The effects of (RS)-MCPG on amphetamine-induced sensitization in neonatal rats

Fiona Yeuk-Lun Choi

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THE EFFECTS OF (RS)-MCPG ON AMPHETAMINE-INDUCED SENSITIZATION IN NEONATAL RATS

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

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

by
Fiona Yeuk-Lun Choi

December 2006
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Approved by:

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Cynthia Crawford, Chair, Psychology
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11/30/06
ABSTRACT

The purpose of this study was to investigate the role of metabotropic glutamate receptors (mGluR) in the ontogeny of amphetamine-induced behavioral sensitization. 11-day-old rat pups were given 5 daily bilateral infusions of the metabotropic glutamate receptor antagonist, (RS)-methyl-4-carboxyphenylglycine (MCPG) (0.0, 2.5, or 25.0 nM) followed by a systemic injection of amphetamine (0.0 or 2.0 mg/kg) and locomotor activity was measured. Following a 48 hr abstinence period, rats were given a challenge injection of amphetamine (0.0 or 0.5 mg/kg) and locomotor activity was again measured. It was hypothesized that rats receiving amphetamine pretreatment and an amphetamine challenge would exhibit a significant increase in activity, indicating short-term behavioral sensitization. In contrast with studies on adult rats, behavioral sensitization in rat pups was not expected to be attenuated by the actions of MCPG on the mGluR system. As predicted, repeated amphetamine administration during the pretreatment phase produced progressively enhanced locomotor activity, indicating the development of behavioral sensitization. Pretreatment with the low dose of MCPG (2.5 nM) significantly attenuated amphetamine-induced locomotor activity while the high dose
of MCPG (25.0 nM) did not significantly affect locomotion. Interestingly, pretreatment with 25.0 nM MCPG significantly increased the locomotor response to a challenge injection of amphetamine in all rats, regardless of amphetamine or saline exposure during pretreatment. The effect of MCPG on locomotor activity appears to be independent from the effects of amphetamine-induced locomotor activity and MCPG pretreatment failed to consistently block the expression of behavioral sensitization in rats pretreated with amphetamine and challenged with amphetamine. Interpretations and possible molecular mechanisms responsible for these results will be explored in the discussion section. In summary, this study demonstrates that contrary to previous studies on adult rats, the mGluR system does not appear to consistently mediate the development of amphetamine-induced sensitization in neonatal rats.
ACKNOWLEDGMENTS

The work contained within this thesis was completed with the help of many people. Those who both directly and indirectly assisted me in the lab are listed alphabetically below. I would like to especially acknowledge the wisdom and expert advice of Drs. Cynthia Crawford, Sanders McDougall, and Yuchin Chien throughout my graduate career. I am grateful for the opportunity to have worked with such an outstanding team of experimental researchers. In addition, I would like to thank Dr. Robert Ricco, Dr. Joanna Worthley, and the faculty and staff of the Psychology Department for all their support and invaluable assistance. Finally, I would like to acknowledge the ASI for research funding.

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CHAPTER ONE
INTRODUCTION

Overview

Abuse of the psychostimulant amphetamine and its analogs, methamphetamine and 3,4-methylenedioxy (MDMA) "Ecstasy", is a major problem of modern society and affects all races and socioeconomic classes. According to the 2000 National Household Survey on Drug Abuse, an estimated 8.8 million people (4.0% of the population) have tried methamphetamine at some time in their lives (National Institute on Drug Abuse [NIDA], 2002). Moreover, there has been an upward trend in the use of psychostimulants, with treatment admissions for the abuse of methamphetamine increasing 294% between 1992 and 1996 (Substance Abuse and Mental Health Services, 1999).

The cause of the recent upsurge of amphetamine abuse is unknown but it may be a consequence of amphetamines' many uses and increased availability. Amphetamines have a number of physiological properties that make them common drugs of abuse. For example, amphetamines are abused recreationally for their ability to elevate mood and increase overall feelings of well-being (Hegadoren, Baker &
Bourin, 1999). In addition, amphetamines are often misused and abused in occupational settings by truck drivers, physicians, students, construction workers, and athletes for their ability to increase stamina, promote alertness and relieve stress (Nencini & Ahmed, 1989; Singh & Jindal, 1980). The availability and low cost of illicit methamphetamine has also increased abuse rates. In contrast to cocaine, which must be imported, methamphetamine can be manufactured very cheaply from ingredients found in over-the-counter cold medications. Thus, as law enforcement has seen some success in preventing the inflow of cocaine, the production and distribution of methamphetamine has risen.

In humans, chronic misuse of amphetamines can lead to long-lasting impairments in brain function and behavior (Robinson & Becker, 1986). Particularly, repeated amphetamine administration can produce paranoia, delusions, hallucinations, and violent behavior (Cretzmeyer, Sarrazin, Huber, Block, & Hall, 2003; Robinson & Becker, 1986; Satel, Southwick, & Gawin, 1991). The cause of these behavioral impairments are unknown, however, positron emission tomography (PET) studies have found that individuals who
are methamphetamine abusers have a lower level of dopamine D₂ receptors (Volkow et al., 2001).

More conclusive evidence for the negative impact of amphetamine exposure on brain functioning and behavior has been yielded by studies in adult rodents. These studies have found that repeated amphetamine exposure induces central nervous system (CNS) cell death and produces a long-term change in behavior, called behavioral sensitization (Cretzmeyer et al., 2003; Robinson & Berridge, 2001; Volkow, Fowler, Wang, & Goldstein, 2002). This change in behavior is characterized by a progressive and enduring enhancement of the drug-induced behavioral effects of amphetamine (Kalivas & Stewart, 1991; Robinson & Berridge, 2001; Sorg & Newlin, 2002). Behavioral sensitization can be observed after as little as one drug exposure and can still be detected for at least one year after the last drug exposure (Kalivas & Stewart, 1991; Robinson & Becker, 1986). Much of the interest generated about behavioral sensitization stems from its utility as an animal analog of human psychosis (Kalivas & Stewart, 1991) and as a model of human drug addiction (Davidson, Gow, Lee, & Ellinwood, 2001; Self & Nestler, 1998).
Given that amphetamine abuse can cause long-term changes in brain functioning, it is alarming that the recent increase in amphetamine abuse includes large numbers of teenagers and young adults (Hegadoren et al., 1999). In 2000, 4.4% of high school seniors reported using methamphetamine at least once in their lifetime and high school students reported having been exposed to methamphetamine by as early as the 8th-grade (NIDA, 2002). The use of MDMA among younger populations has increased dramatically both in the United States (US) and internationally. A 2003 survey of 44,000 US high school students in the 12th grade found that 8.3% reported MDMA use (Johnston, O’Malley & Bachman, 2003). A review of studies conducted in other countries, including Australia and various European countries, found that 1% to 13% of people surveyed reported using MDMA in their lifetime (Parrott, 2001).

Moreover, a large number of young children are also exposed to amphetamine as a treatment for attention-deficit-hyperactivity-disorder (ADHD). It is estimated that 7-16% of all school-aged children in the US suffer from this disorder and the majority of these children are treated with amphetamine or the amphetamine-derivative,
methylphenidate (Solanto, 2002; Teo, Stirling, Thomas, Hobemen, Christian, & Khetani, 2002; Yang, Swann & Dafny, 2003).

Proposal

At present, there have been very few studies examining the effects of chronic amphetamine exposure in human children or teenagers. However, animal studies have revealed that the developing and the adult brain respond differently to repeated psychostimulant exposure, particularly in the expression of behavioral sensitization (McDougall, Duke, Bolanos & Crawford, 1994; Tirelli & Ferrara, 1997; Tirelli, Laviola & Adriani, 2003; Wood, Tirelli, Snyder, Heyser, LaRocca & Spear, 1998). Research now shows that no single neuronal system is entirely responsible for the development of behavioral sensitization (Pierce & Kalivas, 1997; Wolf, 1998). The dopamine system has been identified as being primarily responsible for the rewarding effects of amphetamine (Genova et al., 1997; Pierce & Kalivas, 1997), in addition, an increasing amount of evidence is pointing towards the regulatory role of the metabotropic glutamate receptor system on the functions of
the dopamine system (Ohno & Watanabe, 1995; Saccan et al., 1992).

