into the synapse and an increase in the amount of DA released in response to an action potential (McKim, 2003).

Laruelle et al. (1999) explored DA hyperactivity in schizophrenic patients. Their result demonstrated that patients with schizophrenia have increased DA transmission in response to amphetamine, compared to controls, and when administered to schizophrenic patients, amphetamine would exacerbate positive symptoms. These effects were present in both males and females. Furthermore, these effects were also manifested in schizophrenia patients never exposed to neuroleptic drugs, supporting the claim that the DA change is associated with the illness and not a result of neuroleptic treatment. With respect to the illness phases of schizophrenia, the effect was more pronounced during episodes of illness exacerbation, and is present at the onset, however, it does not worsen as the illness progresses and is not present during periods of remission (Laruelle, 1999). While these important findings offer support to the hypothesis of DA dysregulation being an integral part of schizophrenia, they offer little toward any explanation for the negative symptoms associated with schizophrenia nor do they provide much insight into periods of remission.

More recent hypotheses of the neuropathophysiology of schizophrenia include an interaction between DA and Glu (Laruelle et al., 2005). Laruelle et al., (2005) suggest that schizophrenia

pathology is associated with interconnected dysfunctions and abnormalities in both the Glu and DA systems underlie the pathogenesis. More specifically, one current theory of schizophrenia proposes that NMDA hypofunction within the PFC will result in a pattern of dysregulation in the DA system, which could, in turn, further damage and weaken the glutamatergic connectivity (Laruelle et al., 2005) (see Figure 1.3).

1.8.5. Gender Differences and Co-morbidities in Schizophrenia.

Many gender differences exist in schizophrenia. For instance, women generally have a later onset of illness than men (Häfner, 2003). As well, with respect to social and symptom related course of illness, men fare poorly compared to women during a younger age, however, after menopause, women tend to fare worse than men, suggesting hormonal and age-dependent differences in men and women (Häfner, 2003). In another study, Salokangas et al., (2003) investigated age related differences between genders in the diagnosis of schizophrenia and reported that women had a slightly later age of first admission than males, but with a peak increase much later, approximately 40-44 years of age. In particular, paranoid schizophrenia appears to have a later onset for women than men (Salokangas et al., 2003). Among those diagnosed with schizophrenia, women also have superior social skills than men, and this difference is not present in patients with affective disorder or in non-patient controls (Mueser et al., 1990). Improved social skills and functioning are also related to improved outcome with schizophrenia (Preston, 2000). It is suggested that these improved social skills in females, relative to males, are due to the later onset of the illness (Häfner, 2003).

Others have suggested that estrogen during brain development provides protection for symptoms of schizophrenia in women (Rao & Kölsch, 2003). However, in contrast to Salokangas et al. (2003), data would appear to offer support for the suggestion that estrogen is protective against the negative symptoms of schizophrenia rather than paranoid schizophrenia (Rao & Kölsch, 2003). Finally, other data has been offered to support the idea that estrogen interacts with treatment, with females showing better treatment responses during the menstrual cycle (i.e. when estrogen is high) (Rao & Kölsch, 2003).

Schizophrenia is a disorder which is often associated with co-morbid diagnosis. One of the most commonly reported co-morbid diagnosis with schizophrenia is substance abuse. Further, substance abuse is also more commonly associated with male patients and with positive symptoms of schizophrenia (Talamo et al., 2006). Additionally, Rosen et al., (2006) report the most common co-morbid diagnoses in patients who have met the criteria for the prodromal stage of schizophrenia are major-depressive disorder and cannabis dependence. In this particular study, although there were other co-morbid conditions found in patients meeting the criteria for schizophrenia prodrome, there was not a greater prevalence for any particular co-morbid condition, compared to patients not meeting this criteria (Rosen et al., 2006). Furthermore, cigarette smoking is very common within the schizophrenia population, even when other factors, such as institutionalism, substance use and antipsychotic treatment, are controlled for (de Leon et al., 1995). Others have demonstrated that not only is smoking more prevalent in patients with schizophrenia, but that smokers with schizophrenia also smoke more often and more intensely than do non-psychiatric patients (Tidey et al., 2005).

Schizophrenia is currently believed to be a neurodevelopmental disorder (section 1.8.), with onset typically delayed until adulthood. However, it is also interesting to examine cases where the diagnosis of this condition occurs at a younger age. Interestingly, in childhood-onset schizophrenia there are many co-morbid diagnoses (Ross et al., 2006). In one recent study, data revealed that in 81 children with schizophrenia, 99% presented with a co-morbid disorder. The most commonly associated disorders were ADHD, oppositional defiant disorder, depression, and separation anxiety disorder (Ross et al., 2006). Öner & Monir, (2005) investigated attentional and cognitive skills of children with ADHD and children at high risk (HR) of schizophrenia (parents with schizophrenia) and age matched controls. Within the HR population of children (n=24) a total of 11 were diagnosed with ADHD. Children with ADHD and HR presented with deficits in attention and cognition, however, children with HR and ADHD had an even greater detriment (Öner & Munir, 2005). While such studies are interesting, and important in their own right, it is important to note that childhood-onset schizophrenia is considered more severe than adult-onset and it is therefore difficult to make direct comparisons between the two.

1.9. Animal Models of Schizophrenia

Schizophrenia is a complex mental illness with symptoms that include disorganized thought and speech pattern, hallucinations, delusions and dysfunctions in other solely human behaviors. It appears then, that it would be a daunting, if not an impossible task, to model this disorder in animals. However, for several years this has been the very challenge that many researchers have undertaken (reviews Lipska & Weinberger, 2000; Powell & Miyakawa, 2006; van den Buuse et al.,

2005). The question, however, then becomes: what can actually be modeled in animals, if schizophrenia is indeed a uniquely human mental illness? First, to address this question, it is important to understand the validity of animal models and how they are used to mimic mental disorders.

1.9.1. Validity in animal models

In a recent review paper, Kalueff & Tuohimaa (2004) explored the limitations and benefits of animal models of anxiety and depression. Issues that were raised/addressed in this review are also relevant to animal modeling of schizophrenia, and will therefore be discussed, in brief, here. As pointed out by Kalueff & Tuohimaa (2004), a good animal model should ideally have face validity, predictive validity and construct validity. Face validity means that the model has similarities in phenomena to the human pathology (Kalueff & Tuohimaa, 2004). However, this criterion can be difficult to meet, since disorders often have a wide range of symptoms; symptoms which most often cannot be directly reflected in a single animal model or perhaps even in animals (Powell & Miyakawa, 2006). More often, good animal models are able to demonstrate predictive and construct validity. Predictive validity reflects the ability of the model to predict the therapeutic efficacy of drugs/manipulations that may be effective in humans (Kalueff & Tuohimaa, 2004). This is important, as predictive validity is often the criterion used to verify the usefulness of a novel animal model by using currently available efficacious drugs and demonstrating a beneficial change in the animal in response to this drug. In turn, this animal model may then be used as a tool to screen for novel drug therapies. Construct validity means that the theoretical rationale

behind the model reflects the current theories of the human disorder (Kalueff & Tuohimaa, 2004). An example of an animal model demonstrating construct validity, is the immuno-precipitated neurodevelopmental rodent model of schizophrenia (see section 1.9.3.2.) which reflects one of the current theorized risk factors of schizophrenia. Construct validity is most commonly what is first reflected in animal models, and animal models are often designed to reflect the current theories behind the disorder and the current etiological considerations.

1.9.2. Modeling schizophrenia symptoms in rodents

Which specific behavioral paradigms should be used to mimic symptoms in schizophrenia are not completely agreed upon in the research community. Animal models of schizophrenia often use many different behavioral paradigms to reflect negative/positive symptoms and cognitive deficits that are seen schizophrenia. Behavioral paradigms can reflect symptoms that are seen in the clinical populations either directly (e.g. Pre-pulse inhibition (PPI); see Figure 1.4) (reflective of cognitive deficits) or perhaps indirectly (e.g. reflecting behaviors with some similarity to the population but that cannot be directly mimicked, such as response to novelty which may be indicative of either positive or negative symptoms). Some of the symptoms that are mimicked in animal models are not unique to schizophrenia (e.g. negative and cognitive symptoms) and may be, and often are, reflected in other disorders. This is actually reflective of the clinical population, as some symptoms of schizophrenia are not unique to the disorder in humans and may be seen in disorders such as depression (Candido & Romney, 2002), bi-polar disorder (Murray et al., 2004), epilepsy (Matsuura et al., 2004) and addictions (Kedzior & Martin-Iverson, 2006). There is also a

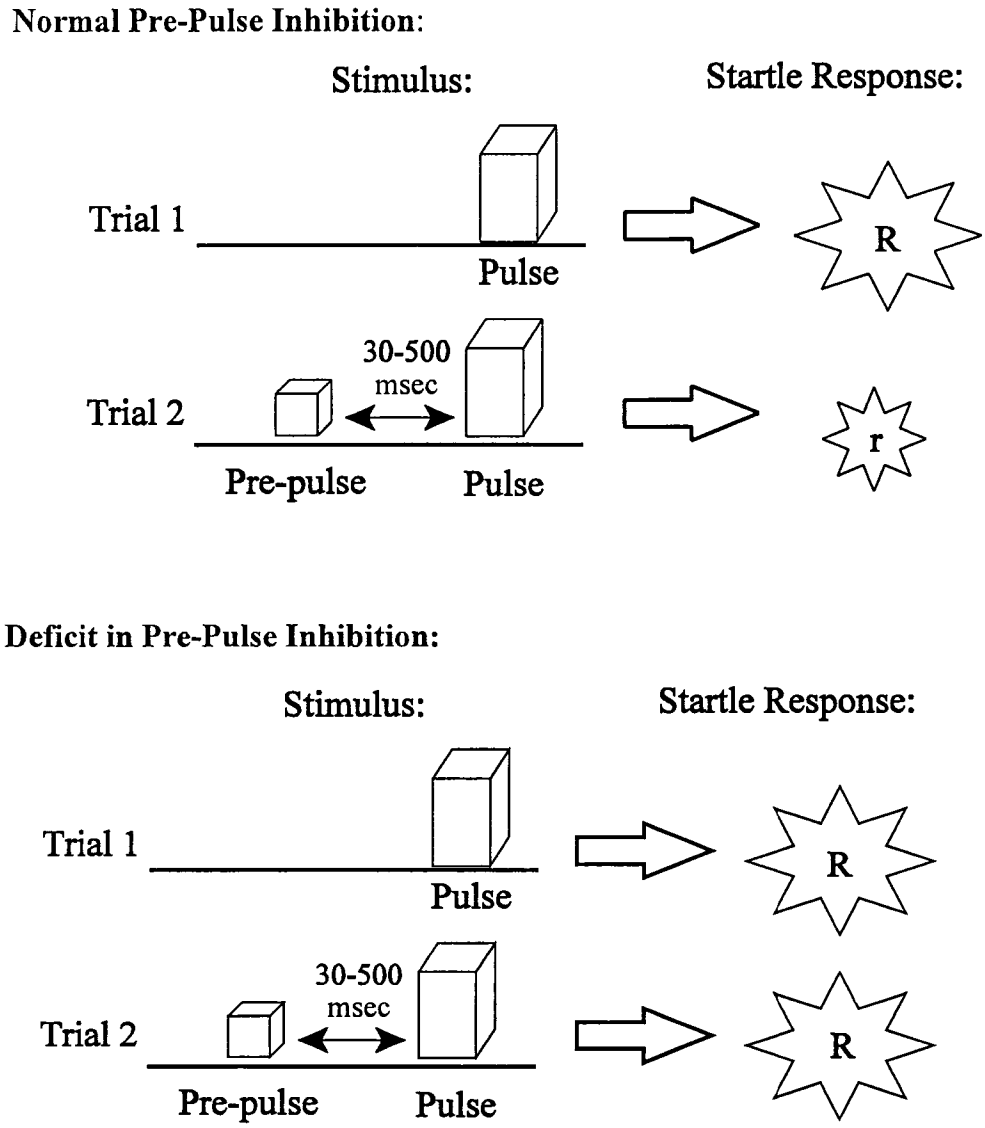


Figure 1.4. Pre-pulse inhibition. Adapted from Swerdlow et al., (2000).

high co-morbidity with schizophrenia and other disorders such as epilepsy (Giatatzis et al., 2004) and drug abuse (Talamo et al., 2006). Therefore, in order to determine if an animal model is relevant to schizophrenia, several behavioral paradigms are typically used. Also there are neurochemical and neuroanatomical similarities that are often investigated, however, this is difficult since there are many confounding factors in the clinical population, such as antipsychotic treatment. The difficulty and complexity of modeling this disorder is however understandable, as it reflects the complexity of the disorder itself.

1.9.2.1. Cognitive Symptoms

Behavioral paradigms directed at cognitive deficits often include PPI, latent inhibition (LI) and the MWM. There are other behavioral paradigms that are used, however, these are perhaps the most common.

Pre-pulse inhibition is commonly used in animal models of schizophrenia since it is disrupted in people with schizophrenia (Braff et al., 2005; Candenhead, 2000; Mackeprang, 2002) and even in relatives who do not have schizophrenia (Candenhead, 2000). Deficits in PPI reflects deficient sensorimotor gating (Swerdlow et al., 2000). Sensorimotor gating is the habituation to repetitious, unimportant stimuli. Essentially, it is a way for the brain to focus on the important stimuli and reserve its resources (Javanbakht, 2006). This is an important brain function, as we are often exposed to simultaneous stimulation in the world and normally we learn to focus our attention on the important stimuli, while filtering (or gating) the irrelevant or less important stimuli

(Javanbakht, 2006). Pre-pulse inhibition is a phenomenon that is present in all mammals, and is one of the simplest measures of sensorimotor gating. Typically, a weak pre-pulse will inhibit the startle reflex to a more powerful stimulus, and the degree to which the reflex is inhibited is measured and can be compared to the reflex without the pre-pulse in the experimental setting (Swerdlow et al., 2000). A deficit in pre-pulse inhibition is manifested as the failure to inhibit the reflex with the presence of a pre-pulse (see Figure 1.4). Deficits found in PPI are reflective of dysfunctions in the neural substrates that modulate PPI such as the hippocampus, mPFC, basolateral amygdala, NAc, and the pedunculopontine nucleus (cortico-striato-pallido-pontine circuitry) (Swerdlow & Geyer, 1998). Therefore alterations in PPI reflect not only an inability to appropriately filter sensory information, but could also reflect mild alterations leading to attentional and cognitive impairments, which could lead to some of the symptoms manifested in schizophrenia (Braff & Geyer, 1990).

The inability to properly filter sensory information, and having dysfunctions in the neural substrates mentioned above, could lead to the misinterpretation of incoming information, resulting in delusions and hallucinations (Javanbakht, 2006). However, deficits in PPI are not unique to schizophrenia and can be seen in other clinical populations such as Parkinson's disease, dementia with Lewy bodies (Perriol et al., 2005), Tourette's co-morbid with ADHD (Castellanos et al 1996), Schizotypal personality disorder (Candenhead, 2000) and chronic cannabis abuse (Kedzior et al., 2006). However, some researchers suggest that deficits in PPI are nonetheless, a reliable trait marker for schizophrenia and that it a candidate phenotype of schizophrenia, but not a primary symptom. Therefore other conditions with alterations in similar neural substrates could also result

in deficits in PPI, but it is reliably found as a deficit in the schizophrenia population. Pre-pulse inhibition has been investigated to determine the genes that are important in PPI and this might be used to help identify genes affected in schizophrenia (Joober, et al., 2002).

Latent inhibition is also a sensorimotor deficit that is found in patients with schizophrenia (Schmajuk, 2005). Latent inhibition is a phenomenon in which pre-exposure to a conditioned stimulus (CS) causes a decrease in the subsequent generation of conditioned responses (CR) when the CS is later paired with a unconditioned stimulus (US) (Barker, 2001). The hippocampus and the NAc are involved in normal functioning of LI, and disruptions within either brain region can cause an impairment in this task (Schmajuk, 2005). A disruption in LI would result in the previous exposure to the CS, not resulting in a decrease in the generation of a CR when it is later paired with a US. This has been demonstrated in patients in the acute phase of schizophrenia, but it is later repaired with the treatment of antipsychotics (Schmajuk, 2005). A decrease in LI has also been reported in various animal models of schizophrenia, including the neonatal ventral hippocampal lesioned (nVH) model (Grecksch et al., 1999) (see section 1.9.3.3.) and the immuno-precipitated neurodevelopmental model (Zuckerman et al., 2003) (see section 1.9.3.2.).

The MWM is a test of spatial processing, and is often used to assess cognitive function (the maze consists of a round pool filled with opaque water). There are at least two general methods for running the maze: (1) the hidden platform version and (2) the visible platform version. Using the hidden platform task, a platform is placed in a fixed location in the pool about 2 cm below the water surface, so that it is not visible. The rat must use room cues to find the platform and escape

the water. Under normal conditions a rat will readily locate the platform, and will do so with increasing ability from any location of the pool, with further trials (i.e. indicates "learning"). The idea is that the rat will construct a spatial map of the pool to find the platform using room cues (Morris, 1984). This test relies on the ability of the hippocampus to form a spatial map, and dysfunctions in hippocampal structure/function will result in poor performance in the maze (Martson et al., 1993). In the visible platform method, the platform is located above the surface of water. This procedure uses different learning strategies (i.e. studies suggest that rats rely on the basal ganglia rather than the hippocampus to solve this task [Packard & Knowlton, 2002]), and is also used to test the rats swimming and visual abilities and degree of motivation (Morris, 1984).

Interestingly, a study by Hanlon et al., (2006) compared patients diagnosed with schizophrenia and control participants in a computer version of the MWM, known as a virtual MWM. The virtual MWM uses the same general procedure as does the standard MWM (used for rodents). Results from this study indicated that patients with schizophrenia traveled farther and took longer to reach the hidden platform than did the control participants. This is similar to results of animal models of schizophrenia, specifically in the nVH model (Silva-Gomez et al., 2003). There was no difference found with the visible platform, however, it is difficult to determine there are no deficits in this learning strategy as it may have actually reflected how easy the task was (Hanlon et al., 2006). The results of this study are important as they offer a more direct link between animal and human studies. However, in human studies, there is the potential confound of treatment, with the schizophrenic patients receiving treatment with anti-psychotics which, in turn, could also impact on spatial processing (Hanlon et al., 2006). Although this confound is present and should be

acknowledged, it is a confound that is present in all clinical research. This fact underscores the importance of and need for good animal models, as they allow for controlled conditions that would not be ethical in research conducted with the clinical population.