Thus, the following study examines the effects of repeated amphetamine treatment during early development using the behavioral sensitization paradigm. Specifically, this thesis will assess the role of the metabotropic glutamate receptor system in the development of behavioral sensitization in the young rat. Identifying the response of the developing brain to psychostimulant exposure will help develop a greater understanding of the neuropharmacological circuitry that is involved in the process of behavioral sensitization and drug addiction.
Dopamine Synthesis and Transmission

Dopamine belongs to a small group of monoamine neurotransmitters called the catecholamines. Dopamine synthesis involves the conversion of the amino acid, tyrosine, to L-dihydroxyphenylalanine (L-Dopa) by the enzyme, tyrosine hydroxylase (TH) (Cooper, Bloom & Roth, 1996). L-Dopa is then converted to dopamine by the enzyme, dopa decarboxylase (Cooper et al., 1996). The release of dopamine from the nerve terminal into the synaptic cleft is calcium-dependent and results from nerve impulse stimulation in the form of an action potential (Chesselet, 1994). The effects of the released dopamine in the synaptic cleft are mediated by activation of dopamine receptors (Meador-Woodruff, 1994). Dopamine receptors are found both presynaptically and postsynaptically on both dopamine and nondopamine cells (Meador-Woodruff, 1994). Dopamine autoreceptors are presynaptic receptors located on the soma, dendrites, and nerve terminals of dopamine cells (Wolf & Roth, 1990). When the autoreceptors on the soma or dendrites are stimulated, the firing rate of the dopamine
neuron decreases (Wu, Reith, Walker, Kuhm, Carroll, & Garris, 2002). When the autoreceptors on the nerve terminals are stimulated, dopamine synthesis and release is diminished (Wu et al., 2002). Postsynaptic receptors are found on nondopamine cells such as GABA and acetylcholine neurons (Cooper et al., 1996).

Inactivation of dopamine after release is accomplished by the dopamine transporter (DAT) in the membrane of presynaptic neurons (Gainetdinov, Jones, Fumagalli, Wightman & Caron, 1998; Giros & Caron, 1993). DAT plays a significant role in the removal of extracellular dopamine, thereby regulating synaptic neurotransmitter concentration (Gainetdinov et al., 1998; Giros & Caron, 1993). After reuptake into the nerve terminal, dopamine may be metabolized by monoamine oxidase (MAO) and converted to DOPAC or repackaged into synaptic vesicles and re-released (Cooper et al., 1996; Meador-Woodruff, 1994). Within the synaptic cleft, dopamine may be converted by catechol-O-methyltransferase (COMT) into homovanillic acid (HVA) (Cooper et al., 1996; Meador-Woodruff, 1994).
Dopamine Receptor System

Dopamine receptors are divided into two families, D₁-like or D₂-like receptors. D₁-like and D₂-like receptors were originally differentiated by their biochemical characteristics. Activation of D₁-like receptors leads to increased activity of adenylyl cyclase and stimulation of phosphoinositide activity (Hille, 1992). D₂-like receptors, on the other hand, inhibit adenylyl cyclase activity, inhibit calcium (Ca²⁺) release into nerve terminals, increase potassium (K⁺) conductance, and decrease the metabolism of phosphoinositide (Steketee, 2003; Surmeier, Bargas, Hemmings, Nairn, & Greengard, 1995).

D₁-like and D₂-like receptors can be further divided into subtypes. There are currently two subtypes of D₁-like receptors, D₁ and D₅, and four subtypes of D₂-like receptors, D₂ short, D₂ long, D₃, and D₄ (Cooper et al., 1996). These subtypes have been identified through gene cloning studies, and are differentiated by their relative distribution in the nervous system. The highest density of D₁ receptors are found in the caudate-putamen of the neostriatum, in mesolimbic structures, which include the nucleus accumbens and olfactory tubercles, in the ventral
tegmental area, and in the substantia nigra pars compacta (Bardo & Hammer, 1991). The level of D₅ receptors are very low in brain, and are only found in the hippocampus, the hypothalamus and the parafascicular nucleus of the thalamus (Lidow, Goldman-Rakic, Gallager, & Rakic, 1991; Meador-Woodruff, Mansour, Grandy, Damask, Civelli, & Watson, 1992). D₂ receptors are found in highest concentration in the caudate-putamen, nucleus accumbens, olfactory tubercle, substantia nigra pars compacta, and olfactory bulbs (Gehlert, Gackenheimer, Seeman, & Schaus, 1992). D₃ receptors are localized in the islands of Calleja, a dense group of small neurons within the olfactory tubercles, and the nucleus accumbens (Gelhert et al., 1992). D₄ receptors are in the highest concentration in the frontal cortex, hypothalamus, thalamus, midbrain, medulla, amygdala and olfactory bulbs (Tarazi, Campbell, Yeghiayan, & Baldessarini, 1998).

All dopamine receptors are G-protein-coupled guanosine triphosphate (GTP-binding protein) receptors (Missale, Nash, Robinson, Jaber, & Caron, 1998; Seamans & Yang, 2004). Stimulation of a dopamine receptor precipitates a sequence of biochemical actions that begin with activation of a G-protein in the membrane. D₁-like receptors are
coupled to stimulating G-proteins (G\textsubscript{s} or G\textsubscript{o1f}) and, when activated, adenylyl cyclase activity is facilitated (Cooper et al., 1996). D\textsubscript{2}-like receptors are coupled to inhibitory G-proteins (G\textsubscript{i} or G\textsubscript{0}) and when they are activated, adenylyl cyclase activity is inhibited (Cooper et al., 1996). The activation and inhibition of adenylyl cyclase activity precipitates a cascade of events in the cyclic adenosine monophosphate (cAMP) system, which plays an important role in neuropharmacological responses to changes in the dopamine system (Tzschentke, 2001).

More specifically, G\textsubscript{s}- and the G\textsubscript{o1f}-proteins activate an effector protein, adenylyl cyclase, which is the catalyst in the conversion of adenosine triphosphate (ATP) to cAMP (Pierce, Premont, & Lefkowitz, 2002). On the other hand, G\textsubscript{i}- and G\textsubscript{0}-proteins inhibit the effector protein, adenylyl cyclase, leading to decreases in cAMP (Pierce et al., 2002). cAMP functions as a second messenger and activates the enzyme, protein kinase A (PKA). PKA is a catalyst for phosphorylation, the transfer of phosphate groups (PO\textsubscript{3}) from ATP to specific sites in the cell, and leads to changes in Ca\textsuperscript{2+} and K\textsuperscript{+} permeability of the cell membrane. The flow of Ca\textsuperscript{2+} and K\textsuperscript{+} across membrane channels is responsible for
neuronal firing, the basis of cell signaling and communication (Grady, Bohm, & Bunnett, 1997).