1.9.2.2. Negative Symptoms

The negative symptoms of schizophrenia can be modeled in animals. However, there are only a couple of symptoms which are commonly reflected in animal models. These symptoms include social withdrawal and the associated negative symptom, anhedonia (reviewed by Ellenbroek & Cools, 2000). Other classic negative symptoms of schizophrenia such as avolition, apathy and alogia are all obviously very difficult to model in animals (Ellenbroek & Cools, 2000).

Ahedonia is listed as associated negative symptoms in the DSM-IV-R (2000) and can be seen in other disorders such as depression. It reflects a reduction in the capacity for pleasure and is very common in schizophrenia (Wolf, 2006). This can be modeled in animals by using appetitive or drug rewards, and measuring their consumption in comparison to control animals. One of the most common measures of appetitive reward in animals is measuring sucrose or saccharin solution consumption (Ellenbroek & Cools, 2000). However, there are several experimental methods used including a basic two choice task (water or sucrose) for either a restricted period of time (Turgeon & Hoge, 2003) or for a 24 hour period (Shimura et al., 2002). Other techniques include more complex methods using conditioning and measuring the motivational levels of the animals by determining the amount of effort the animal is willing to exert for the reward (Merali et al. 2003).

When looking at drug reward, common procedures include the CPP test (LePen et al., 2002) or self administration (Chambers & Self, 2002) using addictive drugs such as amphetamine (LePen et al., 2002) or cocaine (Chambers & Self, 2002). Such tasks are also relevant since appetitive and drug rewards are reliant on the mesocorticolimbic pathway (discussed in section 1.3.), a pathway which also appears altered in the brains of schizophrenic patients (section 1.8.4.2.). However, such differences make it difficult to interpret animal models of anhedonia. For example one study looking a the nVH model (described in section 1.9.3.3.) found that rats demonstrated an increase in sucrose consumption (using pellets instead of a solution) and drug self-administration. These findings could be interpreted to reflect positive symptoms and are reflective of the high rate of drug addiction observed in the schizophrenic population (Chambers & Self, 2002). However, using the same model a decrease in preference for saccharin solution and amphetamine CPP was noted in rats subjected to nVH suggesting that this model could also mimic the negative symptoms of schizophrenia (LePen et al., 2002). Therefore, results from studies need to be looked at critically since different methods used for sucrose consumption and tests such as self-administration and CPP and different drugs could lead to different results in the same animal model.

Social withdrawal is considered to be a negative symptom of schizophrenia (DSM-IV-TR, 2000) and is also a symptom of premorbid functioning (e.g. with social withdrawal in childhood associated with symptoms later in adulthood) (Baum & Walker, 1995). Social withdrawal and functioning is also associated with community survival in patients with schizophrenia, with increased antisocial behavior predicting poorer community survival (Preston, 2000). Social interaction in rodents was characterized by File, (1980) as a measure for detecting anxiolytic

effects of drugs. The optimal procedure involves placing two rodents, matched for sex and treatment, in an unfamiliar environment and observing the interaction between the two animals. In this test, a decrease in social interaction is reflective of social withdrawal. Social interaction has been adapted by many researchers interested in animal models of schizophrenia. The basic method which is commonly used is the consistent in most studies, however specific variables do vary across studies. For instance, social interaction paradigms vary dramatically with respect to the age of the rodents tested (e.g. some studies test adult rodents [Silva-Gomez et al., 2003], others assess adolescent rodents [Venerosi et al., 2006]). The varying ages reflect interesting questions for the clinical population, as social withdrawal is a symptom that is present before the onset of schizophrenia (Baum & Walker, 1995), but direct comparisons between results presented from such studies are difficult to make.

1.9.2.3. Positive Symptoms

The positive symptoms of schizophrenia can also be modeled in animals. Behaviors that are considered to have relevance to positive symptoms include increased locomotor activity, response to novelty, and increased sensitivity to DA agonists (amphetamine) and NMDA antagonists (MK-801 and phencyclidine [PCP]) (Powell & Miyakwa, 2006). Researchers who have used the nVH model (see section 1.9.3.3.) have reported that treated rats show an increase in spontaneous locomotor activity in and increase in responsiveness to a novel environment (Black et al., 1998). This increase in activity levels, seen in treated rats when compared to control rats, is also differentially expressed throughout development and between genders. For instance, while both

male and female rats exhibited this increase in spontaneous locomotion, it was observed only during post adolescence, and this effect was expressed later in development in the female rats when (PND100) compared to the male rats (PND56). These data are suggested to mimic the delayed onset of symptoms seen in the clinical population, which do not typically manifest until the post adolescence period, and the increased delay for onset of symptoms within females ultimately diagnosed with schizophrenia (Black et al., 1998).

Silva-Gómez et al., (2003) reported similar results with the nVH model with respect to increased locomotor activity in response to a novel environment. These authors reported an increase in rodent activity levels in a novel environment during post adolescence, however they did not find a delay in this measure in the nVH female rats. Silva-Gómez et al., (2003) suggest that this difference is because they monitored (and controlled for) the estrous cycle, and locomotor tests were conducted during the estrous stage of the females (a variable that was not controlled for by Black et al., [1998]).

Another animal model of schizophrenia, (i.e. immune activation during pregnancy; see section 1.9.3.2.) used the measure of spontaneous locomotion and locomotion in response to amphetamine as potential symptom of positive symptoms (Zuckerman et al., 2003). However, in this particular study, no differences were found between treated and control rats in activity levels, either with or without amphetamine (Zuckerman et al., 2003).

Within the nVH model, tests using psycho-mimetic drugs have also been implemented. Such

studies have found an increased response to the PCP, using a measure of activity, post adolescence (Kato et al., 2000) and also an increase in responding to the rewarding properties of cocaine, as measured by self-administration (Chamber & Self, 2002). This demonstrates how activity levels and response to DA agonists and Glu antagonists are commonly used as measures of potential positive symptoms. However, the number of measures used to detect schizophrenia symptoms reflected in animals, are expanding as our number of animal models expand. The advancement in our models of the etiology of schizophrenia, will result in an advancement of measures of schizophrenia symptoms (Powell & Miyakwa, 2006).

1.9.3. Modeling Schizophrenia in Rodents

1.9.3.1. Drug Induced (Amphetamine, MK801, PCP) Models

Drug induced schizophrenic-like symptoms in animals was one of the first animal models of schizophrenia (Gambell & Kornetsky, 1976). Such models are used to mimic the alterations in the neurotransmission that is seen in the clinical population. Dopamine agonists, such as amphetamine, result in symptoms which represent the excess in DA transmission (see section 1.8.4.2.). It has been demonstrated that a large enough dose of amphetamine can induce a psychotic episode in humans. Also the schizophrenic population is more sensitive to the effects of amphetamine resulting in increased release of DA and an exacerbation of psychotic symptoms (Ujike, 2002).

Interestingly, Tenn et al., (2005) proposed an amphetamine-induced animal model of the prodromal state of schizophrenia. The study was based on earlier findings which demonstrated an amphetamine-induced sensitized state in the rat which had similarities in neurochemical and cognitive alterations to human schizophrenia patients (Tenn et al., 2003). Using a regimen of a series of escalating doses of amphetamine (1-5 mg/kg) they demonstrated a heightened response to amphetamine, disruptions in PPI, and altered binding in D2 receptors, similar to that seen in the clinical population (Tenn et al., 2003). Tenn et al., (2005) examined not only the amphetamine-induced sensitized state, but also looked at a partial sensitization. Typical and atypical antipsychotics were used on the partial and complete amphetamine-induced animals and compared with appropriate controls. The amphetamine sensitized state included a full regimen of amphetamine, with daily i.p. injections of amphetamine for three weeks, with increasing doses introduced each week (week 1= 1mg/kg, week 2= 2mg/kg, week 3 = 3mg/kg). The partial sensitization included a partial regimen, with only the first week of injections and SAL for the second and third week. They demonstrated that the partial regimen did not produce all of the alterations seen in the full regimen and that intervention with antipsychotics also prevented all of the alterations seen in the full regimen (Tenn et al., 2005). This study is interesting, as it not only postulates an animal model of chronic schizophrenia, but also a model of prodromal stage of schizophrenia and may have predictive validity (see section 1.9.1.) for preventative treatments of schizophrenia psychoses (Tenn et al., 2005).

Glutamate antagonists such as MK801 and PCP are also used to mimic schizophrenic-like symptoms. Both compounds are non-competitive antagonists of the NMDA receptor and actually

bind to the same site actually known as the PCP binding site located inside the pore of the NMDA receptor (Morris et al., 2005). Phencyclidine is a drug that is used illicitly and causes symptoms such as hallucinations, and can cause a profile similar to schizophrenia and exacerbates the symptoms in schizophrenic patients (Morris et al., 2005). Turgeon & Hoge (2003) demonstrated that exposure to PCP (15mg/kg) 20 hours prior to testing resulting in a decrease in sucrose consumption and also decreased responding for food reward in an operant conditioning task in rats. This was hypothesized to be due to PCP-induced attentional deficits and to be reflective of the negative symptom, anhedonia, in schizophrenia. Rung et al., (2005) found MK801 to also produce alterations in behavior reflective of negative symptoms of schizophrenia. It was demonstrated that single injections of MK801 at varying doses not only increased motor activity, but also decreased social interaction in the rat. This was compared to amphetamine, where an increase in motor activity was found but no decrease in social interaction was observed. It is suggested that MK801 administration is a model for both positive symptoms (e.g. represented by increased motor activity) and negative symptoms (e.g. represented by a decrease in social interaction) (Rung et al., 2005). Deficits in PPI have also been detected with the administration of both PCP (Pouzet et al., 2005) and MK801 (Levin et al., 2005).

Based on the evidence presented, drug induced animal models of schizophrenia appear to have face validity. However, the models have only minimal construct validity. Although amphetamine models represent the dysfunction in DA functioning and PCP and MK801 models represent dysfunction in Glu functioning, they are not reflective of the neurodevelopmental theory of schizophrenia.

1.9.3.2. Immuno-precipitated neurodevelopmental Model

The primary aim of immune-based neurodevelopmental animal models of schizophrenia is to mimic a possible risk factor found in the human population. Early exposure to infection, both prenatally, and early postnatally results in an increased risk for the later development of schizophrenia (see section 1.8.1.). Zuckerman & Weiner (2005) developed one such model in rats, using the synthetic cytokine releaser polyriboinsinic-polyribocytidilic acid (poly I:C), which mimics viral infection, in pregnant rats. The pregnant rats were administered 4 mg/kg of poly I:C on gestational day 15 and the offspring were then later tested during adulthood. The infected offspring demonstrated a loss of LI (a sensorimotor gating task, see section 1.8.2.1.), and an enhanced reversal learning in a water-based t-maze. Both tasks represent prior experiences having less influence on current experience as compared to the control animals. These differences were corrected by the atypical antipsychotic clozapine, demonstrating a predictive validity of the this model. There were also differences found with an increase in activity levels with MK-801 administration, however, there were no differences found in the MWM (Zuckerman & Weiner, 2005). This model demonstrates how maternal immune infection results in alterations in sensorimotor gating that can be seen in schizophrenia and can be corrected by antipsychotic treatment, as well as alterations in the Glu system as demonstrated by the sensitivity to MK-801. This provides some face validity for the model and is promising, as the model also is based on current etiological theories of schizophrenia as a neurodevelopmental model that has increased risk with maternal immune response. Another study by Fortier et al., (2004) proposed a similar model using a bacterial endotoxin lipopolysaccharide (LPS) (50 μg/kg) administered on gestational day

18 and 19 of pregnancy in the rat. The results of this study demonstrated an increase in amphetamine induced locomotion and in acoustic startle in PPI in the resulting offspring in adulthood.

Immuno-precipitated animal models of schizophrenia demonstrate face, predictive and construct validity. However evidence is still preliminary, and further characterization of these, and similar models will aid in the validity of the model. The exact mechanisms involved to result in such alterations is not yet understood, therefore only provide preliminary construct validity. Also, exposure to infection is only one potential risk factor for schizophrenia and therefore only provides validity to a subgroup of the clinical population.

1.9.3.3. Neonatal ventral hippocampal lession (nVH) Model

This model was originally developed by Lipska et al. (1993), and is probably one of the most studied and reflects the current theory of schizophrenia as a neurodevelopmental disorder. This model involves lesioning the ventral hippocampus using ibotenic acid, on PND 7 of the rat. This animal model of schizophrenia represents the theory of an alteration in the hippocampus of schizophrenics, and that it is a neurodevelopmental disorder which originates with some insult to the hippocampus during early development.

There have been several behavioral alterations demonstrated in the nVH model. For instance hyperactivity in a novel environment and sensitivity to the locomotor effects of amphetamine were

demonstrated in adulthood (PND56) but were not present at adolescence (PND35) (Lipska et al., 1993). This demonstrates the face validity, such as the post-adolescent onset of symptoms and is reflective of positive symptoms (following the same course as schizophrenia). Silva-Gomez et al., (2003) reported a decrease in social interaction in both the male and female nVH rats, however, there was a deficit found in only the males in the MWM in latency to find the platform. This also reflects the gender differences that are found in schizophrenia. Interestingly, Alquicer et al., (2004) examined DA content in the brain of the nVH rats and also nVH rats which were socially isolated immediately after weaning (PND 21) and appropriate controls. They demonstrated that nVH rats had an elevation in DA content in the hippocampus, while social isolation alone caused an increase in DA content in the limbic regions such as the PFC, NAc and hippocampus. However, the effect of social isolation in nVH was an exacerbation of these increases except in the NAc. This suggests a sensitivity to the alterations in the dopaminergic system to stressors such as social isolation in the nVH model (Alquicer et al., 2004). Such alterations demonstrate face validity for this model of schizophrenia.

1.9.3.4. Genetic based mouse models

There have been several genes identified as susceptibility genes for schizophrenia, including COMT, DTNBP1, NRG1, RGS4, DISC1, and G72 (Harrison & Weinberger, 2005) (reviewed in section 1.8.2.). Therefore, animal studies have been developed which target the susceptibility genes using mice. Results from such studies have yielded inconclusive evidence for a phenotype relevant to schizophrenia (review O'Tuathaigh, 2006). Mouse models targeting the NRG1 and

DISC1 genes, have found alterations in behavioral phenotypes relevant to schizophrenia (O'Tuathaigh, 2006). However, other mouse models targeting the RGS4, DTNBP1, COMT genes have not yielded behavioral phenotypes which are relevant to schizophrenia (O'Tuathaigh, 2006). However, whether or not the genetic mouse models provide behavioral phenotypes relevant to schizophrenia, understanding the roles schizophrenia susceptibility genes play in development, brain metabolism and function of relevant neurotransmitter systems (such as Glu and DA), has provided understanding of the roles these genes could play in the development of schizophrenia (O'Tuathaigh, 2006).

There are other genetic mouse models of schizophrenia, which do not directly target specific schizophrenia susceptibility genes, but rather genes targeting features that have been identified as altered in schizophrenia. An example of one such animal model is the NR1 hypomorphic mouse. This genetic mouse model has chronically and developmentally reduced in NMDA receptors, specifically this results in a large reduction in the NR1 subunit (Duncan et al., 2004). Results from this genetic mouse model has demonstrated a deficit in PPI and a decrease in social affiliation. The social affiliation test was similar to the social interaction test (described in section 1.9.2.2.), however, the test mouse was placed in a covered open field with an unfamiliar mouse and there is no direct interaction between the mice. The NR1 hypomorphic mice spent less time on the side of the arena with the unfamiliar mouse (Duncan et al., 2004). Interestingly, this model was tested for predictive validity using typical and atypical antipsychotics in restoring PPI, however, the results indicated there were no improvements found above what was demonstrated also in the control (wild-type) mice (Duncan et al., 2006). However, it was suggested that the NR1 hypomorphic

mouse model may provide potential as a tool to test novel antipsychotics that do not target the dopaminergic system (Duncan et al., 2006). This model demonstrates face validity and construct validity, mimicking the NMDA hypofunctioning in schizophrenia. However, it failed to provide predictive validity using a few of the existing antipsychotics, but this does not mean there is not future potential for this model in providing predictive validity.

1.9.4. Summary of Animal Models of Schizophrenia

The existing animal models of schizophrenia which model the current theories of the etiology of schizophrenia, demonstrate how much is still to be understood about this disorder. Each of the models reflects at least one aspect of schizophrenia. However, the primary symptoms of schizophrenia such as disorganized thinking and speech, hallucinations, and delusions, cannot be directly modeled in animals. This leaves only symptoms which are often reflected in the prodromal stage of schizophrenia and even in other disorders. However, this does not mean the models are of no importance because they cannot model the primary symptoms of schizophrenia. Each model provides insight into risk factors, neural dysfunctions and provides tools for novel treatments. Therefore, each model provides new insight and understanding and each model has strengths and weaknesses and are actually not in direct competition. There will not be one "ideal" model of schizophrenia, as schizophrenia is a disorder where the exact etiology is most likely different for each individual.

1.9.5. Comparing and contrasting the perinatal domoate rat model to existing rodent models of schizophrenia

In order to begin to look at the perinatal domoate rat model as a potential model of schizophrenia, we must look at the validity of this model and determine what is known and what needs to be explored. This animal model presents with seizure-like behaviors similar to temporal lobe epilepsy (see section 1.6.). Interestingly, there is co-morbidity with schizophrenia and epilepsy. Within epilepsy, the rate of psychoses averages around 2 to 7%, while this rate is much higher than in the general population, ranging around 1%. When looking specifically at temporal lobe epilepsy, the rate of co-morbidity with psychosis raises to 10 to 19%, much higher than the normal population (Giatatzis, 2004). Perhaps by further investigating this model by focusing on behaviors relevant to schizophrenia, we will gain some understanding of the similarity in underlying developmental pathology in schizophrenia and epilepsy.

Interestingly, there are alterations found in the hippocampus of the schizophrenia population (see section 1.8.4.1.). It has been shown that there is a decrease in cell count in the CA1 and CA3 region of the hippocampus in the schizophrenic population (Jönsson et al., 1999 and Luts et al., 1998). This is consistent with Doucette et al., (2004) where a decrease in cells in the CA1 and CA3 of the treated animals was found. Also there has been reported an increase in BDNF mRNA in the hippocampus of schizophrenic patients (Takahashi et al., 2000). This is also consistent with Doucette et al., (2004) where elevations in BDNF mRNA in the hippocampus of these treated animals was noted. This also demonstrates neuroanatomical and neurochemical alterations within

this model that are reflective of the clinical population of schizophrenia, also providing some face validity of the model.

Some of the observations seen in these animals have suggested an alteration in the mesocorticolimbic DA system pathway (see section 1.6). Such observations include the altered response to a novel spatial environment and alterations in the hippocampus (Doucette et al., 2004). Further investigation is needed in order to know if the mesocorticolimbic pathway has been altered (neurochemically, neuroanatomically and/or functionally) in the perinatal domoate rat model. However, if the functional integrity of this pathway has been altered this model would provide construct validity. This model would reflect the neurodevelopmental theory (see section 1.8.), and the alterations seen in the Glu and DA systems (section1.8.4.2.). Therefore the basis of the following studies was to examine the functional integrity of the mesocorticolimbic pathway throughout development in the perinatal domoate rat. This would provide further information as to the face and construct validity and potential of this model as an animal model of schizophrenia.

1.10. The Perinatal Domoate Rat Model: Are There Behavioural Alterations in the Mesocorticolimbic Pathway Relevant to Schizophrenia?

The primary hypothesis of this thesis is that low doses of DOM, during a critical period of development, will result in alterations in behaviours reliant on the functional integrity of the mesocorticolimbic DA pathway. The secondary hypothesis of this thesis is that the perinatal domoate rat model has potential to serve as an animal model of schizophrenia.

In order to test the primary hypothesis, three different behavioural tests were used, the sucrose consumption test, the playground maze, and the nicotine-induced place preference paradigm. The sucrose consumption test was used as an assessment of response to reward. This test has previously shown a decrease in sucrose consumption in an animal model of schizophrenia, reflecting anhedonia, a negative symptom of schizophrenia (Le Pen et al., 2002). The baseline water consumption was measured to determine if there was a difference. An increase in general fluid intake is known as polydipsia, also symptomatic of schizophrenia (Brookes & Ahmed, 2006).

The playground maze was used as a test of response to novelty. This maze was chosen as it can be used to look at alterations in response to novelty independent of alterations in locomotion and spatial processing (Nicholls et al., 1992). This is important because alterations in the dopamine system can result in alterations in activity levels, and we have found differences in the hippocampus of the perinatal domoate rat, which is important in spatial processing. Therefore, the playground maze can be used as a tool for detecting alterations in response to novelty without the mentioned confounds. Also, others have shown the playground maze and the amphetamine place preference paradigm used in conjunction, demonstrated a correlation between response to novelty and drug seeking behaviours (Bevins et al., 2002). This is interesting as response to novelty and reward are both modulated by the mesocorticolimbic pathway (Missale et al., 1998).

Finally the nicotine-induced conditioned place preference paradigm (CPP) will be used to challenge the functional integrity of the dopaminergic system. Nicotine is a drug that has been

determined to be rewarding, because it directly activates the mesocorticolimbic pathway (Ikemoto et al., 2006; Balfour, 2002). Nicotinic administration results in the release of DA and Glu in the NAc (Reid et al., 2000). Interestingly, in schizophrenia there is a higher prevalence of smoking than in the general population (de Leon and Diaz, 2002) (mentioned in section 1.8.5). Also increased and decreased sensitivity to the rewarding properties of drugs has been used as positive and negative symptoms, respectively, of schizophrenia.

Using these tests, which all challenge the dopaminergic system, we will gain a better understanding of the effects of KA receptors in development and their impact on the dopaminergic system (see section 1.3). This will also help determine the potential of the perinatal domoate model as a potential model of schizophrenia, and help direct future directions for further research to characterize this model.

Chapter 2 "Determining the length of retention for object memory using the playground maze: A pilot study" was done to determine the procedure for the playground maze. This chapter was a pilot study looking at variations of inter-trial-intervals in the procedure of the playground maze. This study was done with the primary aim of exploring the reproducibility of the maze when used in or lab. The secondary goal was to determine the maximum length of retention for object recognition in the rat using the playground maze.

Chapter 3 "Drug-seeking and altered responses to novelty in adult rats treated neonatally with domoic acid" uses all three behavioral tests the sucrose consumption, playground maze and

nicotine-induced place preference paradigm during adulthood. Developmental assessments were also conducted including an open field test as an assessment of locomotor activity throughout development (pre-adolescence, adolescence, and adulthood). This chapter explores the role of DOM exposure, during development, on the effects on behaviors mediated by the mesocorticolimbic pathway in the adult rat.

Chapter 4 "Low dose domoic acid in neonatal rats abolishes nicotine induced conditioned place preference during late adolescence" used the behavioral paradigm the nicotine-induced CPP paradigm during adolescence in conjunction with developmental assessments. This chapter deals with developmental differences in drug reward, as adolescence is a period of increased sensitivity to the rewarding properties of nicotine. This will also aid in the understanding of KA receptors in the normal development of behaviors mediated by the mesocorticolimbic pathway.

Chapter 2

Determining the length of retention for object memory using the playground maze: A pilot study

Melissa A. Burt[a], Catherine L. Ryan[b], Tracy A. Doucette[a]

Departments of Biology[a] and Psychology[b], University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI, Canada, C1A 4P3

Abstract

The playground maze was developed to detect responses to novelty, and designed to measure locomotion and exploration separately. The playground maze is a relatively new paradigm and has, to date, only been used in a few studies. The normal procedure for the maze is to have a number of trials to familiarize the rat to the objects on the maze. After the last trial the rat is removed for an inter-trial-interval of 1 minute, then returned to the maze with one of the "familiar" objects replaced with a new "novel" object. However, other paradigms used to detect response to novelty have used varying inter-trial-intervals in order to increase the sensitivity and detect alterations in object recognition in the rat. Therefore, this pilot-study was designed to measure length of retention for object memory using the playground maze in the rat. The inter-trial-intervals used were 1 min, 24 hours, and 48 hours. The results demonstrate a detection of the novel object (measured by an increased exploration of the novel object) in the groups with 1 min and 24 hours, but not 48 hours inter-trial-intervals. This demonstrates when object memory is abolished in the rat on the playground maze (48 hours) and that the playground maze is reproducible in our lab.

2. Introduction

The playground maze was developed by Nicholls et al. (1992) for detecting alterations in response to novelty. This maze was designed to measure directed exploration and locomotion, separately. Previous tests for response to novelty often involved using a novel environment and the level of exploration in the environment was used as a measure of response to novelty. However, Nicholls et al. (1992) suggest that this may not be an accurate measurement of response to novelty, but rather that the level of locomotion may actually reflect the animals attempts to escape the novel environment rather than to explore it. Another problem with existing tests of response to novelty, which use locomotion as an indicator of novelty exploration, is that if drugs or other manipulations are being employed which themselves can alter locomotor levels, it is difficult to isolate the effects on novelty exploration. Therefore, the playground maze was developed in order to detect response to novelty in a familiar environment and to detect novelty directed behaviors in isolation from general locomotion.

Most of the studies to date which have used the playground maze have been interested primarily in altering the dopaminergic system, which modulates behavioral responses to novelty (Klebaur & Bardo, 1999; Nicholls et al., 1992; Mellanby et al., 1999). For instance Mellanby et al. (1999) used the playground maze to detect differences in response to novelty after altering both the dopaminergic system and the hippocampus. This was done by administering a tetanus toxin into the amygdala of some rats and to the hippocampus of other rats. This tetanus toxin resulted in an epileptic seizure originating from these locations. Measures of response to novelty showed that

there was a significant novelty effect in the both the amygdala injected, and control groups, but a novelty effect was not present in the hippocampal injected group. This could be due to the fact that the hippocampus is important in memory (i.e. to respond to novelty, you have to remember the familiar first), and damage could affect memory or it could also be due to the modulation of response to novelty by the hippocampus (i.e. motivational state).

Another study by Klebaur and Bardo, (1999) used the playground maze to discriminate between animals which were high novelty seekers and low novelty seekers, and then compared results between the two groups on the results of CPP using amphetamine. Results from this study revealed that high novelty seekers showed greater amphetamine CPP but do not show a difference in the level of locomotion when compared to the low novelty seekers. This study has implications towards the hypothesis that novelty seeking and drug seeking are neuropharmacologically related.

Nicholls et al., (1992) conducted a study looking at variations of the playground maze and its sensitivity to alterations in response to novelty and locomotion, due to drugs. The drugs used were choldiazepoxide (CDP), which targets the GABAergic system, and amphetamine, which targets the dopaminergic system. Choldiazepoxide (CDP+ increased the novelty effect, while amphetamine did not, and actually abolished it at the highest dose (4 mg/kg). The results of this study demonstrate that the effects of the drugs on locomotion and novelty directed explorations can be separated using the playground maze and can be sensitive to different doses of each drug.

All of these studies show how the playground maze is an effective tool for detecting differences in

response to novelty, and how it can be separated from effects on locomotion, due to an alteration of a system which modulates response to novelty.

The playground maze is routinely conducted using an inter-trial interval of one minute between the last familiarization trial and the novelty trial. However, it has been shown that object recognition is possible with longer retention intervals. Pitsikas et al. (2002) demonstrated that retention intervals for object recognition can last up to an hour, but is abolished after 24 hours. Other studies have also demonstrated longer lasting retention intervals for object recognition, however, these studies used only two objects (Forwood et al., 2005; Pitsika et al., 2002; Puma & Bizot, 1998). Therefore the objective of this study was to determine if object recognition is still possible over such long intervals in the playground maze, considering that the number of items to be remembered is much greater (e.g. a total of eight objects). Knowing the length of object recognition in the playground maze could increase the sensitivity of the maze for future studies, by allowing another measure to be manipulated in studies in which object memory is of interest. Also, another objective of this study was to examine the procedure to determine if it provides optimal object exploration in the rat. This is because one of the major selling points of the maze is the familiarization to the environment and the objects, however the objects are introduced in the first trial. Perhaps having an acclimation trial to the maze without the objects, may increase object exploration during the familiarization trials since the rat has been familiarized to the general environment of the maze first, therefore this is also being investigated in this study.

2.2. Method and Materials

2.2.1. Animals

Sprague-Dawley rats (Charles River Laboratories, St. Constant, PQ, Canada) were used for this study. The rats were adults (>90 days of age) at the time of testing and a total of 28 (20 males, 8 females) were used. All animals were housed with polypropylene caging with wood chip bedding and the colony room was maintained on 22.2°C with a 12 hour light/dark cycle (07:00-19:00h) with food (Purina Lab Chow) and water provided *ad libitum*. All procedures were approved in advance by the UPEI Institutional Animal Ccare Committee and adhered to the guidelines of the Canadian Council on Animal Care.

2.2.2. Playground Maze

The playground maze was a circular platform measuring 100 cm in diameter, and raised 54 cm from the floor. The circular platform had eight 14 cm circles placed evenly around the maze 20 cm from the edge (see Figure 2.1). Eight objects that fit within these circles were used as familiar objects, and one object, a yellow plastic dinosaur (13 cm (length) x 6 cm (height) x 4 cm (width)), which fit inside the circles, was used as the novel object (see Table I). All trials of the procedure were videotaped for later analysis. The time spent exploring each of the objects in the familiarization trials and the novelty trial were determined by visual scoring of the tapes.

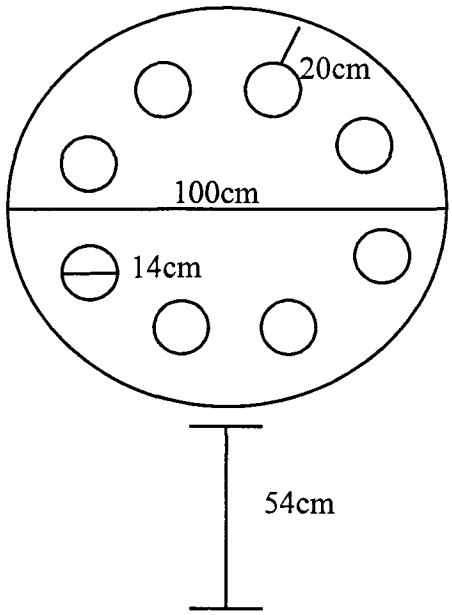


Figure 2.1. Dimensions of Playground Maze.

Object	Color	Approximate Dimensions (cm) (l x w x h) or (di; h)	Mean % time
Sauce Cup (Metal)	Silver	6; 4	16.50
Pencil Sharpener (Plastic)	Clear with black cap	3 x 5 x 7	18.49
Apple (Plastic)	Light Green	4; 6	10.09
Rectangular Dish (Plastic)	Purple	6 x 6 x 5	14.52
(Solved) Rubix Cube®	Orange/Blue/White/ Green/Red/Yellow (Blue facing up, green facing in)	5 x 5 x 5	11.87
Frog (Plastic)	Dark green	6 x 3 x 4	7.31*
Salt Shaker (Plastic with metal top)	Yellow with silver top	3; 7	8.31*
Egg cup (Metal)	Silver	4; 4	12.95

Table I. Description of objects used in playground maze during familiarization trials and mean percentage of time spent with each object in the first trial using all groups. Height (h), length (l), width (w), diameter (di). (* denotes a significant decrease using a one-sample t-test (μ=12.5%), $p<0.05$).

2.2.2.1. Familiarization trials

The procedure for the playground maze was adapted from Nicholls et al., (1994). In brief, it included a total of four familiarization trials followed by a novelty trial. The familiarization trials involved presentation of the same objects at three different locations. The novelty test trial involved replacing one of the now familiar objects with a novel object. Each familiarization trial was three minutes in length and 24 hours apart. The familiarization trials included eight familiar objects, one placed in each of the eight circles on the maze. The rats were each placed in the middle of the maze for a three minute trial and allowed to freely move around the maze. The rat was then removed from the maze, and the maze and objects were wiped clean between animals using Windex®. The location of each of the objects on the maze was changed for each of the four trials, with locations pseudo randomly selected.

2.2.2.2. Novelty trial

The procedure for the novelty trial consisted of removing the rat from the maze after the fourth, and last, familiarization trial. The rat was placed in a holding cage (Groups 1 and 2: see below) or out of view of the maze or returned to home cage (Groups 3 and 4: see below) for the retention interval. During this time, one of the familiar objects was removed, with the remaining seven still in the same location, and the novel object, the "dinosaur," was placed in the location of the object that was removed. The object removed was different for each rat in the group, and pseudo-randomly chosen. After the retention interval, the rat was then placed back in the middle of the

maze facing away from the novel object and allowed to freely move around the maze for a total of three minutes.

2.2.2.2.1. Group 1 (1 minute interval)

This group contained a total of 7 rats (3 males, 4 females). The inter-trial retention interval for the novelty trial was one minute, during this time each rat was placed in a holding cage.

2.2.2.2.2. Group 2 (24 hour interval)

This group consisted of a total of 5 rats (3 males, 2 females). The inter-trial retention interval for the novelty trial was a total of 24 hours. During this interval each rat was returned to its home cage.

2.2.2.2.3. Group 3 (48 hour interval)

This group consisted of 7 male rats. The inter-trial retention interval for the novelty trial was a total of 48 hours. During this interval each rat was returned to its home cage.

2.2.2.2.4. Group 4 (1 minute interval with acclimation)

This group contained a total of 8 rats (4 males, 4 females). The inter-trial retention interval for the

novelty trial was one minute, during this time each rat was placed in a holding cage. However, this group also had an acclimation trial, which consisted of being placed on the maze without any objects for 3 minutes. This trial took place 24 hours before the first familiarization trial. This was to determine if there was an alteration in exploration when familiarized to the environment, before the introduction of the objects.

2.2.3. Analysis

Novelty scores were determined by a percentage, which was the amount of time (sec) spent exploring the novel object / the total possible amount of time (sec) to explore (180 sec). A one-sample t-test (μ=12.5%) was used in order to determine if there was a significant novelty effect (α=0.05). A one-way analysis of variance (ANOVA) was used to explore differences between Group 1 (1 minute interval) and Group 4 (1 minute interval with acclimation) (α=0.05).
Data are presented as (mean ± SEM).

2.3. Results

2.3.1. Group 1 (1 minute interval)

A one-sample t-test (μ=12.5%) revealed there was a significant novelty effect (32.82% ± 4.86) ($t(6)$=5.18, p=0.002) in the 1 minute interval between the last familiarization trial and the novelty trial, common to the literature (see Figure 2.2).

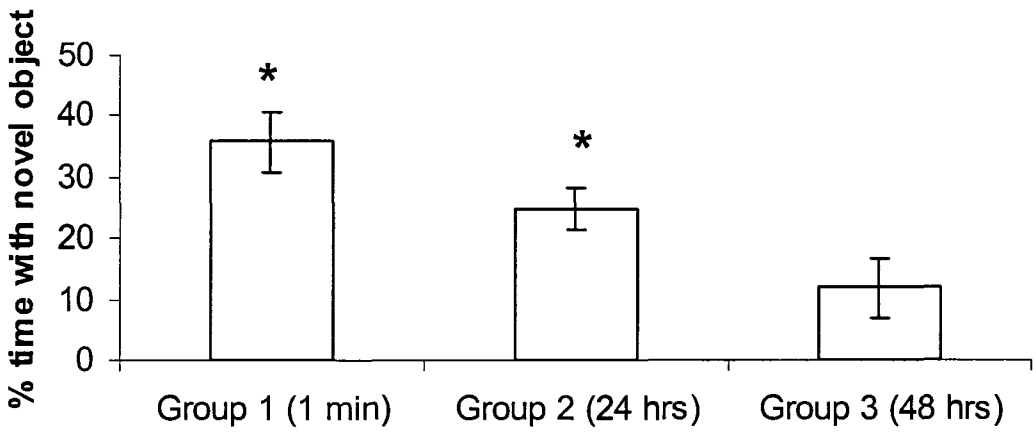


Figure 2.2. Percent time spent with novel object during the novelty trial for Groups 1, 2 & 3. (* denotes a significant novelty effect, $p<0.05$).