The cAMP system can be modified after denervation or prolonged exposure to receptor agonists, resulting in the system being either up- or down-regulated, respectively (Terwilliger, Beitner-Johnson, Sevarino, Crain & Nestler, 1991). Down-regulation is characterized by a decrease in the levels of adenylyl cyclase and PKA activity. The cAMP-dependent PKA pathway is positively coupled to D₁-like receptors and the desensitization of D₁-like receptors leads to a decrease in PKA activity, or down-regulation (Zhuang, Belluscio, & Hen, 2000). Alternatively, the cAMP-dependent PKA pathway is negatively coupled to D₂-like receptors, therefore sensitization of these receptors also lead to down-regulation and a decrease of PKA activity (Dohovics, Janaky, Varga, Saransaari, & Oja, 2003b; Terwilliger et al., 1991). When the number of D₁-like receptors or receptor binding increases, or the number of D₂-like receptors or receptor binding decreases, cAMP is up-regulated and the levels of adenylyl cyclase and PKA activity are enhanced. (Grady et al., 1997; Terwilliger et al., 1991; Tzschtentke, 2001).
Several pharmacological compounds have been developed that are fairly selective for dopamine receptors. Dopamine agonists bind to the dopamine receptor and are able to directly stimulate the receptor, often having a greater affinity for the receptor site than dopamine (Cooper et al., 1996). Dopamine antagonists also bind to the dopamine receptor, but do not stimulate the receptor (Cooper et al., 1996; Seeman & VanTol, 1994). A prototypical agonist for D₁-like receptors (D₁ and D₅) is SKF-38393 and a prototypical antagonist is SCH-23390 (Bischoff, Heinrich, Sonntag & Krauss, 1986; Pierce, Born, Adams, Kalivas, 1996). For the D₂ receptor, apomorphine, bromocriptine and quinpirole are typical agonists and haloperidol, sulpiride, and spiperone are typical antagonists. Quinpirole, pergolide, and 7-OH-DPAT are D₃ agonists and UH232 is a D₃ antagonist. The prototypical agonist for the D₄ receptor is PD168077 and the prototypical antagonist is clozapine (Cooper et al., 1996; Seeman & Van Tol, 1994).
Glutamate Synthesis and Transmission

Glutamate is the primary excitatory neurotransmitter in the central nervous system and is vital for the maintenance and regulation of brain functions and neural development (Bordi & Ugolini, 1999; Conn & Pin, 1997; Nakanishi & Masu, 1994; Wolf, 1998). There are two sources from which glutamate is synthesized. The first involves the transformation of glucose through the Krebs cycle and provides the main source of glutamate within the central nervous system. The second is from the synthesis of glutamine in glial cells, which is transported into nerve terminals where glutaminase converts it into glutamate (Cooper et al., 1996).

Glutamate is stored in synaptic vesicles of glutamate nerve terminals (Conn & Pin, 1997; Cooper et al., 1996). A Ca$^{2+}$-dependent exocytotic process releases glutamate into the synapse upon depolarization of the nerve terminal (Cooper et al., 1996). Glutamate in the synaptic cleft is mostly removed by glial-type (GT$\_g$) glutamate transporters located on glial cells (Chaudhry, Lehre, van Lookeren
Campagne, Otterson, Danbolt, & Storm-Mathisen, 1996). A smaller fraction of extracellular glutamate is removed by neuronal-type (GT\(_n\)) glutamate transporters, located on the plasma membrane of the presynaptic nerve terminal (Chaudhry et al., 1996). Upon reuptake by GT\(_g\) transporters into glial cells, glutamate is converted by glutamine synthetase into glutamine and is transported back into a glutamate nerve terminal where it can be synthesized into glutamate (Cooper et al., 1996). Glutamine can also be oxidized into \(\alpha\)-ketoglutarate and transported into the neuron to replace the \(\alpha\)-ketoglutarate that is used during the synthesis of neuronal glutamate through the Krebs cycle (Cooper et al., 1996).

Glutamate Receptor System

There are two classes of glutamate receptors, ionotropic and metabotropic. Ionotropic glutamate receptors (iGluRs) are ligand-gated cation channels that are involved in fast excitatory neurotransmission (Wolf, 1998). These ion channel-linked receptors are further divided into N-methyl-D-aspartate (NMDA) receptors, kainate (KA) receptors, and amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Conn & Pin, 1997; Dale,
Originally, iGluRs were thought to be the only glutamate receptors and the long-term effects of the glutamatergic system were thought to require activation of other neurotransmitter receptors such as dopamine, acetylcholine, serotonin, and norepinephrine (Mao & Wang, 2002a). It was later discovered that there existed a class of glutamate receptors that are coupled to G-proteins. These receptors mediate slower glutamate responses and are called metabotropic glutamate receptors (mGluRs) and constitute a large family of receptors coupled to various intracellular signal transduction systems (Conn & Pin, 1997; Lopez-Bendito, Shigemoto, Fairen, & Lujan, 2002; Mao & Want, 2002b). MGlusRs are located at pre-synaptic and post-synaptic sites and regulate both the release of glutamate and the excitation of the post-synaptic membrane (Dale et al., 2002).

Our understanding of glutamatergic neurotransmission has been greatly altered by the discovery of mGluRs, because these receptors have the ability to transform short neuronal activation into long-lasting changes in synaptic activity. Glutamate, through mGluRs, can modify or fine-tune activity at the same synapses responsible for fast
synaptic responses. This characteristic makes them an essential mechanism in synaptic plasticity (Conn & Pin, 1997; Dale et al., 2002).

There are currently a total of eight known subtypes within the mGluR family, labeled mGluR1 through mGluR8 (Conn & Pin, 1997). These subtypes are classified into three groups based on sequence homology, pharmacology and G protein-coupling specificity, which define the transduction mechanism (Conn & Pin, 1997; Cooper et al., 1996; Lopez-Bendito et al., 2002). Group I mGluRs, which include mGluR1 and mGluR5, are linked through Gq to phosphoinositide hydrolysis and Ca\(^{2+}\)-mediated signal transduction. Phosphoinositide hydrolysis involves the stimulation of phospholipase C, which increases intracellular inositol 1,4,5-triphosphate (InsP\(_3\)) concentrations, releases intracellular Ca\(^{2+}\), and activates protein kinase C (PKC) (Pickering et al., 1993). Through Gs, Group I mGluRs activates adenylyl cyclase which results in cAMP formation, activating protein kinase A (PKA) activity (Dohovics, Janaky, Varga, Hermann, Saransaari, & Oja, 2003a). Both Group II mGluRs, consisting of mGluR2 and mGluR3, and Group 3 mGluRs, consisting of mGluR4, mGluR6, mGluR7, and mGluR8, are negatively coupled to
adenylyl cyclase (Conn & Pin, 1997; Dale et al., 2002; Lopez-Bendito et al., 2002).

The development of drugs that specifically target mGluRs and their various subtypes has led to a greater understanding of the function of mGluRs in the nervous system. The prototypic agonists for the Group I mGluRs are quisqualate and 3,5-dihydroxyphenylglycine (3,5-DHPG) and the prototypic antagonists are α-methyl-4-carboxyphenylglycine (MCPG) and two derivatives of phenylglycine, (S)-4-carboxyphenylglycine ((S)-4CPG) and (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3HPG) (Ito et al., 1992; Hayashi et al., 1994). Compared to mGluR1, mGluR5 is less sensitive to the antagonists (S)-4CPG and (S)-4C3HPG, but shows similar affinity to MCPG (Hayashi et al., 1994). For the Group II mGluRs, a prototypic agonist is 2R, 4R-4-aminopyrrolidone-2,4-dicarboxylate (APDC) (Hayashi et al., 1994). Phenylglycine derivatives are competitive antagonists for this receptor group, with the most potent being α-methyl-4-phosphonophenylglycine (MPPG) and α-methyl-4-sulfonophenylglycine (MSPG) (Hayashi et al., 1994). The prototypic agonist for Group III mGluRs is L-amino-4-phosphonobutyrate (L-AP4) and the prototypic
The antagonist is α-methyl-L-amino-4-phosphonobutyrate (MAP4) (Hayashi et al, 1994).
CHAPTER 4