2.3.2. Group 2 (24 hour interval)

A one-sample t-test (μ=12.5%) revealed there was a significant novelty effect (24.85% ± 3.40) ($t(4)$=3.536, p=0.024) with a 24 hour interval between the last familiarization trial and the novelty trial (see Figure 2.2).

2.3.3. Group 3 (48 hour interval)

A one-sample t-test (μ=12.5%) revealed there was no significant novelty effect (11.90% ± 4.86) with an interval of 48 hours between the last familiarization trial and the novelty trial (see Figure 2.2).

2.3.4. Group 4 (1 minute interval with acclimation)

Using a one-way ANOVA no significant differences were found compared to Group 1 in the mean time (sec) spent exploring out of total time (180 sec) in the first familiarization trial. There were no significant differences found compared to Group 1 in meant time (sec) spent exploring out of total time (180) in the novelty trial.

2.4. Discussion

The results of this study demonstrate consistent findings with those present in the literature when

the similar procedures were used (e.g. Group 1 data). The results from group 2 (1 minute interval with acclimation) demonstrated that an acclimation trial to the maze, without any objects, did not alter exploration with the objects and is not necessary. These results are important in determining whether the procedure for the playground maze was reproducible in our lab.

The results from Group 2 (24 hour interval) demonstrated that the retention for object memory using the playground maze can last as long as 24 hours in the rat. This was demonstrated by a significant novelty effect in Group 2. However, there was no significant novelty effect found for Group 3 (48 hour interval) demonstrating that the retention for object memory in the playground maze in the rat is abolished by 48 hours. Previous studies looking at object recognition did not find retention for object memory to last up to 24 hours (Pitsika et al., 2002). However most studies looking at when memory for objects is abolished often use less trials and fewer objects (Forwood et al., 2005; Pitsika et al., 2002; Puma & Bizot, 1998). The procedure for the playground maze requires several familiarization trial, each spaced 24 hours apart (Nicholls, et al. 1994), this would lead to a longer retention for object memory. If each trial is spaced 24 hours and the rat becomes familiarized with the objects, then it is reasonable to expect that memory for the objects would last up to 24 hours. However, the results from Group 3 (48 hour interval) reveal that retention for object memory is abolished by 48 hours. Therefore, the novel object is not detected as novel and results in a decrease in investigation with the object. The results of Group 3 (48 hour interval) are important because it demonstrates the novel object (dinosaur) used for the study was not inherently more interesting than the familiar objects. Group 3 (48 hour interval) did not spend more than significantly more time with the novel object than 12.5%, which is the average amount of time

spent with all eight objects. Therefore, after 48 hours the objects in the familiarization trials are not "familiar" during the novelty trial, and the novel object is not detected as "novel".

These results are important as they not only show the playground maze is reproducible in our lab, but that the procedure can include an interval of 24 hours between the last familiarization trial and the novelty trial. This is important because it could increase the sensitivity of the playground maze to different memory problems. The sensitivity of the maze is increased by knowing when object memory is abolished (e.g. after 48 hours). Increasing the latency between last familiarization trial and test trial is a test of longer term retention and could be sensitive to different memory alterations than that of the standard procedure. Therefore, future studies could include such manipulations in the procedure of the playground maze, to alter its sensitivity.

Chapter 3

Drug-seeking and altered responses to novelty in adult rats treated neonatally with domoic acid.

Melissa A. Burt[a], Catherine L. Ryan[b], Tracy A. Doucette[a]

Departments of Biology[a] and Psychology[b], University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI, Canada, C1A 4P3

Abstract

Activity in the mesocorticolimbic DA system is linked to responses to novelty, reward, and drug-seeking behaviours. Glutamate signalling, through KA receptors, has been shown to modulate DA release in this pathway. In the present study, a low, non-convulsive dose of the KA receptor agonist, DOM was administered to rat pups over PND 8-14. As juveniles and adolescents, rats were assessed in the open field. During adulthood, rats were tested in an open field, a sucrose consumption task, the playground maze and in a nicotine-induced CPP paradigm. Drug-induced differences were found in open field behavior at each time point assessed. Male rats treated neonatally with DOM were more responsive to novelty, as indicated by an increase in time spent exploring objects during the novelty trial of the playground maze. In nicotine-induced CPP, DOM-treated females developed a conditioned place preference for the nicotine-paired compartment of the test arena, an effect that was maintained for at least a month following the final drug-compartment pairing. The results of this study underscore the importance of the Glu system in the ontogeny of behaviors that rely on the functional integrity of the mid-brain DA system.

3. Introduction

The development of the mammalian CNS is a complex process, as the CNS of the young animal is much more vulnerable and reactive to insult than is that of the mature organism (Dobbing & Sands, 1979; Dobbing & Smart, 1974; McDonald & Johnston, 1990; Vernadakis & Woodbury, 1969; Vorhees, 1986b). For instance, given any particular neurotoxicant, an adult CNS will respond with characteristic patterns of behavioural responses and lesions, with the timing of exposure resulting in no fundamental differences (Kaufmann, 2000), except for those which are attributed to the normal aging processes (Vernadakis & Woodbury, 1969; Vorhees, 1986b). However, the immediate effects of a neurotoxicant may not be the same as the delayed effects (Vorhees, 1986b). This is especially true in early development. Various agents have potential to induce delayed neurotoxicity well after exposure has ceased. The possibility of delayed neurotoxicity stems from at least two separate scenarios. Firstly, the ontogeny of a particular function may occur late in development. Consequently, the manifestation of the pathological change would not be revealed until the function would ordinarily be present in the "normal" animal. Secondly, developmental insults which affect both anatomy and/or function may be masked or attenuated due to neuronal compensation or plasticity and could result in apparently transient effects, none-the-less having later sequella (Rice & Barone, 2000).

A particularly critical period of CNS development is the brain growth spurt which, in the rat, commences around the day of birth and extends until the third week of postnatal life (Dobbing & Smart, 1974). Characterized by axonogenesis, dendritic arborization, development of

neurotransmitter systems, developmental cell death, synapse formation (as well as trimming of extraneous synapses), and myelination, the brain growth spurt is of considerable consequence when assessing potential developmental neurotoxicants, as it is known that systems becoming functionally mature are most susceptible to teratogenetic agents (Dobbing & Sands, 1979; Vorhees, 1986b). Significantly, all processes which characterize the brain growth spurt rely on appropriate glutamate receptor signalling (McDonald & Johnston, 1990).

Glutamate mediates most of the excitatory neurotransmission in the mammalian CNS. In addition to its role as a neurotransmitter, Glu is intimately involved in regulating CNS maturation, and plays a critical role in a wide variety of both physiological and pathological processes (McDonald & Johnston, 1990; Meldrum, 2000; Meldrum & Garthwaite, 1990; Parsons et al., 1998). The effects of Glu are mediated through a complex family of receptors which include both ionotropic and metabotropic variations (Bleakman & Lodge, 1998; Ozawa et al., 1998). Ionotropic receptors are directly linked to ion channels while metabotropic receptors are coupled to G proteins. Ionotropic Glu receptors are tetrameric and the various combinations of protein subunits, specific for each receptor type, combine to form the receptor complex. Ionotropic receptors are further divided into NMDA and non-NMDA receptors. Kainate and AMPA receptors make up the non-NMDA class (Bettler & Mulle, 1995; Bleakman & Lodge, 1998). Molecular cloning has revealed the existence of five KA receptor subunits that are commonly known as GluR5, GluR6, GluR7, KA-1 and KA-2 (Bettler & Mulle, 1995; Ozawa et al., 1998).

Kainate receptor complexes can form homomeric and heteromeric assemblies. For all known

functional recombinant KA receptors, KA elicits a fast onset and rapidly desensitizing response, however, other pharmacological and functional properties differ depending on subunit composition. Kainate receptors are located both presynaptically and postsynaptically (Chittajallu et al., 1999; Lerma, 2003; Lerma et al., 2001; Malva et al., 1995; Malva et al., 1996; Paternain et al., 2000; Paternain et al., 1998; Xt et al., 2006). In addition, the diversity of KA receptors is furthered by post-transcriptional subunit modifications which include alternative splicing and RNA editing, with both processes capable of altering the gating characteristics of the resulting receptor complex (Bettler & Mulle, 1995; Gregor et al., 1993; Grigorenko et al., 1998; Grigorenko et al., 1997; Kohler et al., 1993; Ozawa et al., 1998; Paschen et al., 1996; Schiffer et al., 1997; Sommer et al., 1991). Finally, many developmental shifts occur with respect to regional subunit expression and post-transcriptional modification (Bernard et al., 1999; Bernard et al., 1994; Monyer et al., 1991; Paschen et al., 1997), any of which, have potentially important implications for plasticity and neurotoxicity.

Rather than producing a transient pharmacological effect, receptor acting compounds administered to the immature organism, especially during the brain growth spurt, have the potential to cause permanent, irreversible insult. This insult can be expressed as either a permanent dysfunction to the neurotransmitter system involved, or may result in "irreversible imprinting" of receptor densities, which in turn results in lasting functional and/or structural changes to the nervous system (Kaufmann, 2000). Thus, alterations in glutamate receptor signalling at particular receptor subclasses may potentially be of significant consequence to the developing organism, but as such, may also reveal important physiological roles for particular receptor classes in ontogenetic

processes.

Domoic acid, and the related kainoid, KA are excitatory amino acids, structurally similar to the endogenous excitatory neurotransmitter, Glu. At low concentrations, both compounds are selective for the KA class of ionotropic glutamate receptors (Tasker et al., 1996; Verdoorn et al., 1994). Domoic acid has greater affinity for the so-called "low-affinity kainate receptors" (comprised of GluR5-7), while KA binds with greater affinity to the KA1, KA2 subunits, which when incorporated into a KA receptor assembly form "high-affinity kainate receptors" (Ozawa et al., 1998; Ritter et al., 2002; Tasker et al., 1996; Verdoorn et al., 1994). However, at increasing concentrations, this distinction is lost and other Glu receptors may be activated by these ligands.

To date, the majority of studies using these agonists to study the role that KA receptors play in early development, have used relatively large, seizure-producing doses. However, recent work in our lab has shown that repeated subcutaneous (s.c.) injections of low (non-convulsive) equi-efficacious doses of DOM (5 or 20μg/kg) or KA (25 or 100μg/kg) (Doucette et al., 2000) during a critical period of development (PND 8-14) (i.e. the brain growth spurt) produced differences in pre-weaning physical and neurobehavioural assessments, as well as long-lasting behavioral changes, and neurochemical and neuroanatomical anomalies within the hippocampus (Doucette, et al., 2004; Doucette et al., 2003; Tasker et al., 2005).

Glutamate receptors (including KA receptors) are located throughout dopaminergic systems, including within the mesocorticolimbic pathway, and can modulate DA release (Crowder &

Weiner, 2002; Legault & Wise, 2001; Mathé et al., 1998). For instance, functional KA receptors have been found on neurons in the rat NAc core, and can have pre- and postsynaptic consequences upon activation (Crowder & Weiner, 2002). It has also been demonstrated that DA release is modulated by non-NMDA receptors (Mathé et al., 1998) and NMDA receptors (Legault & Wise, 2001) in the VTA. Not only do Glu receptors modulate DA release in the mesocorticolimbic pathway, but they can also modulate behaviors mediated by this pathway such as response to novelty (Bevins et al., 2002; Legault & Wise, 2001). The mesocorticolimbic pathway is also known to have critical involvement in modulating behavioral responses to appetitive reward and to drug reward and drug seeking behaviors (Salamone et al., 2005; Spanagel & Weiss, 1999). It is therefore reasonable to speculate that alterations in Glu signalling during development could then, in turn, affect dopaminergic function.

By assessing a variety of behaviors which are largely modulated by DA, the current study was designed to determine whether early exposure to DOM would alter the functional integrity of the mesocorticolimbic system. Based on a previously reported procedure, rat pups were treated with a low dose of DOM from PND 8-14 (Doucette et al., 2003, Doucette et al., 2004). These animals were subsequently evaluated in behavioral paradigms reflective of mesocorticolimbic DA integrity; the open field as a measure of activity, sucrose consumption and nicotine-induced CPP as a measure of reward sensitivity, and the playground maze as a measure of novelty responsiveness.

3.1. Materials and Methods

3.1.1. Experimental Animals

Experimental manipulations were conducted on the offspring of untimed pregnant female SD rats (Charles River Laboratories, St. Constant, PQ). Dams and litters were housed in individual cages, under standard laboratory conditions with food and water provided *ad libitum* and a 12 hour light/dark cycle (lights on at 07:00). The day of birth of the litter was designated as PND 0. Within 24 hours, the litters were culled to 10 pups per litter (5 males and 5 females, where possible). Pups were weaned at 21 days of age (PND 21) with two to three rats per cage and then individually housed on PND 49. The total number of rats used for the experimental procedures was 48 rats, 20 females and 28 males. All procedures were conducted according to the guidelines established by the Canadian Council on Animal Care and in accordance with the Animal Care Committee at the University of Prince Edward Island.

3.1.2. Toxin Treatment

Domoic acid was obtained from Diagnostic Chemicals Ltd. (Charlottetown, PE). The drug was dissolved in sterile SAL and injections were administered s.c. The pups received a single daily injection of either 20 μg/kg DOM or equal volume of SAL, over PND 8-14. Within each litter, half the males and half the females received DOM and the other half received SAL; this was quasi randomly assigned in each litter. A total of 24 rats were treated with DOM (10 females, 14 males)

and 24 rats served as saline control (SAL) (10 females, 14 males). The experimenter was blind to the individual treatment conditions of each animal throughout the experiment. To individuate pups within the litter, rat pups were marked daily until weaning, using a non-toxic ink marker.

3.1.3. Developmental Measures

Several developmental measures were assessed starting on PND 8, as described below.

3.1.3.1. Weight Gain

Weights were assessed daily until weaning on PND 21. Rats were then weighed weekly until the end of the experiment.

3.1.3.2. Auditory Startle

Animals were tested daily until criterion was reached. Auditory startle was defined as a visible startle response to a noise made by a small clicker held approximately 14 cm above the pup's head.

3.1.3.3. Eye Opening

Eye opening, as defined as a break in the suture of the eye, was assessed daily. Pups were examined for this developmental milestone until a break in the suture of both eyes was noted.

3.1.3.4. Sexual maturation

Testes descent or vaginal opening was assessed in all rats starting on PND 19 until criterion was reached. In order to ensure consistency of handling, these assessments were continued daily for each rat until all rats reached criterion.

3.1.4. Behavioural Testing

3.1.4.1. Open field

Animals were placed in an open field for 10 minutes and activity was measured as number of grid crosses and rearing. A grid cross was defined as both fore paws crossing into the next grid, and rearing was defined as only the hind paws touching the ground. Rats were tested in the open field at each of three different developmental ages: PND 18 (pre-adolescence), PND 36 (adolescence) and again at approximately PND 150 (adulthood). The open field arena used for testing at both PND 18 and PND 36 measured 77 x 44 cm, however, with grid sizes measuring 4.5 x 4.5 cm (8 inner and 16 outer grids) for PND 18, and 16 x 16 cm (3 inner and 12 outer grids) for PND 36. The open field arena used for testing at PND 150 measured 105 x 105 x 40 cm, with grids measuring 25 x 25 cm (4 inner and 12 outer grids). Each trial was video taped for later scoring and the dependent measures included total number of grid crosses, total inner and total outer crosses, number of rearing episodes, as well as a defecation score. Additionally, a habituation score was calculated. This consisted of the number of grid crosses in the first five minutes minus the number

of grid crosses in the last five minutes.

3.1.4.2. Playground Maze (PND 56)

The playground maze was a circular platform measuring 100 cm in diameter, and raised 54 cm from the floor. The circular platform had eight 14 cm circles placed evenly around the maze 20 cm from the edge. Eight objects that fit within these circles were used as familiar objects, and one object, a plastic "dinosaur", was used as the novel object. Each trial was videotaped for later scoring. In brief, testing included a total of four familiarization trials followed by a novelty trial.

3.1.4.2.1. Familiarization trials

The procedure for the playground maze was adapted from procedures outlined elsewhere (Nicholls et al., 1992) (also see Chapter 1). Each familiarization trial was three minutes in length and 24 hours apart. The familiarization trials used eight familiar objects (see Table II), one placed in each of the eight circles on the maze. For a single trial, each rat was placed in the middle of the maze for 3 min and allowed to freely move around the maze. While the objects remained constant, the

Object	Color	Approximate Dimensions (cm) (l x w x h) or (di; h) as appropriate
Sauce Cup (Metal)	Silver	6; 4
(Solved) Rubix Cube®	Orange/Blue/White Green/Red/Yellow	5 x 5 x 5
Pencil Sharpener (Plastic)	Clear with black cap	3 x 5 x 7
Salt Shaker (Plastic with metal top)	Yellow/Silver	3; 7
Egg cup (Metal)	Silver	4; 4
Rectangular Dish (Plastic)	Purple	6 x 6 x 5
Frog (Plastic)	Dark Green	6 x 3 x 4
Apple (Plastic)	Light Green	4; 6

Table II. Description of objects used in the playground maze during familiarization trials. Height (h), length (l), width (w), diameter (di).

location of each of the objects on the maze was changed for each of the four trials, this was pseudo randomly chosen.

3.1.4.2.2. Novelty trial

The procedure for the novelty trial consisted of removing the rat from the maze after the fourth (i.e. last) familiarization trial. The rat was placed in a holding cage, out of view of the maze, for 1 min. During this time, one of the familiar objects was removed, with the remaining seven still in the same location, and the novel object, the "dinosaur," was placed in the location of the object that was removed. The object to be replaced was pseudo-randomly chosen, and balanced across treatment groups. Following this, the rat was then placed back in the middle of the maze facing away from the novel object and allowed to freely move around the maze for a total of three minutes.

All trials of the procedure were videotaped for later analysis. The time spent exploring each of the objects in the familiarization trials and the novelty trial were determined by visual scoring of the tapes and time spent in locomotion and distance travelled were determined using Ethovision (v.2.0, Noldus).