EFFECTS OF AMPHETAMINE ON DOPAMINE AND GLUTAMATE SYSTEMS

Effects of Amphetamine

Dopamine System

Amphetamine is a psychostimulant that increases the synaptic release of the monoamines, dopamine, norepinephrine and serotonin (Koob & Bloom, 1998; Self & Nestler, 1995). Amphetamine primarily works by reversing the transport of monoamines by binding to monoamine reuptake transporters, especially the dopamine transporter (DAT) (Kalivas & Stewart, 1991; Pierce & Kalivas, 1997; Weiss & Koob, 2001). In addition, amphetamine also blocks monoamine reuptake and inhibits monoamine oxidase (Genova, Berke & Hyman, 1997; Vanderschuren & Kalivas, 2000). The increased release of monoamines, primarily dopamine, is believed to be the reason amphetamines are pleasurable and abused by humans (Pierce & Kalivas, 1997; Robinson & Berridge, 1993; Self & Nestler, 1995). A single administration of amphetamine is capable of stimulating extracellular dopamine release and causes dose-dependent elevations of dopamine in the brain (Pierce & Kalivas,
Amphetamine, at doses of 1.0, 3.0, and 9.0 mg/kg, are capable of elevating extracellular dopamine levels to 700%, 800%, and 1300% of basal levels, respectively (Kankaanpaa, Meririnne, Lillsunde, & Seppala, 1998). The displacement of dopamine from storage vesicles, inhibition of monoamine oxidase, and the blocking of reuptake transporters suggests that amphetamine is capable of producing neurotoxic effects on brain pathways (Pierce & Kalivas, 1997; Weiss & Koob, 2001). In addition, the massive release of serotonin from presynaptic vesicles leads to an eventual depletion of serotonin in cells and further contributes to the neurotoxic effects of amphetamine (Labarca et al., 1995; Kankaanpaa et al., 1998). Behavioral studies show that acute low to medium doses of amphetamine administered to rats lead to an increase in locomotor activity, while high doses of the psychostimulant can induce stereotypy, such as compulsive licking and gnawing (Kalivas & Stewart, 1991; Robinson & Becker, 1986). Acute administration of amphetamine can also lead to an enhanced sensitivity to stressful stimuli, a decreased response to natural reinforcers, and a decreased threshold for helplessness (Volkow et al., 2002).
Glutamate System

Amphetamine also causes an increase in extracellular glutamate (Karler, Chaudhry, Calder & Turkanis, 1990; Labarca et al., 1995; Wolf & Xue, 1998). There are several explanations for the increase in glutamate activity after systemic administration of amphetamine. Glutamate reuptake may be affected by D₁ receptor stimulation of sodium (Na⁺) and K⁺ gated channels in neuronal cells (Wolf & Xue, 1998). Amphetamine may also increase Ca²⁺-independent glutamate release through "hypoxia-induced reversal" of the glutamate reuptake transporter (Del Arco, Gonzalez-Mora, Armas, & Mora, 1999; Vanderschuren & Kalivas, 2000). In addition, the reuptake of glutamate may be blocked or impeded by a number of agents such as: oxygen radicals, nitric oxide, or arachidonic acid, which remains in the extracellular space following amphetamine administration (Wolf & Xue, 1998).

The role of mGluR5 in psychostimulant-induced effects has been examined using mGluR5-null mutant mice. In these mice, cocaine does not increase locomotor activity, suggesting that mGluR5s contribute significantly to psychostimulant-induced locomotor activity (Chiamulera et al., 2001). Interestingly, the absence of mGluR5 receptors did not affect either basal dopamine levels or cocaine-
induced increases in dopamine levels. In addition, the
distribution and expression of dopamine receptors and DAT
did not differ between wild-type and mGluR5-null mutant
mice. These results call into question the functional
relationship between dopamine receptors, mGluR5 receptors,
and the effects of psychostimulants on these systems
(Chiamulera et al., 2001).

Interactions Between mGluR and
Dopamine Systems

Interestingly, it appears that many of the behavioral
effects of mGluR activation are dependent on the dopamine
system. For instance, activation of dorsal striatal mGluRs
induce dopamine-mediated contralateral turning behavior.
This is based on the observation that dose-dependent
activation of mGluRs enhances the release of dopamine in
the striatum. During periods of hyperstimulation, however,
activation of mGluRs result in a reduction of dopamine
release (Smith & Beninger, 1996; Verma & Moghaddam, 1998;
Wolf, 1998). Additional evidence of this mGluR/dopamine
interaction is the finding that dopamine antagonists
attenuate locomotor activity induced by the mGluR agonist,
1-aminocyclopentane-1,2-dicarboxylic acid (1S,3R-ACPD) (Kim
& Vezina, 1997; Kim & Vezina, 1998a; Meeker, Kim, & Vezina, 1998). These findings, along with the fact that output neurons in the nucleus accumbens express both dopamine and mGluR receptors, suggest that the mGluR system works in synergy with dopaminergic inputs through intracellular signaling pathways to influence the effects of psychostimulants (Kim, Beeler, & Vezina, 2000; Kim & Vezina, 1998a; Kim & Vezina, 1999; Mao & Wang, 2002c; Vezina & Kim, 1999).
CHAPTER 5

BEHAVIORAL SENSITIZATION

Overview

When a psychostimulant (e.g., amphetamine, methylphenidate, and methamphetamine) is repeatedly administered to rodents, distinct enduring behavioral changes occur. One type of change that can occur is a decline in the effectiveness of the psychostimulant to affect behavior, a phenomenon referred to as tolerance (Kalivas & Stewart, 1991; Robinson & Becker, 1986). Alternatively, repeated psychostimulant treatment can cause an enhanced response known as reverse tolerance, or behavioral sensitization. Behavioral sensitization typically occurs with repeated, intermittent exposure to a psychostimulant, especially with doses in the low to moderate range (Kalivas & Stewart, 1991). This enhanced behavioral response is usually progressive, and can persist for days, weeks, months, and even years after the last drug administration (Kalivas & Stewart, 1991; Robinson & Becker, 1986; Steketee, 2003). This response persistence has been associated with the craving related to drugs of abuse, which may lead to relapse despite a prolonged period of
abstinence (Kalivas & Stewart, 1991; Robinson & Becker, 1986).

The process of behavioral sensitization involves two stages referred to as induction and expression (Kalivas & Stewart, 1991; Pierce & Kalivas, 1997; Robinson & Becker, 1986; Vanderschuren & Kalivas, 2000). In the induction phase, the sensitized response develops and is indicated by a progressive increase in behavioral responding after each repeated drug administration. The expression of behavioral sensitization is indicated by an enhanced behavioral response to an acute drug administration (i.e., challenge injection) after repeated pre-exposure to the drug (Pierce & Kalivas, 1997; Robinson & Becker, 1986).

Neural Basis of Behavioral Sensitization

Role of the Dopamine System

The behavioral change to repeated psychostimulant administration is, in part, due to the neuroadaptive nature of the mesolimbic and nigrostriatal dopamine system (Pierce & Kalivas, 1997). The mesolimbic dopamine pathway extends from the ventral tegmental area (VTA) of the midbrain to the nucleus accumbens (Nac), prefrontal cortex (PFC) and amygdala, and has been demonstrated to partially mediate
the expression of behavioral sensitization (Genova et al., 1997; Pierce & Kalivas, 1997). It has recently been shown that repeated administration of amphetamine may completely block long-term depression (LTD), a persistent decrease in excitatory synaptic transmission, in the excitatory synapses of VTA neurons, and may contribute to the initiation of the sensitization process (Jones, Kornblum, & Kauer, 2000). In addition, the long lasting expression of sensitization has been attributed to adaptations in the neurons of the nucleus accumbens as well (Pierce & Kalivas, 1997; Robinson & Kolb, 1997).