3.1.4.3. Sucrose Consumption: Two choice task (commencing on PND 63)

Water was measured every 24 hr for three consecutive days, to determine baseline water

consumption. Each bottle was filled with 450 ml of water, and total amount (ml) consumed within each 24 hr interval was measured. On subsequent days, two water bottles were placed in the home cage, one containing 450 ml water and the other containing 450 ml of sucrose solution (5%). The amount consumed from each bottle was measured and then replaced with 450 ml of fresh tap water and 450 ml of fresh sucrose solution every 24 hours for three consecutive days. The locations of the bottles (suspended from the left or right side of the cage lid) was alternated each day.

3.1.4.4. Nicotine Induced Conditioned Place Preference: Unbiased procedure (approximately PND 200-240)

(-)-nicotine hydrogen tartrate salt (obtained from Sigma, Canada) was diluted in SAL (0.5 ml/kg) for a dose of 0.6 mg/kg, and pH was adjusted to 7.0 using NaOH. The CPP chamber was constructed from 1/4 inch plexi-glass, to the dimensions of 110 x 40 x 45 cm with a removable partition in the center (i.e. which could divide the maze into two compartments measuring 55 x 40 cm). In turn, the two compartments were visually and tactually distinct with one compartment consisting of a smooth floor (plexiglass) with two horizontal stripes (6.5 cm in width) across each of the four walls, and with the other compartment consisting of a ridged (each ridge 2 mm) plastic floor, with 3 vertical strips (4 cm width) equi-spaced on the 55 cm long walls and 2 vertical stripes spaced equi-distant on the 40 cm long walls. The walls and floor were dark green and the stripes were a light brown.

The nicotine induced CPP procedure involved an initial injection-habituation phase, followed by

an habituation trial, eight conditioning trials and finally, a test trial. The injection-habituation phase involved administering a SAL injection (s.c.) in the room in which the injections during conditioning would take place. This was followed by a habituation trial in which the rats were injected with saline and placed in the CPP maze with the central partition removed, such that the rat was allowed access to both maze compartments, for a total of 15 minutes. The rats were then removed from the maze and returned to their home cage for 24 hrs until the start of the conditioning trials. The conditioning trials included administering either SAL or nicotine (s.c.; experimenter blind) to the rat and then placing it in the appropriate compartment for 30 minutes. Following this, the rat was then removed and placed in the home cage for 24 hours until the next trial, for a total of eight trials (four paired with nicotine and four with SAL).

The procedure used was an unbiased procedure, with half of the rats in each treatment condition (DOM and SAL) receiving nicotine always paired with one compartment, and the remaining rats receiving nicotine always paired with the other compartment. This was done to control for any biases that could exist in inherent preference for either of the two compartments. Each rat received four pairings of nicotine and four pairings of SAL, with drug (either nicotine or saline) on the initial conditioning day, counter balanced across conditions. Each day the pairing was alternated. After the eight conditioning trials, the rats were then placed in the CPP maze again for the test trial.

The test trial was conducted 24 hrs after the last conditioning trial and was 15 min in duration. The rat was placed in the middle of the maze, with the door removed, and allowed access to both compartments. A second test trial (Test Trial 2) was conducted approximately one month after the

first test trial (Test Trial 1).

All procedures (i.e. habituation, conditioning trials, test trials) were videotaped for later scoring. In order to determine a compartment preference, the test trial was scored for time spent in each compartment and number of times the rat crossed from one compartment to the other. Criterion for compartment entry was determined once the head and front shoulders passed the centre line into that compartment.

3.1.5. Data Analysis

Initially, all data were analyzed using 2-way (gender x treatment) ANOVAs, and with repeated measures where applicable. If a significant main effect was found for gender, or if an interaction was present with this variable, then separate analyses were conducted on data obtained from male and female rats. Data analyses were conducted using SPSS (SPSS v.11.2, Chicago), and for all analyses, the significance level was set at α=0.05.

3.2. Results

3.2.1. Physical and neurobehavioural assessments

Various indices of physical and neurobehavioural development were assessed in the rat pups, commencing on PND 8 and continuing daily until weaning.

3.2.1.1. Weight Gain

Weight gain was assessed over 24 hr intervals from PND 8-14. An initial analysis revealed a significant interaction between day and gender ($F(6,264)=2.269$, $p=0.037$). For data obtained from male rats, a subsequent repeated measures ANOVA revealed a significant main effect for day ($F(6,156)=2.915$, $p=0.010$), but a comparable effect was not found with the data obtained from female counterparts. There was no significant effects or interactions found for the between-subjects variable (i.e. treatment) in any of the analyses conducted.

3.2.1.2. Pre-weaning Weight

Body weights were assessed on PND 18 using a 2 way-ANOVA. No significant effects or interactions were found.

3.2.1.3. Auditory Startle

Analysis for data reflecting the PND on which an acoustic startle reflex was first observed in rat pups revealed no significant main effects or interactions.

3.2.1.4. Eye Opening

A two-way ANOVA on data obtained for eye-opening (defined as the PND on which a break in the

suture of both eyes was present) revealed a significant effect for gender, with female pups opening their eyes prior to their male counterparts (14.25 ± 0.18 and 14.79 ± 0.14, for females and males respectively)($F(1,47)=6.135$, $p=0.017$). Consequently, separate independent samples t-tests were conducted on data obtained from male and female rat pups. Analyses revealed that female rats treated with DOM reached criterion at a younger age than those treated with SAL ($t(18)= -2.178$, $p=0.022$) (DOM: 13.9 ± 0.38 and SAL: 14.6 ± 0.16), an effect not present in male rat pups (DOM: 14.19 ± 0.19 and SAL: 14.19 ± 0.21).

3.2.1.5. Sexual Maturation

Independent samples t-test were done to determine whether treatment effects were present for the PND on which testes descent / vaginal opening occurred. No significant effects were present in either male (DOM: 22.50 ± 0.33 and SAL: 22.64 ± 0.31) or female (DOM: 31.1 ± 0.41 and SAL: 31.3 ± 0.62) rats for these indices of sexual maturation.

3.2.2. Behavioural Testing

Behavioural assessments were conducted with each rat at juvenile, adolescent and adult time points.

3.2.2.1. Open Field

Each rat was tested for 10 min in an open field arena on PND 18, 36, and 150. Measures obtained included habituation scores, grid crosses, rearing, and fecal boli counts (see Table III).

3.2.2.1.1. PND 18 (pre-adolescent)

A habituation score was calculated for each rat by subtracting the number of total grids crossed during the last 5 min of a 10 min trial from the total number of grids crossed during the first 5 min. Analysis of habituation scores revealed that unlike SAL-treated rats, those rats exposed to DOM as neonates did not habituate to the open field arena during the 10-min trial ($F(1, 47)=4.479$, $p=0.040$) (-5.21 ± 8.10 and 12.46 ± 4.48, for DOM-treated and SAL-treated rats, respectively).

No significant differences were found for total number of grid crosses over the 10 min interval, total grid crosses during the first 5 min of the trial, total number of inner grids crossed or total number of outer grids crossed. A significant main effect was found for treatment, in the total number of grid crosses which occurred during the last five minutes of the 10 min trial (e.g. as reflected in the habituation score) ($F(1,47)=5.024$, $p=0.030$) with the DOM-treated rats crossing more grids (50.42 ± 9.61) than SAL-treated rats (29.38 ± 5.46).

While significant main effects for gender were found for total number of rears during the 10 min trial (males: 71.96 ± 8.93 and females: 100.00 ± 17.19) ($F(1,47)=6.003$, $p=0.018$) and number of

	Males		Females	
Measure	Saline	DOM	Saline	DOM
PND 18				
Grid (#)	70.07 (±11.82)	73.86 (±13.82)	72.80 (±18.25)	127.20 (±27.39)
Grid Block 1 (#)	40.71 (±6.83)	38.86 (±5.99)	43.40 (±9.56)	54.10 (±14.71)
Grid Block 2 (#)	29.36 (±6.64)	35.00 (±9.14)*	29.40 (±9.69)	72.00 (±17.59)*
Habituation	11.36 (±6.46)	3.86 (±6.94)*	14.00 (±6.21)	-17.90 (±16.57)*
Inner Grid (#)	14.93 (±2.78)	17.36 (±2.69)	18.90 (±4.47)	26.80 (±7.51)
Outer Grid (#)	55.14 (±9.75)	56.50 (±12.67)	53.90 (±14.25)	100.40 (±20.84)
Rearing (#)	9.14 (±2.13)	10.86 (±2.16)	14.00 (±4.50)	21.90 (±4.62)
Fecal Boli (#)	0.93 (±0.41)	1.21 (±0.39)	0.60 (±0.27)	1.10 (±0.38)
Grooming (#)	3.36 (±0.45)	3.36 (±0.31)	3.00 (±0.54)	1.70 (±0.37)
Grooming (s)	28.16 (±5.36)	28.18 (±4.37)	32.37 (±6.77)	15.56 (±4.10)
PND 36				
Grid (#)	198.86 (±9.26)	189.07 (±11.84)	196.90 (±15.52)	230.80 (±13.67)
Grid Block 1 (#)	126.80 (±6.02)	125.00 (±7.09)	127.30 (±7.67)	138.40 (±11.00)
Grid Block 2 (#)	72.00 (±6.67)	64.07 (±6.17)	68.80 (±9.26)	88.20 (±7.06)
Habituation	54.86 (±8.71)	69.93 (±10.53)	58.50 (±7.01)	56.60 (±12.19)
Inner Grid (#)	30.57 (±2.74)	31.36 (±3.07)	25.40 (±2.99)	33.70 (±2.68)
Outer Grid (#)	161.14 (±11.81)	157.71 (±10.26)	171.50 (±13.09)	197.10 (±12.93)
Rearing (#)	63.29 (±5.05)	51.86 (±4.84)	50.00 (±5.34)	69.00 (±5.68)*
Fecal Boli (#)	5.36 (±0.63)	4.79 (±0.59)	4.90 (±0.96)	3.08 (±0.92)
Grooming (#)	3.93 (±0.67)	3.71 (±0.61)	3.40 (±0.62)	2.70 (±0.34)
Grooming (s)	24.94 (±5.15)	29.69 (±6.05)	26.96 (±4.96)	21.66 (±4.78)
PND 150				
Grid (#)	151.21 (±9.69)	118.92 (±7.21)*	137.90 (±11.16)	166.70 (±8.19)*
Grid Block 1 (#)	86.86 (±5.58)	70.54 (±5.32)*	76.00 (±7.25)	97.00 (±5.86)*
Grid Block 2 (#)	64.36 (±5.92)	48.38 (±3.75)*	61.90 (±5.47)	71.10 (±5.58)
Habituation	22.50 (±6.19)	22.15 (±5.71)	14.10 (±6.36)	25.90 (±6.17)
Inner Grid (#)	27.64 (±2.42)	17.38 (±1.97)	21.90 (±3.23)	31.50 (±4.85)
Outer Grid (#)	123.57 (±8.10)	101.54 (±6.07)	115.90 (±8.87)	136.60 (±6.16)
Rearing (#)	23.64 (±2.79)	21.92 (±3.37)	35.10 (±4.85)	31.00 (±2.65)
Fecal Boli (#)	4.21 (±0.66)	3.85 (±0.68)	1.60 (±0.76)	0.40 (±0.40)
Grooming (#)	2.79 (±0.32)	3.23 (±0.67)	3.90 (±0.31)	3.30 (±0.63)
Grooming (s)	19.79 (±3.61)	23.18 (±4.42)	23.30 (±3.12)	15.10 (±3.57)

Table III. Data obtained in the open field arena for male and female rats treated with either saline or 20 μg/kg DOM. Variables are quantified as latency, in seconds (s) or number (#). Values in parentheses represent standard errors. (PND) Postnatal day; (Grid) Total grid crosses; (Block 1) Minutes 1-5; (Block 2) Minutes 5-10; (Habituation) Total grid crosses during Block 1 - Total grid crosses during Block 2. * denotes $p < 0.05$, relative to saline.

grooming episodes (males: 3.36 ± 0.27 and females: 2.35 ± 0.35) ($F(1,47)=5.627$, $p=0.022$), no significant effects were present for treatment, nor were any interactions present. Finally, analysis of variance revealed no significant effects or interaction for the number of fecal boli present at the end of the 10 min open-field trial.

3.2.2.1.2. Open Field PND 36 (adolescent)

No significant main effects or interactions were found in separate analyses conducted for total grid crosses during the 10-min trial, habituation scores, total grid crosses during either the first or last 5 min of the 10 min trial, or for total inner grid crosses. However, a significant gender effect was present for total outer grid crosses (males: 159.43 ± 7.68 and females: 184.30 ± 9.42) ($F(1,47)=4.238$, $p=0.045$), Subsequent analyses on data obtained from male and female rats using separate independent samples t-tests revealed no significant differences between DOM- and SAL-treated animals among either gender.

A significant gender x condition interaction was found for total number of rears occurring during the 10 min trial ($F(1,47)=8.003$, $p=0.007$). Independent samples t-tests conducted separately for data from male and female rats demonstrated a significant effect for treatment in females ($t(18)=2.375$, $p=0.015$) with the DOM-treated rats rearing more frequently (69.00 ± 5.68) than those neonatally treated with SAL (50.50 ± 5.34).

Finally, no significant differences were found for defecation, number of grooming episodes, or

time (sec) spent in bouts of grooming.

3.2.2.1.3. Open Field PND 150 (adult)

Analysis of the total number of grid crosses in the total 10 min trial revealed a significant gender x treatment interaction ($F(1,47)=10.946$, $p=0.002$). Independent samples t-tests were therefore conducted for data obtained from males and females separately. Significant, and opposite treatment effects were found for both males ($t(26)= -2.674$, $p=0.007$) and females ($t(18)=2.082$, $p = 0.026$), with male DOM-treated rats crossing fewer grids than did their saline counterparts (118.92 ± 7.21 and 151.21 ± 9.69), and with female DOM-treated rats crossing more grids than did those treated with SAL (166.70 ± 8.19 and 137.90 ± 11.16).

Similar effects were found when data obtained during the first 5 min of the trial were analyzed, revealing a significant gender x treatment interaction ($F(1,47)=9.601$, $p=0.003$).
Again, subsequent analyses demonstrated comparable main effects for treatment in both male and female rats, with DOM-treated males crossing fewer grids than their saline counterparts ($t(26)= -2.118$, $p=0.022$) (70.54 ± 5.32 and 86.86 ± 5.58), while DOM-treated females crossed significantly more grids than did those treated with SAL ($t(18)=2.254$, $p=0.018$) (97.00 ± 5.86 and 76.00 ± 7.25).

With respect to the total number of grids crossed in the last five minutes of the trial, there was a significant interaction between gender and treatment ($F(1,47)=6.026$, $p=0.018$). Subsequent

analysis using an independent samples t-test difference revealed that DOM-treated male rats crossed fewer grids than did SAL-treated males ($t(26)$=-2.282, p=0.016) (48.38 ± 3.75 and 64.36 ± 5.92). A comparable effect was not found for treatment among female rats.

No significant main effects or interactions were found when habituation scores were analyzed using a 2-way ANOVA.

A gender x treatment interaction was found to be present for the number of inner grid crossings ($F(1,47)$=10.509, p=0.002). Independent samples t-tests were therefore done for females and males separately. Analyses revealed that a treatment effect was present only for male rats, with DOM-treated animals crossing significantly fewer grids than those treated with SAL ($t(26)$= -3.295, p=0.002) (DOM: 17.38 ± 1.97; SAL: 27.64 ± 2.42).

In the number of outside grid crosses in the 10 min trial, there was a significant effect for interaction between gender and condition ($F(1,47)$=8.049, p=0.007). Further analyses demonstrated that in female rats, DOM-treatment resulted in significantly more outer grid crosses ($t(18)$=1.917, p=0.036) (136.60 ± 6.16 and 115.90 ± 8.87 for DOM- and SAL-treated rats respectively), whereas in male rats, DOM-treatment produced rats who demonstrated fewer outer grid crosses ($t(26)$= -2.178, p=0.020) (101.54 ± 6.07 and 123.57 ± 8.10 for DOM- and SAL-treated rats respectively).

While a significant main effect was present for gender with respect to total number of rears ($F(1,47)$=8.693, p=0.005) (males: 22.78 ± 2.15 and females: 33.05 ± 2.73), no significant treatment

effects were found for either males or females on this variable. Similarly, a significant gender effect was present for number of fecal boli ($F(1,47)=18.792$, $p<0.001$) (males: 4.03 ± 0.48 and females: 1.00 ± 0.50), but again no treatment effect was found.

Finally, no significant effects or interactions were present for number of grooming episodes or for the total time spent grooming.

3.2.2.2. Sucrose Consumption

The average water consumption over three days was analyzed using a 2 way-ANOVA (gender x treatment). There was a significant effect for gender ($F(1,48)=38.448$, $p<0.001$) with male rats consuming more water, on average, than female rats (males:48.58 ± 1.62; females: 34.76 ± 1.25). Analyses, however, revealed no effect for treatment.

The amount of sucrose consumed over three consecutive days was analyzed using an ANOVA with the repeated measure of day. While a significant between subjects effect for gender ($F(1,44)=11.911$, p=0.001) was present with males consuming more than female counterparts (males: 152.32 ± 9.92; females: 99.27 ± 11.74), subsequent analyses demonstrated that treatment effects were not present.

3.2.2.3. Playground Maze

3.2.2.3.1. Novelty Effect

To determine whether a significant response to novelty was present, a one sample t-test (μ=12.5%), using the percent of time spent with the novel object for each treatment group, was used (see Nicholls et al., 1992). Data obtained from female rats demonstrated significant effects for both SAL-treated ($t(9)=2.164$, $p=0.03$) (22.63% ± 4.68) and DOM-treated ($t(9)=3.054$, $p=0.007$) (29.19% ± 5.46) rats.

Similarly, in male rats, both DOM- and SAL-treated rats spent a significant proportion of the total time interacting with novel objects (DOM: $t(13)=3.310$, $p=0.003$, 21.56% ± 2.74; SAL: $t(14)=3.648$, $p=0.002$, 24.43% ± 3.27)

Independent samples t-tests were done to compare the percent of time spent with the novel object between the conditions in the females and males. No significant differences were found.

3.2.2.3.2. Novelty Trial

A significant treatment x gender interaction was found in an analysis of the total time (sec) spent with all eight objects ($F(1,47)=7.624$, $p=0.008$). While no treatment effects were found for female rats, analysis revealed that DOM-treated males spent significantly more time with the objects than

did their SAL-treated counterparts ($t(26)=2.534$, $p=0.009$) (78.41 ± 4.16 and 58.65 ± 6.18) (Figure 3.1). There was no significant difference between the conditions in the females (Figure 3.1).

No significant effects or interactions were found for mean distance (cm) moved between two time points taken by the program during the trial, for velocity (cm/sec), for movement frequency, nor for total duration (sec) of movement.

3.2.2.4. Nicotine-induced condition place preference

3.2.2.4.1. Test Trial 1

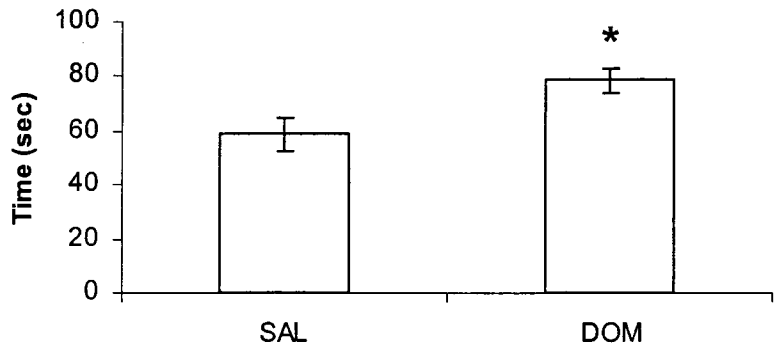
Paired samples t-tests were used to compare the time (sec) spent in the nicotine paired compartment with the time spent in the saline paired compartment in each of the treatment groups. Only the DOM females demonstrated a nicotine-induced conditioned place preference, as evident in the time (sec) spent in the nicotine paired compartment (522.00 ± 35.54) relative to the time (sec) spent in the saline paired compartment (390.00 ± 35.13) ($t(9)=1.871$, $p=0.047$) (Fig. 3.2).

In the number of center crosses, a 2-way ANOVA (treatment x condition) revealed no significant differences.

3.2.2.4.2. Test Trial 2

The nicotine-induced place preference was maintained, as evident by a reassessment approximately

A



B

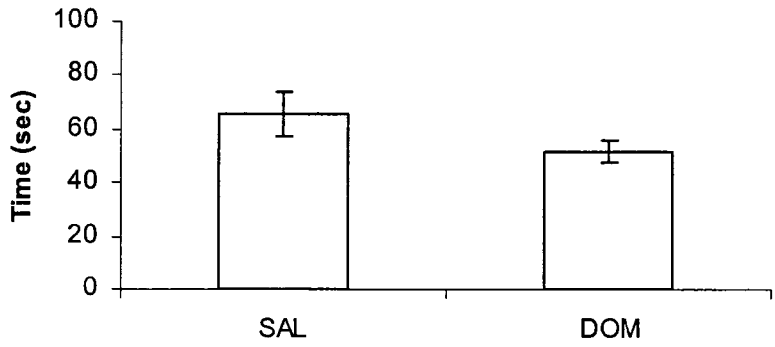


Figure 3.1. Mean time (sec) spent with objects during the novelty trial on the playground maze for male (A) and female (B) rats. Error bars represent standard errors. (* indicates $p < 0.05$ relative to saline group).

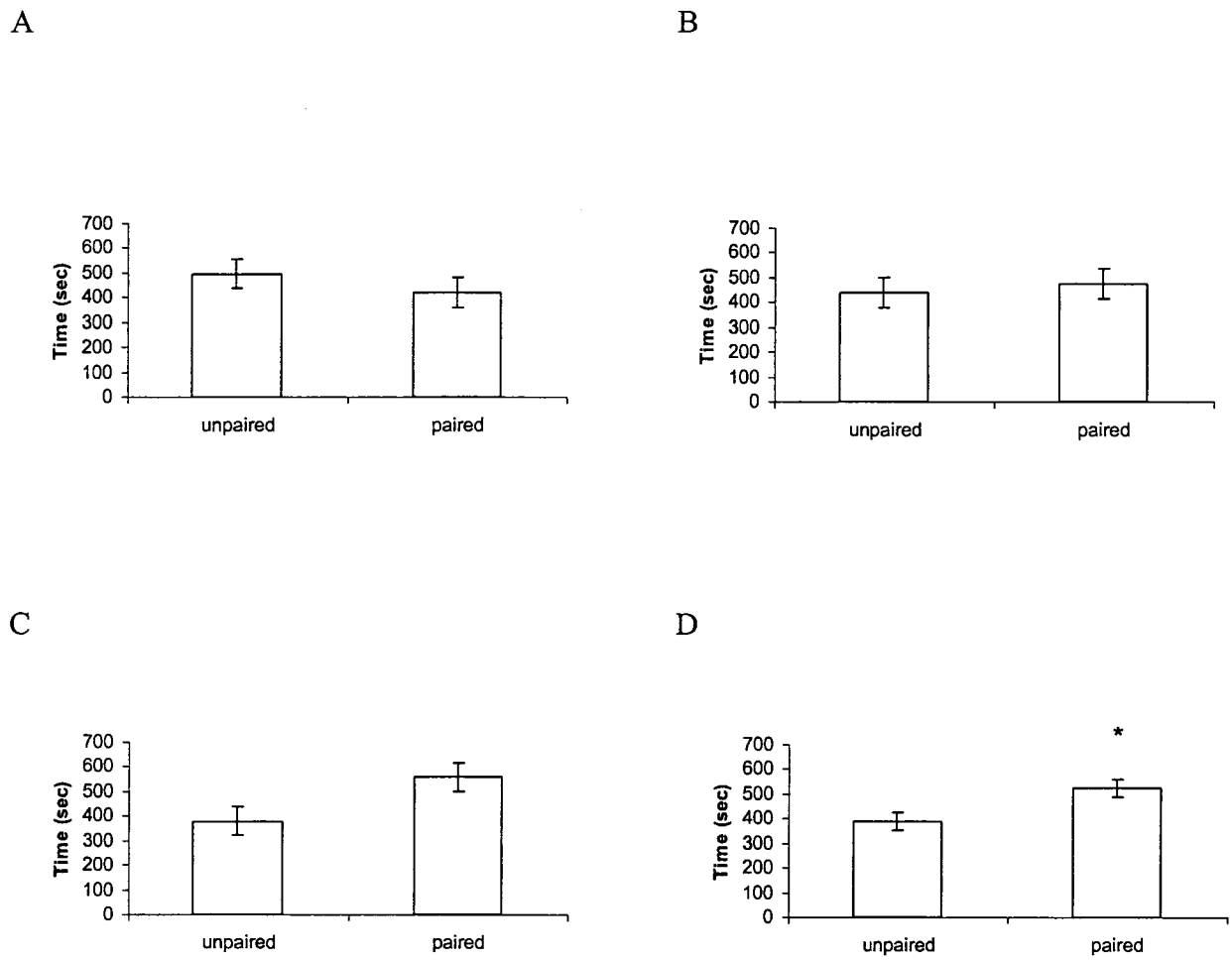


Figure 3.2. Mean time (sec) spent in nicotine-paired and unpaired chambers in the 24 hr following the final conditioning trial for male saline (A) and DOM-treated (B) rats and for female saline (C) and DOM-treated (D) rats. Error bars represent standard errors. (* indicates $p < 0.05$ relative to unpaired compartment).

1 month later. Again, DOM-treated females spent significantly more time (sec) in the compartment previously paired with nicotine (563.30 ± 27.64), than in the compartment previously paired with saline (372.60 ± 29.25) ($t(9)=3.372$, $p=0.004$) (Figure 2.3). This effect was not found for male rats or SAL-treated females (Figure 3.3).

Again, no significant differences were found for the number of center crosses, as evidenced using a 2-way ANOVA.

3.3. Discussion

Results of the present study support the idea that DOM exposure, presumably via early low-level activation of KA receptors, during a critical period of development results in alterations in behaviors that are reliant on the functional integrity of the mesocorticolimbic DA pathway. Data have shown that while no overt signs of toxicity were present in rat pups following DOM-treatment were (as assessed by weight gain), the dose used was of physiological relevance (as evidenced by accelerated eye opening in female DOM-treated pups).

Differences between the treated and control rats varied across the stages of development in the open field arena. During pre-adolescence (PND 18), the DOM treated groups demonstrated a decrease in habituation to the open field, as evidenced by grid crosses, with an increase in activity found only in the last five minutes of the 10 min trial. During adolescence (PND 36) an increase in rearing was found in the female DOM treated rats. During adulthood (PND 150) DOM treated

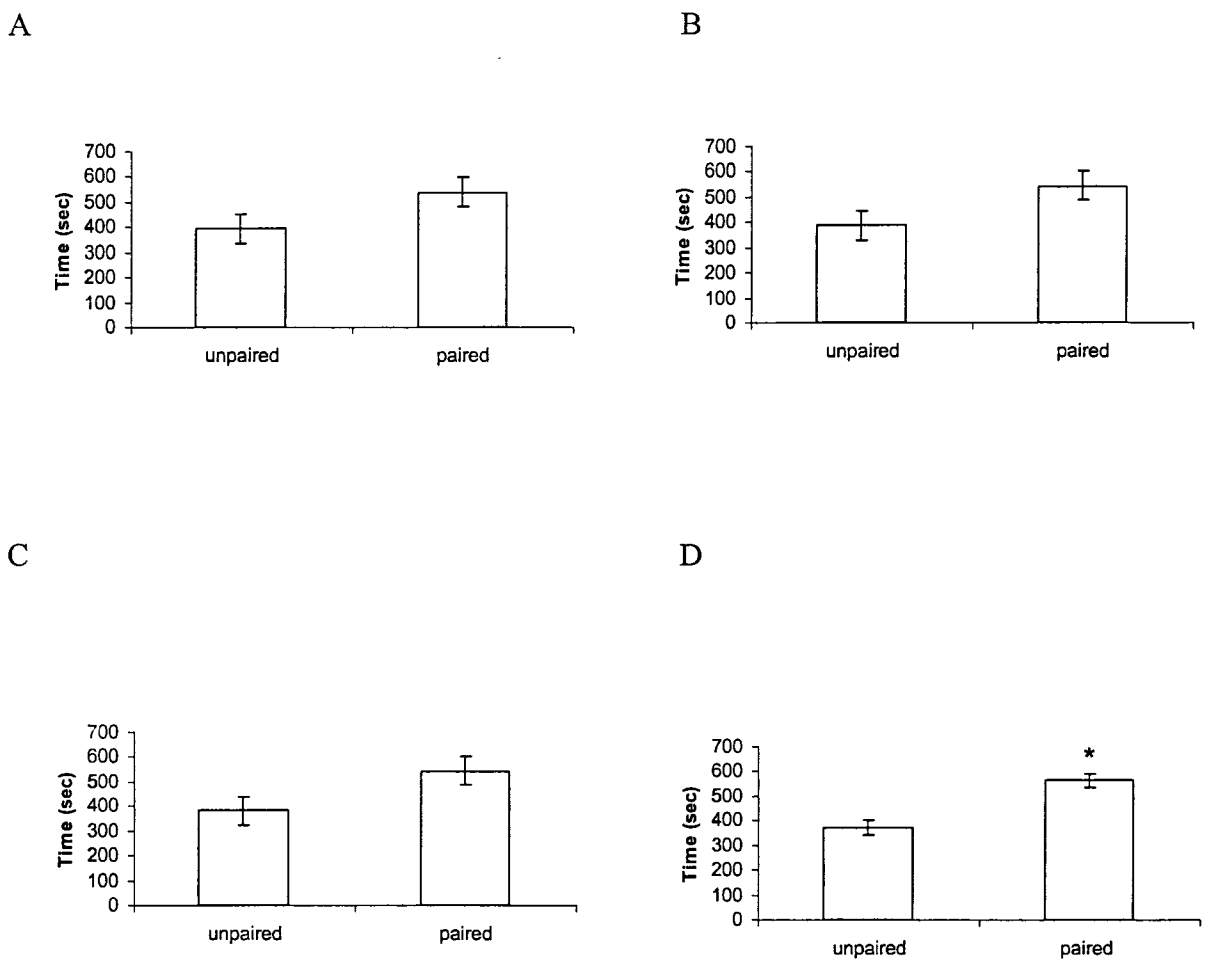


Figure 3.3. Mean time (sec) spent in nicotine-paired and unpaired chambers approximately 30 days following the final conditioning trial for male saline (A) and DOM-treated (B) rats and for female saline (C) and DOM-treated (D) rats. Error bars represent standard errors. (* indicates $p < 0.05$ relative to unpaired compartment).

males demonstrated a decrease in grid crosses relative to saline controls, while an increase in grid crosses was found in the DOM treated females when compared to their SAL counterparts. These results are interesting since they demonstrate not only alterations in activity in treated animals, but that changes manifested are gender related and different at various stages of ontology.

Dopamine is important in modulating activity in the open-field, as evidenced by studies looking at neonatal-6-hydroxydopamine lesioned rats (Breese et al., 2005). This model has been shown to produce a selective reduction of brain DA and results in hyperactivity during adolescence and impaired habituation (Breese et al., 2005). Therefore, one possible explanation for our present findings is that the alterations in activity found in the open field reflect an alteration in DA function.

In the playground maze, DOM-treated male rats demonstrated an altered response during the novelty trial (i.e. increased exploration of all objects), suggesting that when novelty is introduced, DOM-treated male rats increase generalized exploration to familiar objects, while still detecting the novel object. However, as compared to saline controls, no differences were found with respect to directed exploration of the novel object. This finding suggests an intriguing parallel to the negative symptoms of schizophrenia, whereby the introduction of novelty causes a retreat to the familiar. For example, it has been shown that a tetanus toxin-induced epilepsy (i.e. by injecting a tetanus toxin in the ventral hippocampus) abolishes the novelty response, but does not alter exploration of the familiar objects in the playground maze (Mellanby et al., 1999). It has been proposed that the rats with tetanus toxin-induced epilepsy using the ventral hippocampus, may be

demonstrating behaviors parallel to the negative symptoms of schizophrenia (Mellanby et al., 1999). The novel object is not detected as "new" and receives similar attention as the familiar objects.

Perhaps similar mechanisms may be involved in the male DOM rats, demonstrating that although the novel object is detected as "new" there is also an increase in exploration of the "familiar". Either this could be due to a lack of habituation to the familiar objects, or a gravitation to familiarity in a novel situation. While this effect was not found for females in the present study, others have reported that estrogen may be protective against the negative symptoms of schizophrenia (Rao & Kölsch, 2003).

Previous literature has demonstrated how difficult it can be to achieve nicotine-induced place conditioning, and important considerations include age, dose, and procedure. For instance, a dose that is too high will cause an aversion, whereas a dose too low will not induce conditioning; a biased procedure is far more sensitive to conditioning (LeFoll & Goldberg, 2005); and juvenile rats are far more sensitive to developing a conditioned place preference than are adult rats (Vastola et al., 2002). In comparison, although our protocol employed a relatively low dose in adult animals and used an un-biased procedure, we were none-the-less able to show a nicotine-induced conditioned place preference in DOM-treated female rats, suggesting an increased sensitivity to the rewarding properties of nicotine, which was maintained for at least one month.

It is interesting to note that mild alterations in Glu signaling during development resulted in an

increased sensitivity to the rewarding properties of nicotine in the adult female rats, as nicotine dependence is proposed to be dependent on the plasticity of the mesocorticolimbic pathway (Balfour, 2002). The results of the nicotine-induced CPP would therefore suggest that the functional integrity of the DA system has been altered due to the perinatal treatment with DOM.

While a similar effect was not found for male rats, other studies report gender differences in an operant conditioning task with nicotine. For instance, in a self-administration study it has been shown that female rats responded more to both the nicotine-reinforced and non-reinforced lever when the signal light was not present, and also responded more to the reinforced lever when the signal light was present, than did males (Chaudri et al., 2005). Still others have shown gender differences in brain metabolism in response to nicotine (Fallon et al., 2005), and it has also been reported that females are more susceptible to drugs of abuse and have enhanced behavioral sensitivity, and the that higher rates of use reported in males may be due to social constraints (Lynch, 2006).

Of interest to note is the strong connection between smoking and schizophrenia (de Leon & Diaz, 2005). Although there has been much research exploring the cognitive benefits of nicotine with respect to schizophrenia (Levin et al., 2006), there has been little looking at the rewarding properties of nicotine in animal models of schizophrenia. Therefore, it is interesting to speculate that perhaps the conditioning to nicotine, found in the female DOM treated rats, may reflect positive symptoms of schizophrenia, similar to that reported for amphetamine-induced place preference conditioning (Le Pen et al., 2002).

Because schizophrenia is a heterogeneous disorder, no single "ideal" animal model can represent the entire population of schizophrenic patients (Lipska, 2004). As pointed out by others (Powell & Miyakawa, 2006) it is likely that with the advent of additional models, each new model might represent a subpopulation or even a particular aspect or endophenotype of schizophrenia. Using this model, we have, presently and in previous work, reported behavioural, neurochemical, and neuroanatomical consistencies with both clinical and experimental literature, which includes i) seizure susceptibility [e.g. There is co-morbidity with schizophrenia and epilepsy. Within epilepsy the rate of psychoses averages around 2 to 7%, while this rate is much higher when looking specifically at temporal lobe epilepsy, the rate raises to 10 to 19%, much higher than the normal population (Giatatzis et al., 2004)], ii) alterations in DA-related behaviours, including activity levels (Black et al., 1998), and altered responses to reward and to novelty, iii) elevated hippocampal BDNF mRNA (Takahashi et al., 2000) and iv) decreased hippocampal cell counts (Grigorenko et al., 1998; Grigorenko et al., 1997) leaving this to be an intriguing speculation worthy of further study. However, whether or not our model with have further utility in schizophrenia-related research remains to be determined.

In conclusion, we believe that the results of the current study provide evidence that early exposure to low doses of DOM during a critical period of development, will produce a variety of anomolous behaviours that would suggest that KA receptors play an important role during early ontogeny with respect to establishing the functional integrity of the midbrain DA system.