Neuroadaptations caused by repeated amphetamine treatment include a transient decrease in the sensitivity of dopamine autoreceptors found on the cell body and dendrites of dopaminergic neurons, and a longer lasting supersensitivity of dopamine D₁ receptors in striatal neurons (Kalivas & Stewart, 1991; Robinson & Becker, 1986). It has been demonstrated that dopamine D₁ receptors are critical in the induction of behavioral sensitization to amphetamine, because amphetamine sensitization is inhibited when D₁ receptor antagonists are administered systemically or intra-cranially to the VTA, (Hamamura et al., 1991; Ujike, Onoue, Akiyama, Hamamura, & Otsuki, 1989;
Vanderschuren & Kalivas, 2000). However, most of these studies have used SCH-23390 to block D₁ receptors, leaving open the possibility that non-specific actions of SCH-23390 (i.e., its effects on serotonin (5-HT₂) receptors) may contribute to the prevention of amphetamine sensitization (Bischoff et al., 1986). D₁ receptor agonists have been used to further examine the importance of D₁ receptors for the development of sensitization. When SKF-38393, a D₁ receptor agonist, is repeatedly administered into the VTA, behavioral sensitization to cocaine and amphetamine is produced (Pierce et al., 1996). When cholera toxin, a compound which activates adenylyl cyclase much like the stimulation of D₁ receptors is administered intra-VTA, the behavioral effects caused by acute amphetamine administration are heightened and the development of amphetamine-induced sensitization is magnified (Byrnes, Weinstein, & Wallace, 1997).

Alternatively, sensitization to amphetamine has been demonstrated to occur through mechanisms not involving dopamine D₁ receptors. Evidence for D₁ receptor-independent amphetamine sensitization has been demonstrated through the use of D₁ receptor knockout mice. Although lacking
functional D₁ receptors, these animals readily develop amphetamine sensitization (Karper et al., 2002).

Dopamine D₂ receptors may also be involved in the development of amphetamine-induced sensitization, but their function is less clearly defined. For example, amphetamine sensitization is blocked when D₂ antagonists, such as haloperidol, clozapine, YM-09151-2 and nemonapride, are administered (Bjijou, Stinus, Le Moal, & Cador, 1996; Hamamura et al., 1991). Other D₂ receptor antagonists, such as pimozide and spiperone, did not block amphetamine sensitization, and studies using sulpiride have provided inconclusive results (Karler et al., 1990; Vanderschuren & Kalivas, 2000).

Role of the Glutamate System

Although there is a substantial amount of evidence suggesting that the dopamine system plays a pivotal role, modifications in the dopamine system are not solely responsible for the development of behavioral sensitization (Wolf, White, & Hu, 1994). For instance, the enhanced behavioral effects of amphetamine can be blocked by administration of glutamate NMDA antagonists such as MK-801 (Karler et al., 1990; Wolf & Xue, 1998). Moreover, repeated psychostimulant administration elevates glutamate
transmission in both the Nac and the VTA (Churchill, Swanson, Urbina, & Kalivas, 1999; Sesack, Deutch, Roth, & Bunney, 1989; Vanderschuren & Kalivas, 2000).

MGlurRs also play an integral part in the induction and expression of psychostimulant-induced behavioral sensitization (Attarian & Amalric, 1997; Darracz, Drouin, Blanc, Glowinski & Tassin, 2001; Kim & Vezina, 1998b; Kim & Vezina, 1998c; Nicoletti et al., 1999; Vezina & Kim, 1999; Wolf, 1998). Repeated psychostimulant administration increases the expression of mGlur5 in the nucleus accumbens, the VTA, and the caudate of the dorsolateral striatum (Ghasemzadeh, Nelson, Lu, & Kalivas, 1999). This elevated level of mRNA encoding mGlur5 suggests that metabotropic glutamate receptors may have a significant role in psychostimulant-induced sensitization; however, little information currently exists on the regulation of mGlur5 function after repeated psychostimulant administration (Wolf & Xue, 1998; Xue, Ng, Li, & Wolf, 1996).

Recent studies have found that the development of amphetamine sensitization can be blocked by intracranial-VTA administration of an mGlur antagonist (Darracz et al., 2001; Kim & Vezina, 1998b; Kim & Vezina, 2000; Vezina &

These studies indicate that the development of psychostimulant-induced sensitization involves the glutamate system, more specifically, activation of metabotropic glutamate receptors, in addition to the dopamine system. Demonstrating that metabotropic glutamate receptors play a role in mediating psychostimulant-induced behavioral sensitization has revealed an interesting functional interaction between the dopamine system and metabotropic glutamate receptors (Ohno & Watanabe, 1995; Sacaan, Bymaster & Schoepp, 1992; Sacaan, Monn & Schoepp, 1991).
Ontogeny of Dopamine Receptors

Dopamine receptors undergo substantial changes across the neonatal period (Gelbard, Teicher, Faedda & Baldessarini, 1989; Murrin & Zeng, 1990; Rao, Molinoff & Joyce, 1991). In general, both dopamine D₁ and D₂ receptors are at low levels at birth and are first detectable in regions such as the prefrontal cortex, striatum, caudate-putamen, and olfactory tubercles (Murrin & Zeng, 1990). In these brain regions, dopamine receptor levels increase sharply after birth, reaching adult levels at approximately postnatal day 25 (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000). Receptor density then continues to increase, rising above adult levels, until the onset of puberty at postnatal day 40 (Andersen et al., 2000; Tarazi & Baldessarini, 2000). Following this overexpression, dopamine receptors undergo the process of pruning in which receptor levels gradually decline to adult levels by postnatal day 60 and remain relatively constant throughout adulthood (Tarazi & Baldessarini, 2000). In the nucleus
accumbens, low levels of dopamine receptors are also detectable at birth. Dopamine receptors in these regions begin to increase around postnatal day 10 and reach maximum levels by postnatal day 40 (Teicher, Andersen, & Hostetter, 1995). Receptor levels remain constant thereafter, without the process of pruning (Anderson et al., 2000; Teicher et al., 1995).

Ontogeny of Metabotropic Glutamate Receptors

Studies using in situ hybridization and autoradiographic binding techniques have revealed that mGluR5 is present at birth and reaches peak levels at approximately postnatal day 10 in the striatum and frontal cortex (Catania, Landwehrmeyer, Testa, Standaert, Penney, & Young, 1994; Romano, Smout, Miller & O’Malley, 2002). The expression of mGluR5 protein then gradually decreases over the course of development until the animal reaches adulthood (postnatal day 60), at which point the level of mGluR5 mRNA stabilizes (Romano et al., 2002; Romano, Vanden Pol, & O’Malley, 1996). This change is most pronounced in the cortex, hippocampus, midbrain/thalamus and striatum (Romano et al., 1996). Developing mGluR5 receptors function similarly to adult receptors, however, the
stimulation of phosphatidyl inositol hydrolysis is more enhanced in the developing (postnatal day 10) brain than in the adult (postnatal day 60) brain (Palmer, Nangel-Taylor, Krause, Roxas, & Cotman, 1990; Sacaan, Santori, & Rao, 1998; Sortino, Nicoletti, & Canonico, 1991; Van den Pol, Romano, & Ghosh, 1995).

Ontogeny of Behavioral Sensitization

The acute administration of psychostimulants causes a similar dose-response curve in developing and adult rats (Tirelli et al., 2003). Low to moderate doses (0.2 - 1.0 mg/kg) of amphetamine produce an increase in locomotor activity and a high dose (5.0 mg/kg) produces stereotypic licking and gnawing (Porrino, Lucignani, Dow-Edwards, & Sokoloff, 1984; Tirelli et al., 2003). In contrast, repeated psychostimulant treatment has different effects in developing and adult rats. Early studies found that rats tested prior to and during weaning were unable to express behavioral sensitization to repeated cocaine or amphetamine administration (Barr & Wang, 1993; Fujiwara, Kazaway, Nakashima, Sato, & Otuki, 1987; Kolta, Scalzo, Ali, & Holson, 1990). Later it was discovered that psychostimulant-induced sensitization can be achieved in
developing rats by: (1) consistently pairing the drug administration and testing environment; (2) extending the number of pre-exposure days; and (3) minimizing the length of the drug abstinence period (McDougall et al., 1994; Tirelli & Ferrara, 1997; Tirelli et al., 2003; Wood et al., 1998; Zavala, Nazarian, Crawford, & McDougall, 2000). These later studies found that young rats can readily express sensitization if testing occurs within 24 hr to 48 hr after the last pretreatment injection (i.e., short-term sensitization). However, the expression of long-term sensitization (testing occurring 1 week or more after the last pretreatment injection) requires an extended pretreatment period and is much weaker than that observed in adult rats (Tirelli & Ferrara, 1997; Zavala et al., 2000). The less robust long-term effects of repeated drug administration in the young rat has been attributed to age-related neurobiological maturation (i.e. receptor density and functioning), which may contribute to the change in response to the psychopharmacological action of drugs (Tirelli et al., 2003).
Summary

Dopamine receptor levels appear to mature quickly, therefore the involvement of dopamine receptors in the developmental differences of behavioral sensitization between young and adult rats may be minimal. However, the differential expression of mGluR5 across ontogeny may play an important role in the developmental changes of behavioral sensitization. Although mGluR5 is important for the development of sensitization in the adult rat (Attarian & Amalric, 1997; Darracz et al., 2001; Kim & Vezina, 1997; Kim & Vezina, 1998a; Kim & Vezina, 1998b; Kim et al., 2000; Wolf, 1998), the role mGluR5 plays in behavioral sensitization in the preweanling rat has not yet been studied. Further investigation is needed to understand the possible involvement of mGluR5 in the neuroadaptations that follow repeated psychostimulant administration in the developing rat.
CHAPTER 7
SUMMARY AND EXPERIMENTAL HYPOTHESES

Summary and Purpose

The induction and expression of psychostimulant-induced behavioral sensitization differs between developing and adult rats. Both short- and long-term sensitization can be readily induced and expressed in adult animals, but in developing animals, the induction and expression of long-term sensitization cannot be consistently reproduced and is often only weakly detected (Tirelli & Ferrara, 1997; Zavala et al., 2000). Although the cause of this developmental difference is unknown, it is possible that immaturity in the glutamate systems is responsible. Specifically, metabotropic glutamate receptors have been shown to be immature at birth and undergo substantial changes during the early postnatal weeks. These changes in the density and efficacy of glutamate binding sites on metabotropic glutamate receptors may contribute to the ontogenetic differences of behavioral sensitization. In adult rats, administration of an mGluR5 antagonist (i.e., MCPG) blocks the development of behavioral sensitization. At present, the effect of MCPG on behavioral sensitization in the

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developing rat has not yet been investigated. Therefore in the present study, MCPG was administered to rat pups to determine if amphetamine-induced behavioral sensitization would be affected by metabotropic glutamate receptors.

Experimental Overview

A behavioral dosing paradigm previously shown to induce short-term behavioral sensitization in young rats was used. During the pretreatment phase of the experiment, rats received amphetamine or saline in combination with a low dose of MCPG (2.5 nM), a high dose of MCPG (25.0 nM) or no MCPG (0.0 nM). Two doses of MCPG were used to determine whether this antagonist has a dose-dependent effect on amphetamine-induced behavior. All animals received the pretreatment for 5 consecutive days and behavioral activity was recorded on each day following drug administration. A test day occurred 48 hours after the last pretreatment (i.e., after 1 drug abstinence day). Animals pretreated with saline received a challenge injection of either saline or amphetamine, and animals pretreated with amphetamine were challenged with a challenge injection of amphetamine only, and locomotor activity was measured. Groups are summarized in Table 1.
### Table 1: Summary of Treatment Groups

<table>
<thead>
<tr>
<th>Pretreatment (5 days)</th>
<th>2 AMPH doses (SAL, AMP (2mg/kg))</th>
<th>Challenge injection (SAL, AMP (0.5mg/kg))</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL</td>
<td>SAL</td>
<td>SAL</td>
<td>G1 (n=10)</td>
</tr>
<tr>
<td>AMP</td>
<td>AMP</td>
<td>AMP</td>
<td>G2 (n=10)</td>
</tr>
<tr>
<td>MCPG (2.5nM)</td>
<td>SAL</td>
<td>SAL</td>
<td>G3 (n=10)</td>
</tr>
<tr>
<td>AMP</td>
<td>AMP</td>
<td>AMP</td>
<td>G4 (n=10)</td>
</tr>
<tr>
<td>MCPG (25nM)</td>
<td>SAL</td>
<td>SAL</td>
<td>G5 (n=10)</td>
</tr>
<tr>
<td>AMP</td>
<td>AMP</td>
<td>AMP</td>
<td>G6 (n=10)</td>
</tr>
</tbody>
</table>

**Hypotheses**

During the pretreatment phase it was predicted that the induction of behavioral sensitization would occur in rats receiving repeated amphetamine treatment and no MCPG, as demonstrated by a progressive increase in locomotor activity after each drug exposure day. It was also expected that, in contrast with studies on adult rats, the induction of behavioral sensitization would not be attenuated in rat pups receiving repeated amphetamine treatment in combination with a low or a high dose of MCPG. During the challenge phase it was expected that rat pups pretreated with amphetamine and no MCPG and challenged with amphetamine would exhibit a significantly higher level of activity relative to rats receiving an acute amphetamine...
challenge (i.e., pretreated with saline and no MCPG and challenged with amphetamine), indicating behavioral sensitization. It was also predicted that developing rats, in contrast to what is seen in adults, would express behavioral sensitization when challenged with amphetamine following pretreatment with amphetamine in combination with either a low or high dose of MCPG. The inability of MCPG to block sensitization in preweanling rats would provide evidence that the immaturity of metabotroptic receptors is important in the ontogenetic differences seen in behavioral sensitization.
CHAPTER 8

METHODS

Subjects

Subjects were 90 (n=10 per group) 10-day-old male and female rats of Sprague-Dawley descent (Harlan), born and raised at California State University, San Bernardino. Rat pups were kept with dams throughout behavioral testing. No more than one rat from each litter was placed into a particular group to control for litter effects. The colony room was maintained at 21-23°C and kept under a 12-hr light/dark cycle. All the experiments were approved by the Institutional Animal Care and Use Committee and met the International Animal Guide for the Care and Use of Laboratory Animals.

Drugs

(RS)-Methyl-4-carboxyphenylglycine (MCPG; Tocris Cookson, Inc., Ellisville, MO) was dissolved in saline and microinjected at a volume of 0.25 μl/side. (+)-Amphetamine (AMPH; Sigma Aldrich; St. Louis, MO) was mixed in saline and injected i.p. at a volume of 5 ml/kg.
Apparatus

Behavioral testing was conducted in commercially available (Coulbourn Instruments, Allentown, PA) activity monitoring chambers (25.5 x 25.5 x 41 cm), consisting of acrylic walls, a plastic floor, and an open top. A photobeam array, with 16 photocells and detectors, was used to measure distance traveled (horizontal locomotor activity).

Surgery

On postnatal day 10, rats received a 1 ml/kg dose (i.p.) of a commercially available solution of ketamine hydrochloride and xylazine hydrochloride. Once a surgical plane of anesthesia was achieved, rats were placed in a Cunningham Neonatal Rat Adapter attached to a standard Kopf stereotaxic apparatus. A single incision was made midsagittally along the skull and the skin retracted. Two small holes (1.5 mm diameter) were drilled in the skull using a Dremel Moto tool and stainless steel guide cannulas (26 gauge) were implanted bilaterally 1.0 mm above the lateral ventricles (1.0 mm lateral, -2.0 mm ventral, and +4.4 mm anterior to lambda). Stereotaxic coordinates were obtained from Sherwood & Timiras, 1970. Commercially
available super glue gel was used to secure the cannulae in place. The skin was then glued together with a small amount of super glue gel. Surgical tools were sterilized after each surgery. After surgery, stainless steel stylets were used to seal the cannulae to prevent occlusion until time of testing. Rats were allowed to recover post-surgery away from the dam in a temperature-controlled chamber (30°C). After each rat pup became fully responsive it was placed back with the dam. All rats began behavioral testing 24 hr after surgery. Immediately after behavioral assessment was finished on the last test day, rats were sacrificed, and injection sites were verified.