Chapter 4

Low dose domoic acid in neonatal rats abolishes nicotine induced conditioned place preference during late adolescence.

Melissa A. Burt[a], Catherine L. Ryan[b], Tracy A. Doucette[a]

Departments of Biology[a] and Psychology[b], University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI, Canada, C1A 4P3

Abstract

The mesocorticolimbic DA system is important in mediating behaviors in response to reward, including the rewarding properties of nicotine. The Glu system has been demonstrated to modulate DOM release within the mesocorticolimbic pathway. The current study used low, non-convulsive doses of the KA receptor agonist, DOM (20 μg/kg), administered in rat pups over PND 8-14, a critical period of development in the rat. The rats were later tested in the nicotine-induced CPP paradigm during late adolescence. Peri-adolescence is a period of increased sensitivity to the rewarding properties of nicotine, and a period of maturation for the mesocorticolimbic pathway. The results of this study demonstrated nicotine-induced conditioning in the control rats, and an abolishment of conditioning in the rats treated neonatally with DOM. This study demonstrates the importance of KA receptors in the normal development of behaviors mediated by the mesocorticolimbic pathway, specifically response to the rewarding properties of nicotine during adolescence.

4. Introduction

Glutamate is an excitatory amino acid and an important signaling molecule in the mammalian CNS. An optimal level of Glu activity is needed for normal CNS development, as it plays a key role in many physiological processes that underlie CNS development, including neuronal survival, dendritic and axonal structure, synaptogenesis and synaptic plasticity (for review see McDonald & Johnston, 1990). Glutamate functioning is mediated by a family of receptors that include both ionotropic and metabotropic receptors (Bleakman & Lodge, 1998; Ozawa et al., 1998). The ionotropic receptors are further subdivided into NMDA, AMPA and KA receptors (Bettler & Mulle, 1995; Bleakman & Lodge, 1998). To date five KA receptor subunits, GluR5, GluR6, GluR7, KA-1 and KA-2, have been identified through molecular cloning (Bettler & Mulle, 1995; Ozawa et al., 1998). These subunits are classified as either "high affinity" (KA1, KA2) or "low affinity" (GluR5-GluR7) based on their affinity to the naturally occurring toxin KA. However, the naturally occurring toxin DOM has shown affinity to the low affinity subunits (GluR5-GluR7). Both KA and DOM are agonists for kainate receptors (Lerma et al., 2001). The pharmacological properties and functioning of KA receptors depend on the subunit composition, however, all functional KA receptors elicit a fast onset and a desensitizing response, and can be located both pre and postsynaptically (Chittajallu et al., 1999; Lerma, 2003).

Few studies, to date, have been directed at exploring the role that Glu and its receptors play in early development without focusing on the excitotoxic properties of this transmitter. This is especially true with respect to the involvement of KA receptors in CNS development. However, some studies

have looked at the effects of very low equi-efficacious non-convulsive doses of DOM (5 or 20μg/kg) and the related kaniod, KA (25 or 100μg/kg) at a critical period of development (Doucette et al., 2004, Doucette et al., 2003). In these studies, low doses of DOM and KA were administered to rat pups during PND 8-14, a period known as the brain growth spurt. Changes in development and in the resulting adult were subsequently observed. The brain growth spurt is a period of rapid CNS development, and is therefore a period when there is great susceptibility to alterations in normal development (Dobbing & Smart, 1974). Therefore, although the doses used in these studies were low non-convulsive doses, there were long lasting alterations seen in development, behavior, neurochemistry and neuroanatomy within the hippocampus (Doucette et al., 2004, Doucette et al., 2003).

Some of the changes seen in these animals suggest that treatment with DOM produces an alteration in the dopaminergic system. Such observations include the altered response to a novel spatial environment and alterations (e.g. anatomical and neurochemical) in the hippocampus (Doucette et al., 2004). The dopaminergic system, especially the mesocorticolimbic pathway, is important in the modulation of response to novelty (Bevins et al., 2002, Legault & Wise, 2001) and has connections with the hippocampus (Pennertz & Kitai, 1991). A previous study was conducted to address this question and examined the response to novelty and reward in animals treated perinatally with DOM (see Chapter 3). Using 20μg/kg of DOM from PND 8-14, differences were found in both response to novelty and reward in the treated animals. Observed differences include alterations in activity levels in the open field, which varied depending on the developmental time point. Also, alterations in response to novelty were detected in the DOM-treated males in the

playground maze. With the introduction of a novel object, these animals manifested an increase in the investigation of familiar objects, compared to the control males. Of particular interest are the results found in the nicotine induced CPP paradigm. The DOM-treated females were the only treatment group to develop conditioning in adulthood to the dose of 0.6 mg/kg of nicotine. These results demonstrate that alterations in Glu signalling during development can alter behaviors reliant on the mesocorticolimbic DA pathway.

Glutamate receptors, including KA receptors, are expressed throughout the mesocorticolimbic pathway and have been shown to modulate the release of DA (Crowder & Weiner, 2002, Legault & Wise, 2001, Mathé et al., 1998). Kainate receptors have been located in the rat NAc and functional KA receptors are located both pre and postsynaptically (Crowder & Weiner, 2002). Kainate receptors also potentiate GABAergic synaptic transmission in the NAc, an effect which appears to be mediated presynaptically (Crowder et al., 2006). It has also been demonstrated that AMPA/KA antagonist (LY293558) applied to the VTA results in an increase in DA levels in the NAc and a decrease in DA levels in the PFC (Takahata & Moghaddam, 2000). There is also evidence to support the idea that non-dopamine neurons in the VTA, perhaps GABAergic interneurons and projection neurons, contain NMDA, AMPA and KA receptors (Wang & French, 1995). NMDA and AMPA/KA receptors appear to play a role in the rewarding properties of drugs which produce their reinforcing effects by activating the mesocorticolimbic pathway (Biondo et al., 2005; Suto et al., 2003). For instance receptor blockade of NMDA and AMPA/KA receptors has been shown to prevent the acquisition of D 2/3 dopamine receptor stimulation CPP (Biondo et al., 2005). The sensitizing effects of administration of amphetamine in the VTA, to the acquisition of cocaine self-

administration, appears to require the activation of NMDA and AMPA/KA receptors and even mGluRs (Suto et al., 2003). This demonstrates not only the functional presence of glutamate in the mesocorticolimbic pathway, but also the importance of Glu in the rewarding properties of some drugs which activate this pathway.

Nicotine is an addictive drug, and the rewarding properties of nicotine appears to mediated through the mesocorticolimbic pathway (for reviews see Ikemoto et al., 2006; Balfour, 2002). Interestingly, nicotine actually causes the release of Glu, in measurements of extracellular Glu levels, in the NAc of the rat when administered either 0.3 mg/kg s.c., causing a 30% increase, or 0.6 mg/kg s.c, causing a 50% increase in Glu levels (Reid et al., 2000). Doses of nicotine (0.15, 0.3, 0.6 mg/kg i.p.) not only cause an increase in microdialysate content of Glu and other amino acids in the NAc, but has also been shown to induce CPP (Kashkin & DeWitte, 2005). Nicotine will induce conditioning in the CPP paradigm, but is only effective at optimal doses, too little or too much will not result in a preference for nicotine or could cause an aversion (Le Foll & Goldberg, 2005a). Nicotine will also be self administered by rats (Chaudri et al., 2005, Le Foll & Goldberg, 2005b, Le Foll & Goldberg, 2006) further demonstrating the rewarding properties of nicotine.

The peri-adolescent period for the rat spans from approximately PND 28-60 and encompasses early adolescence (PND 28-35), late adolescence (PND 38-45) and young adulthood (PND 53-60) (Badanich et al., 2006). During peri-adolescence the mesocorticolimbic pathway is maturing, with the DA autoreceptors becoming functionally mature (Spear & Brake, 1983).

The adolescent rat is more susceptible than the adult rat to the conditioning effects of nicotine (Vastola et al., 2002) and other addictive drugs such as cocaine (Badanich et al., 2006). Not only does the adolescent rat condition more readily than the adult rat, but the adolescent rat has distinct behavioral and neuroendocrine adaptations from the adult rat (Cruz et al., 2005). Cruz et al., (2005) found that the adolescent rat had an increased locomotor response to nicotine compared to adults with an acute dose, however, repeated injections of nicotine caused behavioral sensitization to a subsequent injection, 3 days later, resulting in an increase in locomotion in the adult which was not detected in the adolescent rat. Also Adriani et al., (2003) reported that the peri-adolescent rat had enhanced neurobehavioral vulnerability to nicotine in comparison to the post-adolescent rat. The peri-adolescent rat had increased self-administration to nicotine in adulthood, when pretreated with nicotine during peri-adolescence. However, there was no such increase in self administration of nicotine in adulthood in the animals pretreated with nicotine during post-adolescence (Adriani, et al., 2003). Also, the rats pretreated with nicotine during peri-adolescence had an increase in nicotinic acetylcholine receptors (nAChR) that are DA neuron specific subunits ($\alpha 5$, $\alpha 6$ and $\beta 2$ acetylcholine (ACh) receptor subunits) in adulthood. This effect was not found in post-adolescent pretreated rats (Adriani, et al., 2003). This study demonstrates the sensitivity of the adolescent rat to the rewarding properties of nicotine, and also characterizes a difference in the dopaminergic brain pathways mediating the underlying rewarding properties. Such findings show that the adolescent has very different responses to nicotine and that there is a developmental shift in the effects of such drugs which may be due to the maturation of the mesocorticolimbic pathway. Understanding how the adolescent is affected is important in the understanding of the development of the brain throughout the entire life span.

The purpose of this study was to examine the effects of early exposure to low doses of DOM on the development of reward seeking behaviors in the late adolescent rat. Treatment with DOM could ultimately alter the functional integrity of the mesocorticolimbic pathway, and in turn alter the conditioning of the treated animals during peri-adolescence, to nicotine in the CPP. Understanding how nicotine conditioning is altered in the DOM treated animals, can in turn aid in understanding the role KA receptors may play in the development of the mesocorticolimbic pathway.

4.1. Materials and Methods

4.1.1. Experimental Animals

All experimental manipulations were conducted on the offspring of untimed pregnant SD rats (Charles River Laboratories, St. Constant, PQ, Canada) with the day of parturition designated as PND 0. The litters were culled to 10-11 pups (5 males, 5 females where possible) within 24 hours of birth. The pups were weaned at 21 days of age (PND 21) with two to three rats (of same gender) per cage. All animals were housed within polypropylene caging with wood chip bedding, and the colony room was maintained at 22.2°C with a 12 hour light/dark cycle (07:00-19:00h) with food (Purina Lab Chow) and water provided *ad libitum*. A total of 60 rats were used in the experimental procedure (31 males, 29 females).

4.1.2. Toxin treatment

Domoic acid was obtained from Diagnostic Chemicals Ltd. (Charlottetown, PE, Canada). The test chemical was dissolved in sterile SAL and injections were administered s.c. The pups received a single daily injections of either 20 μg/kg DOM or equal volume of SAL, from PND 8 until PND 14. Within each litter, DOM and SAL injections were administered equally amongst the males and females, this was quasi randomly assigned in each litter. A total of 30 rats were treated with DOM (15 males, 15 females) and a total of 30 rats served as control (SAL) (16 males, 14 females). The experimenter was blind to the individual treatment conditions of each animal throughout the entire experiment. The pups were marked daily with non-toxic permanent marker, until weaning, for identification.

4.1.3. Developmental Measures

Developmental measures were assessed daily starting on PND 8, the assessments were taken prior to injections on PND 8- 14. The weights of the animals were assessed daily until weaning on PND 21. Auditory startle was assessed until criterion was reached, which was defined as a visible startle response to a noise made by a small clicker held approximately 14 cm above the pups head. Eye opening, defined as a break in the suture of the eye, was also assessed daily. The pups were examined for this developmental milestone until a break in the suture of both eyes was noted.

4.1.4. Nicotine induced Conditioned Place Preference

The animals were conditioned starting at 40 days of age and were tested on PND 52 (i.e. during late adolescence in the rat). (-)-nicotine hydrogen tartrate salt (obtained from Sigma, Canada) was diluted in physiological saline (1ml/kg) for a dose of 0.6 mg/kg, an was adjusted to pH 7.0 using NaOH. The CPP chamber was constructed from 1/4 inch plexi-glas, to the dimensions of 83 (l) x 30.5 (w) x 46 (h) cm, with a removable partition in the center (i.e. which could divide the maze into two compartments of 41 (l) x 30.5 (w) x 46 (h) cm). In turn, the two compartments were visually and tactually distinct, with one compartment having a smooth floor with two horizontal stripes (6.5 cmin width) across each of the four walls, the other compartment having a ridged (each ridge 2 mm) plastic floor with 3 vertical strips (4 cm width) equi-spaced on the 41 cm wall and 2 vertical stripes equi-spaced on the 30.5 cm walls. The walls and floor were dark green and the stripes were light brown.

The nicotine induced CPP procedure involved an initial injection-habituation phase, followed by an habituation trial, eight conditioning trials and finally, a test trial (as previously described in section 2.1.4.4.). In brief, the injection-habituation phase involved administering a saline injection in the room in which the injections during conditioning would take place. This was done to habituate the rats to the injection procedure and reduce anxiety before the conditioning trials began. This was followed by an habituation trial in which the rats were injected with saline and placed in the CPP maze with the door removed and allowed access to both maze compartments for a total of 15 minutes. The rats were then removed from the maze and returned to their home cage for 24

hours until the start of the conditioning trials. The conditioning trials included administering either saline or nicotine to the rat and then placing it in the appropriate compartment for 30 minutes. Following this, the rat was then removed and placed in the home cage for 24 hours until the next trial, for a total of eight trials (four paired with nicotine and four with saline). The procedure was an unbiased procedure with half of each treatment condition (DOM and SAL) receiving nicotine paired with compartment one and the other half receiving nicotine paired with compartment two. This was done to control for any biases that could exist in inherent preference for either of the two compartments. Each rat received four pairings of nicotine and four pairings of saline, with drug (either nicotine or saline) on the initial conditioning day (counter balanced across conditions). Each day the pairing was alternated. After the eight conditioning trials, the rats were then placed in the CPP maze again for the test trial. This trial was conducted 24 hours after the last conditioning trial and was 15 minutes in duration. The rat was placed in the middle of the maze, with the door removed, facing the wall, and allowed access to both compartments.

All testing was videotaped for later scoring. In order to determine a compartment preference, the test trial was scored for time spent in each compartment and number of times the rat crossed from one compartment to the other. The rat was determined to be in a compartment once the head and front shoulders passed the centre line into the compartment.

4.1.5. Adherence to guidelines

All procedures were approved in advance by the UPEI Institutional Animal Care Committee and

adhered to the guidelines of the Canadian Council on Animal Care.

4.2. Results

4.2.1. Developmental Measures

Weight gain was assessed over 24 hr intervals from PND 8-14. Repeated measures ANOVA (days x gender x treatment) revealed no significant differences in weight gain over PND 8-14. Using 2-way ANOVA (gender x treatment) no significant differences in auditory startle or eye opening was revealed in the rats.

4.2.2. Nicotine Induced Conditioned Place Preference

Paired samples t-tests were used to compare the time (sec) spent in the in the nicotine paired (paired) compartment with the saline paired (un-paired) compartment in each of the treatment groups. Only the SAL males and SAL females demonstrated nicotine induced conditioned place preference. Interestingly, the DOM males and DOM females did not demonstrate conditioning. The SAL males spent more time (sec) in the paired compartment (570.00 ± 46.50) relative to the un-paired compartment (355.81 ± 46.53) ($t(15)=2.308$, $p=0.036$). The SAL females spent more time (sec) in the paired compartment (642.86 ± 62.66) relative to the un-paired compartment (312.79 ± 10.44) ($t(13)=2.508$, $p=0.026$) (see Figure 4.1). In the number of center crosses, a 2-way ANOVA (gender x treatment) revealed no significant differences.

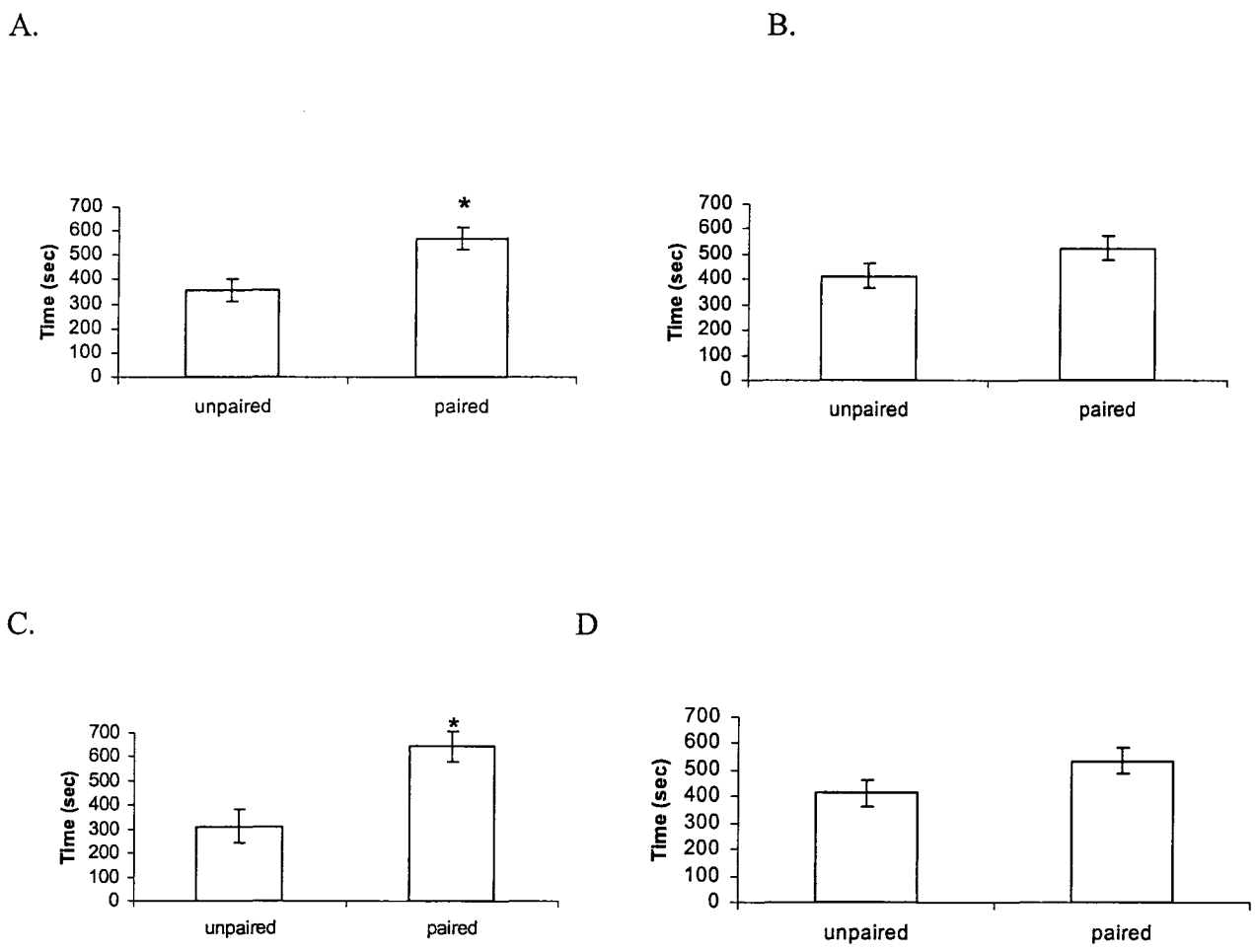


Figure 4.1. Mean time (sec) spent in nicotine-paired and unpaired chambers in the 24 hr following the final conditioning trial for male saline (A) and DOM-treated (B) rats and for female saline (C) and DOM-treated (D) rats. Error bars represent standard errors.(* indicates $p < 0.05$ relative to unpaired compartment).