Behavioral Procedure

During the pretreatment phase, injection cannulae (26 gauge) were lowered through the guide cannulae, extending 1.0 mm beyond the guide, into the lateral ventricles. Injection cannulae were connected with PE20 tubing to a Hamilton microsyringe which was used to bilaterally microinject the glutamate antagonist, MCPG (0, 2.5, or 25 nmol) at a volume of 0.25 μl per side. MCPG was delivered at a constant rate over a 30 s period. Infusion cannulae were left in place for an additional 60 s. Fifteen minutes
following the MCPG infusion, rats were injected with saline or amphetamine (2 mg/kg) and placed in the testing apparatus. Locomotor activity was measured for 60 min and the mean distance traveled was recorded per 5 min time block. On pretreatment days 1-5, rats received daily treatments of one intracranial (i.c.) infusion and one intraperitoneal (i.p.) injection. A single test day occurred 48 hr after the last pretreatment day. On the test day, rats pretreated with saline received a challenge injection of saline or amphetamine (1 mg/kg) and rats pretreated with amphetamine received a challenge of amphetamine (0.5 mg/kg). Locomotor activity was measured for 120 min and mean distance traveled was recorded per 5 min time block. In summary, 9 groups (n=10 per group) of rats received the following sequence of drugs during the pretreatment i.c./i.p. – test day i.p. phases (doses are in parentheses): SAL/SAL – SAL, SAL/SAL – AMP(0.5), SAL/AMP(2) – AMP(0.5), MCPG(2.5)/SAL – SAL, MCPG(2.5)/SAL – AMP(0.5), MCPG(2.5)/AMP(2) – AMP(0.5), MCPG(25)/SAL – SAL, MCPG(25)/SAL – AMP(0.5), MCPG(25)/AMP(2) – AMP(0.5).
Histology

At the end of behavioral testing, rats were sacrificed and the brains removed and fixed in 10% formalin for a minimum of 7 days. Following the fixation period, brains were sucrose-protected in a 20% sucrose solution for 24 hr. Coronal sections (75 μm) were cut in a temperature-controlled cryostat maintained at -25°C. Sections were mounted and stained with thionin and cannulae placements were verified.
CHAPTER NINE

RESULTS

Locomotor Activity

Pretreatment Phase

Mean distance traveled (i.e., locomotor activity) by rats pretreated with MCPG (0.0, 2.5, or 25.0 nM, i.c.) and amphetamine (2.0 mg/kg) or saline for five consecutive days starting on PD 11 are summarized in Table 2. Locomotor activity is also represented in Figure 1.

Rats that received no amphetamine and no MCPG treatments (G1 and G2, in reference to Table 2) and rats that received repeated amphetamine and no MCPG treatments (G3), are the relevant groups for testing the hypothesis regarding amphetamine-induced behavioral sensitization in the absence of MCPG. A 2 (AMPH dose) x 5 (pre-exposure days) ANOVA for mixed design (with AMPH dose as a between-subjects variable and pre-exposure days as a within-subjects variable) involving G1, G2, and G3 was conducted. A progressive increase in locomotor activity after each drug exposure day was observed in both G1, G2, and the G3 rats. [Main effect of pre-exposure days: $F(4, 112) = 34.89; p = .000$]. However, a significantly higher level of
locomotor activity was observed in the G3 rats ($M = 1940.40$), when compared to the G1 and G2 rats ($M = 1165.28$). [Main effect of AMPH dose: $F(1, 28) = 20.98; p = .000$]. This suggests that the induction of behavioral sensitization occurred following repeated amphetamine treatments.

| Table 2: Mean Distance Traveled (Standard Error) During Pretreatment Period |
|-----------------------------|------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| 3 MCPG dose                | 2 AMPH dose      | D1        | D2        | D3        | D4        | D5        | Mean D1-D5 |
| SAL                         | SAL              | 371.77 (102.74) | 826.95 (192.28) | 1377.93 (250.10) | 1614.94 (261.46) | 1634.83 (243.37) | 1165.28 (130.88) |
| AMP (2)                     | SAL              | 732.47 (91.83)  | 1522.56 (240.66) | 1896.91 (161.36) | 2785.39 (285.32) | 2764.69 (307.28) | 1940.40 (151.31) |
| MCPG (2.5)                  | AMP (2)          | 426.48 (100.68) | 683.15 (138.11) | 1308.42 (306.03) | 1599.09 (351.05) | 1239.06 (214.61) | 1051.24 (174.65) |
| SAL                         | AMP (2)          | 532.47 (78.85)  | 1106.10 (158.42) | 1716.78 (136.56) | 2092.04 (176.37) | 2007.55 (228.06) | 1490.99 (113.17) |
| MCPG (25)                   | MCPG (2.5)       | 303.15 (62.78)  | 655.43 (138.75) | 946.76 (232.94) | 1278.60 (244.43) | 1070.26 (264.42) | 850.84 (814.37) |
| SAL                         | AMPH (2)         | 550.60 (77.93)  | 1180.23 (165.87) | 1773.11 (159.44) | 2356.19 (96.32)  | 2467.88 (175.85) | 1666.80 (76.77)  |
| AMPH (2)                    |                 |             |           |            |            |            |             |

Group
- G1 n=10
- G2 n=10
- G3 n=10
- G4 n=10
- G5 n=10
- G6 n=10
- G7 n=10
- G8 n=10
- G9 n=10
Figure 1. Locomotor Activity During Pretreatment Period. Mean (±SEM) distance traveled for entire testing period of rats pretreated with 0.0 nM MCPG (top panel), 2.5 nM MCPG (middle panel), or 25.0 nM MCPG (bottom panel) followed 15 min later by an injection of 2.0 mg/kg amphetamine (filled symbols) or saline (open symbols) on five consecutive days. Behavioral testing lasted 60 min and occurred immediately after amphetamine or saline injections. (a) Significantly different from rats given saline (open symbols).
Further analysis of G3 animals (rats receiving repeated amphetamine and no MCPG treatments) and the progressive increase in locomotor activity after each drug exposure was made using repeated-measures pairwise comparisons (Refer to Table 3). The activity level on D5 is approximately equal to the activity level on D4; D5 and D4 activity levels are significantly greater than D1, D2, and D3. D3 activity level is greater than D2, but not significantly greater; while D3 and D2 activity levels are significantly greater than D1. This set of results further supports the occurrence of amphetamine-induced behavioral sensitization.

Table 3: Repeated-Measures Pairwise Comparisons of Rats Receiving Repeated Amphetamine and no MCPG Treatments

<table>
<thead>
<tr>
<th>Day Mean (Std. Error)</th>
<th>2: 1522.56 (240.66)</th>
<th>3: 1896.91 (161.36)</th>
<th>4: 2785.39 (285.32)</th>
<th>5: 2764.69 (307.28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p = .005</td>
<td>p = .000</td>
<td>p = .000</td>
<td>p = .000</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>p = .095</td>
<td>p = .011</td>
<td>p = .011</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>p = .003</td>
<td>p = .007</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>p = .927</td>
</tr>
</tbody>
</table>

p = .005
p = .000
p = .000
p = .000

p = .095
p = .011
p = .011

p = .003
p = .007

p = .927

49
To test the hypothesis that induction of behavioral sensitization would not be attenuated in rat pups receiving repeated amphetamine treatment in combination with a low or high dose of MCPG, two 2 (AMPH dose) x 5 (pre-exposure days) ANOVA for mixed design (with AMPH dose as a between-subjects variable and pre-exposure days as a within-subjects variable) were conducted (one for G4 & G5 vs. G6 and the other for G7 & G8 vs. G9). In addition, G3, G6, and G9 animals were tested using a 3 (MCPG dose) x 5 (pre-exposure days) ANOVA for mixed design (with MCPG dose as a between-subject variable and pre-exposure days as a within-subjects variable). To confirm the hypothesis, the relative behavioral pattern contrasting G4 & G5 with G6 and the relative behavioral pattern contrasting G7 & G8 with G9 should resemble the relative behavior pattern observed in contrasting G1 & G2 with G3. Moreover, when G3, G6, and G9 animals are compared, a significant main effect of pre-exposure days should be observed; while no significant main effect of MCPG dose should be observed.