4.3. Discussion

The results of this study demonstrate that early exposure to low doses of DOM result in an altered response to reward during adolescence. This was illustrated by the DOM-treated rats failing to demonstrate a place conditioning preference to nicotine, which was present in the control animals. Adolescent rats are sensitive to nicotine (Cruz et al., 2005) and condition to the drug under normal circumstances (Vastola et al., 2002). We not only demonstrated a sensitivity to the conditioning properties of nicotine that spans into late adolescence in the rat, but also that this sensitivity is altered in rats treated perinataly with low doses of DOM. Therefore, there would appear to be something fundamentally different about the mesocorticolimbic pathway, which mediates the rewarding properties of nicotine (reviews Ikemoto et al., 2006; Balfour, 2002), in the DOM-treated animals.

There are several different possible interpretations of the results of this study. Perhaps the DOM rats may have faster CNS development in the mesocorticolimbic pathway. This could be possible based on evidence in previous studies which demonstrated an accelerated eye opening in female DOM-treated rats (Doucette et al., 2003, see Chapter 3) which suggests an acceleration in CNS maturation. Perhaps, the conditioning results from the DOM treated rat's are similar to those from adult rats during nicotine conditioning, with the failure to condition to the nicotine. Previous studies demonstrate that an adult rat does not normally condition to 0.6 mg/kg of nicotine (Vastola et al., 2002, see Chapter 2). However, in order to make this conclusion, further evidence would be needed. It would be important to characterize the DOM rat's response to the other properties on

nicotine such as self administration, activity levels, receptor densities (e.g. KA, nACh, and DA receptors) and neurotransmitter levels (e.g. DA and Glu in the VTA and NAc) in comparison to control adolescents and control adults. Also, it would be important to look at nicotine induced CPP during early adolescence in theDOM-treated animals to further understand the development of the mesocorticolimbic pathway and the rewarding properties of nicotine in the treated animals.

At low doses DOM stimulates the GluR 5-7 subunits in KA receptors (Lerma et al., 2001). Perhaps stimulating these receptors during the brain growth spurt has resulted in an alteration in the functioning of the mesocorticolimbic pathway, where KA receptors are located. Perhaps this stimulation has resulted in and increase in KA receptors located on the GABAergic neurons in the NAc (Crowder et al., 2006) during adolescence. Nicotine's rewarding effects are thought to be due to the activation on the VTA resulting in an increase in DA release in the NAc (reviews Ikemoto et al., 2006; Balfour, 2002) and also causes an increase in Glu levels in the NAc (Reid et al., 2000). The release of Glu could in turn activate the KA receptors on the GABAergic neurons in the NAc and this could result in a greater inhibitory tone of the mesocorticolimbic pathway in the DOM-treated animals during adolescence and could potentially account for the inability of the DOM-treated animals to condition to nicotine. Looking at the KA receptor densities in the VTA and the NAc would be helpful in understanding if this is a potential reason for the DOM-treated animals failure to condition to the nicotine.

Results form this study strongly suggest that there has been a fundamental difference in the DA system during adolescence in rats treated perinataly with DOM. This demonstrates the importance

of glutamate (especially KA receptors) in the normal development of the CNS, specifically the mesocorticolimbic pathway.

Chapter 5: General Discussion

5. Discussion

The primary hypothesis of this thesis was to determine if low doses of DOM administered early in development would result in the manifestation of later behavioral alterations that rely on the functional integrity of the mesocorticolimbic DA pathway. The secondary aim of this thesis was to begin determining the potential for the perinatal DOM rat model to serve as an animal model of schizophrenia.

5.1. Addressing the Primary Hypothesis: Have behaviors mediated by the mesocoticolimbic dopamine pathway been altered?

The results from Chapter 3 and Chapter 4 have demonstrated alterations between DOM-treated and controls rats in activity levels throughout development, in response to novelty in the adult males, and in conditioning to nicotine in the adult females and adolescent males and females. As addressed in Chapter 3, results from the open field demonstrated alterations in activity levels in the DOM-treated animals. Dopamine is important in modulating activity levels, and alterations in the DA system during development can result in altered activity levels (Breese et al., 2005).

The mesocorticolimbic pathway is important in modulating responses to novelty (Bevins et al., 2002; Legault & Wise, 2001), and to the rewarding properties of nicotine (Ikemoto et al., 2006;

Balfour, 2002). The results from the playground maze demonstrate an altered response to the introduction of a novel object in the male DOM-treated rats. The results from the nicotine-induced CPP paradigm, demonstrated an increased sensitivity to the rewarding properties of nicotine in the female DOM-treated rats. The sensitivity was manifested as a conditioning to the nicotine as an adult, a period of development when this dose does not normally induce conditioning in the adult rat. Although there are gender differences in the manifestation of altered behaviors, the perinatal DOM-treated rat does demonstrates alterations in behaviors mediated by the mesocortiolimbic pathway.

In Chapter 4, this was further supported by the failure of the adolescent DOM-treated rats to condition to nicotine in the nicotine-induced CPP, unlike the control rats which did condition to nicotine. The adolescent rat is more susceptible to the conditioning properties of nicotine than adult rats, and normally demonstrates conditioning (Vastola et al., 2002). One possible explanation for the DOM-related differing in nicotine conditioning could be due to a possible delay in maturation of the DA mesocoritcolimbic pathway. Thus the adolescent DOM rats fail to condition while manifesting changes sensitivity to nicotine in adulthood, a time when control rats sensitivity has passed. Therefore, looking at the results from both Chapter 3 and 4, the DOM-treated rats may indeed have functional alteration in the mesocorticolimbic pathway that is manifested differently throughout development and between genders.

It is also difficult to determine whether the results from the nicotine-induced CPP are reflecting changes to the rewarding properties of nicotine, and not potentially difference in learning

(conditioning). For instance a failure of the adolescent animals to condition to nicotine could also be reflective of an inability/delay to learn the place-conditioning task. The basal ganglia in particular is important in procedural learning, such as place-conditioning (Packard & Knowlton, 2002). Interestingly, the DA content does not reach adult concentrations until PND 50-60 in the rat (Broening, 1998). Perhaps the DOM-treated rats have an altered ability to learn the place-conditioning task, due to an alteration in the basal ganglia, during adolescence. A self-administration task would be beneficial in answering this question, as mentioned in the discussion of Chapter 4 (section 4.3). A self-administration task can often have different results compared to the CPP (Le Foll & Goldberg, 2005b), and would provide insight to the alterations in the mesocorticolimbic pathway that appear to be manifested in the behaviors of the DOM-treated rodents. This would be important to administer both during adulthood and during adolescence, in order to determine if the results remain consistent with what was found in the CPP. It would also be interesting to conduct the nicotine-induced CPP during early adolescence (starting from PND28), to see if the conditioning is still abolished in the DOM-treated rats, or if this is a transient effect limited to late adolescence. In order to understand the precise mechanisms involved in the alterations to nicotine in the DOM-treated rats, other measures could aid in understanding. Firstly, measuring alterations in activity levels after nicotine administration would be interesting. If nicotine did not result in an increase in activity levels in the adolescent DOM-treated rat, then perhaps the decrease in exploration during the nicotine conditioning trials, could account for the abolishment of conditioning. Also interesting would be to do an *in vivo* microdialysis study looking at extracellular levels of Glu and DA in the NAc after nicotine administration, similar to a study by (Reid et al., 2000). Such further investigations would provide insight into precisely what

behaviors are altered and how the mesocorticolimbic pathway has been altered.

5.2. Addressing the Secondary Hypothesis: What is the potential for the perinatal domoate rat model as an animal model of schizophrenia?

The results from Chapter 3 demonstrate face value for the perinatal DOM rat model as a potential animal model for schizophrenia. The alterations in activity levels in the DOM-treated rats throughout development not only suggest an alteration in the DA system, but also provides support for the potential of the perinatal DOM rat model as an animal model of schizophrenia. The decrease in habituation to the open field, during pre-adolescence (PND18) is very interesting. Early in development the using 6-hydroxy dopamine in the rodent, generally results in an increase in activity levels and impaired habituation (Breese et al., 2005, Shaywitz et al., 1977). This model has been used as an animal model of ADHD (Breese et al., 2005) and ADHD has been linked to early development in children with parents with schizophrenia and considered at high-risk (Öner & Munir, 2005), and is also a co-morbid condition commonly found in child-onset schizophrenia (Ross et al., 2006). The results in the open-field in the adult rats are also of considerable interest. Previous animal models of schizophrenia have interpreted decreases in activity and increases in activity to negative and positive symptoms of schizophrenia, respectively (Powell & Miyakawa, 2006). The DOM-treated male rats demonstrated a decrease in activity, which could be interpreted as potentially representative of negative symptoms of schizophrenia. This decrease in activity levels was not present in the adolescent (PND 36) DOM-treated male rats, perhaps indicative of a delayed onset of symptoms. The DOM-treated female rats demonstrated an increase in activity

levels, perhaps representative of positive symptoms. There was only a mild increase in activity found in the adolescent DOM-treated female rats, with an increase in rearing, which could also represent a delayed onset of symptoms similar to the development of schizophrenia. This is also an interesting gender difference, as estrogen has been considered to be protective of the negative symptoms of schizophrenia (Rao & Kolsch, 2003). Therefore results in the open field, throughout development demonstrate not only alterations in the DA system dependent of the stage of development, but also lend face value for potential as an animal model of schizophrenia.

In Chapter 3, the results from the playground maze also lend support to the male DOM-treated rats potentially being representative of predominately negative symptoms of schizophrenia. The increase in exploration of the familiar objects in these animals, could reflect negative symptoms. As discussed in Chapter 3, it has been suggested that an increase in exploration of familiar objects could potentially represent negative symptoms (Mellanby et al., 1999). However, the DOM-treated male rats still had a significant novelty effect, determining the ability to detect the novel object, but also an increase in exploration of the familiar objects. This could potentially represent either a decrease in the ability to habituate to the familiar objects, or a revert to the familiar when novelty is introduced. Which ever reason for the increase in exploration of the familiar, it represents an alteration in response to novelty in the DOM-treated males. This is important since response to novelty is often altered in schizophrenia (Laurens et al., 2005). There was no comparable difference found in the DOM-treated females, which might again reflect the protective effect of estrogen against the negative symptoms of schizophrenia (Rao & Kölsch, 2003) and correspond to the potential of the male DOM-treated rats reflecting negative, rather than positive symptoms.

The results for the nicotine-induced CPP in both Chapter 3 and 4 provide interesting results. The conditioning to the nicotine in the adult DOM-treated females could be reflective of positive symptoms in schizophrenia. It has been suggested that increased conditioning to addictive drugs could be representative of positive symptoms of schizophrenia (Chambers & Self, 2000) and a decrease in conditioning reflective of negative symptoms (LePen et al., 2002). Also, this result is interesting since the rate of smoking is so high in the schizophrenia population (de Leon et al., 2005). There was no conditioning found in the control males and females or the DOM-treated males. Therefore, the behaviors reflective of positive symptoms are predominately found in the DOM-treated female rats. During adolescence, neither the DOM-treated males or females demonstrated conditioning, unlike the control males and females. This could reflect a delayed onset of positive symptoms in the DOM-treated female rats. The failure to condition to nicotine in adolescence could also potentially reflect anhedonia in the adolescent DOM-treated rat, which is classically displayed during adolescence in patients with schizophrenia (Lieberman et al., 2001). However, further investigation is necessary for complete interpretation of the results found in both studies from the nicotine-induced CPP.

Although, results from the two studies provide some support for the perinatal DOM rat model, as a potential model for schizophrenia, further characterization is necessary. Future directions for furthering the face value of this potential model, would include measures in PPI and/or LI, as these are commonly used in current animal models of schizophrenia (see section 1.9.2.1.). This would be interesting to do at different developmental time points, first to look at if deficits are apparent during adulthood, then if deficits are apparent during adolescence. This would be interesting as it

has been suggested that a delay in LI deficits, could represent the delay in symptoms seen in schizophrenia pathology (Zuckerman & Weiner, 2005). However, deficits in sensorimotor gating (PPI deficits) are also considered trait markers for schizophrenia, and could be manifested before the onset of schizophrenia (Joober, et al., 2002). If deficits are present in the DOM-treated animals, it would add face value, as an animal model of schizophrenia. Potentially future studies could look at the use of antipsychotics such as clozapine in the correction of disruptions if they are present, this has been demonstrated in previous animal models of schizophrenia (Zuckerman & Weiner, 2005). It would also provide predictive validity to the perinatal DOM rat model.

Another important test, would be to conduct the social interaction task which is a behavioral test used to mimic negative symptoms of schizophrenia in animal models (see section 1.9.2.2.). Since DOM-treated rats have symptoms which resemble negative symptoms in the males, it would be interesting to determine if there is an alteration in social interaction in the DOM-treated rats. This would be interesting to first look at differences in the adults and then in adolescence. If a decrease in social interaction is present in adulthood and not in adolescence, then this could reflect a delay in onset of symptoms. However, this would not be necessary in order to mimic symptomtology of schizophrenia, as social withdrawal is present prior to the onset of schizophrenia (Baum & Walker, 1995). It would be interesting to look at differences in gender, since the current results from the two studies demonstrate strong differences in the manifestation of behaviors depending gender, with females demonstrating predominately positive-like symptoms and the males negative-like symptoms.

It would also be interesting to determine whether there are differences in response to amphetamine or MK801 in the DOM-treated rats. These compounds have been previously used in animal models of schizophrenia (see section 1.9.2.3.). MK801 would be a particularly interesting compound to investigate, as previous results in the DOM-treated rats demonstrated alterations in behaviors mediated by the NMDA receptor (Tasker et al., 2005). Pharmacological challenges in the form of dose-response curves to either amphetamine or MK801, would allow a functional assessment of the integrity of the DA and Glu systems, respectively.

Future investigations looking at alterations in the neurochemistry and neuroanatomy in the adult domoate treated rats, could include hippocampal analysis. The previous studies already give face validity for alterations in the hippocampus of the DOM-treated rats (Doucette et al., 2004). These previously reported decreases in hippocampal cell count (Jönsson et al., 1999; Luts et al., 1998) and elevations in BDNF mRNA (Takahashi et al., 2000) have been demonstrated in the brain of schizophrenics. However, it would be interesting to look at alterations in the organization of the pyramidal neurons in the hippocampus. This is a classic alteration demonstrated within the schizophrenia population (Jönsson et al., 1999). It would also be important to look at the GluR expression in the hippocampus, particularly the GluR 5-7, which are the receptors in which DOM has affinity (Lerma et al., 2001) and have already been altered in the brain of schizophrenic population (Benes et al., 2001).

Based on results of the two studies it would be relevant to investigate GluR in the NAc and the VTA. Initial investigations should focus on GluR 5-7 KA receptor subtypes, as not only are they

the target receptors during treatment, but results indicate they are potentially being altered. The results from Chapter 4 suggest a possible increase in KA receptors during adolescence in the NAc, and potentially, the VTA. Therefore it would be important to look at receptor concentrations during adolescence and adulthood. Also, since the results from the two studies indicate alterations in the mesocorticolimbic DA system, therefore alterations in DA may be relevant. Preliminary investigations could look at dopamine transporter (DAT) concentration, since DAT is a good measure of DA neurons (Cooper et al., 1996). DA receptors could also be investigated, potentially looking at D1 and D2/3 rceptors as they are commonly represented in structures located within the mesocorticolimbic pathway (Missale et al., 1998). Investigations could also look at NMDA receptor concentrations, this would be especially relevant if alterations were found in response to MK801. Another potential future investigation could look at the dendritic sprouting in the PFC. This has been demonstrated to be altered in animal models of schizophrenia, specifically the nVH model (Flores et al., 2005) and the schizophrenia population (Kolluri et al., 2005). Additionally alteration in laminar organization of the cortex of schizophrenia patients has also been reported and could be investigated in this animal population.

5.3. Conclusions

We have had demonstrated not only face value for the model, warranting further investigation as to the potential of the perinatal DOM rat model as a potential animal model of schizophrenia, but also provided further construct validity. We have demonstrated that mild alterations in the Glu system early in development, results in alterations in behaviors mediated by the mesocorticolimbic

pathway. This is congruent with schizophrenia as a neurodevelopmental model, and the dysfunction in DA/Glu interaction found in schizophrenia. We have not only succeeded in the primary aims of the thesis, but also the secondary aims. These studies suggest that further investigations in to the alterations in the mesocorticolimbic pathway of the DOM-treated rats, and into the potential of the perinatal DOM rat model as a model for schizophrenia are warranted.

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