As a control, a 3 (MCPG dose) x 5 (pre-exposure days) ANOVA for mixed design (with MCPG dose as a between-subject variable and pre-exposure days as a within-subjects variable) was also conducted to see how MCPG dose would
influence rats receiving no repeated amphetamine treatment (i.e., G1+G2 vs. G4+G5 vs. G7+G8). As can be seen from the second and third panels in Figure 1 (and the data presented in Table 2), similar to the G3 rats (that received repeated amphetamine treatment and no MCPG), a progressive increase in locomotor activity after each drug exposure day was observed for the G6 rats (animals that received repeated amphetamine and a low, 2.5nM, dose of MCPG) as well as for the G9 rats (animals that received repeated amphetamine and a high, 25nM, dose of MCPG). [Main effect of pre-exposure days: $F(4,108)= 74.05; p = .000$]. Moreover, as illustrated in Figure 1, when compared to their corresponding counterparts (i.e., rats receiving the same amount of MCPG, but with no repeated amphetamine treatments, G6 vs. G4+G5; G9 vs. G7+G8), the rat pups receiving repeated amphetamine treatments showed significantly higher levels of locomotor activity, indicating the development of behavioral sensitization. [Main effect of AMPH dose: For rats with low, 2.5nM MCPG—G6 vs. G4+G5, $F(1,28)= 5.20, p = .030$; for rats with high, 25nM MCPG—G9 vs. G7+G8, $F(1,28)= 27.90, p = .000$.]

Interestingly, the induction of behavioral sensitization in rat pups receiving repeated amphetamine
treatments was, to a certain degree, influenced by the amount of MCPG given in combination with the amphetamine treatments. [Main effect of MCPG: $F(2,27) = 3.70, p = .038$]. As can be seen in Table 4, although the G9 rats (those receiving high, 25nM, MCPG, $M = 1666.80$) expressed a lower locomotor activity level than the G3 rats (those with no MCPG, $M = 1940.40$), the difference was not significant. On the contrary, the G6 rats (those with low, 2.5nM, MCPG, $M = 1490.99$) exhibited a significantly lower locomotor activity level than the G3 rats (those with no MCPG, $M = 1940.40$). No significant differences in locomotor activity levels were observed between the G6 rats (those with low, 2.5nM, MCPG, $M = 1490.99$) and the G9 rats (those receiving high, 25nM, MCPG, $M = 1666.80$), although, in general, the G6 rats showed a lower level of activity than the G9 rats. This set of results indicates that the induction of behavioral sensitization is attenuated, to a certain degree, in rat pups receiving repeated amphetamine treatment in combination with a low dose of MCPG (refer to Figure 1).
Table 4: Tukey HSD Tests for Rats Receiving Repeated Amphetamine Treatments in Combination with no MCPG, a low dose (2.5nM) of MCPG, or a high dose (25nM) of MCPG

<table>
<thead>
<tr>
<th>MCPG Pretreatment</th>
<th>Homogeneous Subset</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Set 1</td>
<td>Set 2</td>
</tr>
<tr>
<td>MCPG (2.5)</td>
<td>1490.99</td>
<td></td>
</tr>
<tr>
<td>MCPG (25)</td>
<td>1666.80</td>
<td>1666.80</td>
</tr>
<tr>
<td>MCPG (0)</td>
<td></td>
<td>1940.40</td>
</tr>
</tbody>
</table>

When rats with no repeated amphetamine treatment (i.e., G1+G2 vs. G4+G5 vs. G7+G8) were compared, the results indicated that low or high MCPG lowered the rats’ locomotor activity levels; however, the influence was not statistically significant, $F(2, 57) = 2.22, p = .118$.

Test Day: Amphetamine Challenge

Mean distance traveled during the entire testing period for rats receiving a challenge injection of AMPH (0.5 mg/kg) or saline 48 hr after five consecutive days of MCPG (0.0, 2.5, or 25.0 nM, i.c.) and AMPH (2.0 mg/kg) or saline pretreatment is presented in Table 5. Depending on the type of challenge injection (saline or amphetamine) received, rat pups pretreated with no MCPG (in combination with amphetamine or saline (G1, G2, and G3)) significantly differed in locomotor activity levels on the test day.
[Main effect of Treatment combination: $F(2,26) = 21.57; p = .000$].

<table>
<thead>
<tr>
<th>Pretreatment (5 days)</th>
<th>Test day</th>
<th>Challenge injection</th>
<th>Mean</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 MCPG doses</td>
<td></td>
<td>SAL</td>
<td>6155.79 (1637.18)</td>
<td>G1 (n = 10)</td>
</tr>
<tr>
<td>2 AMPH doses</td>
<td></td>
<td>AMP (0.5mg/kg)</td>
<td>25299.80 (2898.56)</td>
<td>G2 (n = 10)</td>
</tr>
<tr>
<td>SAL</td>
<td></td>
<td>AMP (0.5mg/kg)</td>
<td>29783.61 (1415.29)</td>
<td>G3 (n = 10)</td>
</tr>
<tr>
<td>2.5nM MCPG</td>
<td></td>
<td>SAL</td>
<td>8070.03 (1661.18)</td>
<td>G4 (n = 10)</td>
</tr>
<tr>
<td>2 AMPH doses</td>
<td></td>
<td>AMP (0.5mg/kg)</td>
<td>24906.19 (2130.68)</td>
<td>G5 (n = 10)</td>
</tr>
<tr>
<td>25nM MCPG</td>
<td></td>
<td>AMP (0.5mg/kg)</td>
<td>27972.65 (1841.00)</td>
<td>G6 (n = 10)</td>
</tr>
<tr>
<td>SAL</td>
<td></td>
<td>SAL</td>
<td>9731.50 (3516.75)</td>
<td>G7 (n = 9)</td>
</tr>
<tr>
<td>2 AMPH doses</td>
<td></td>
<td>AMP (0.5mg/kg)</td>
<td>32033.41 (3473.58)</td>
<td>G8 (n = 10)</td>
</tr>
<tr>
<td>25nM MCPG</td>
<td></td>
<td>AMP (0.5mg/kg)</td>
<td>41693.01 (3729.51)</td>
<td>G9 (n = 10)</td>
</tr>
</tbody>
</table>

Further analyses with Tukey HSD tests are presented in Table 6. Without MCPG, a higher level of locomotor activity was observed for the G3 rats (those pretreated with AMPH and challenged with AMPH, $M = 29783.61$) than the G2 rats (those pretreated with saline and challenged with...
Table 6: Tukey HSD Tests for Rats Challenged with 0.5 mg/kg Amphetamine After One Drug Abstinence Day

<table>
<thead>
<tr>
<th>MCPG Pretreatment</th>
<th>Homogeneous Subset</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Set 1</td>
<td>Set 2</td>
</tr>
<tr>
<td>MCPG (0)+saline-saline</td>
<td>G1</td>
<td>6155.79</td>
<td></td>
</tr>
<tr>
<td>MCPG (0)+saline-AMPH</td>
<td>G2</td>
<td>25299.80</td>
<td></td>
</tr>
<tr>
<td>MCPG (0)+AMPH-AMPH</td>
<td>G3</td>
<td>29783.61</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MCPG Pretreatment</th>
<th>Homogeneous Subset</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Set 1</td>
<td>Set 2</td>
</tr>
<tr>
<td>MCPG (2.5)+saline-saline</td>
<td>G4</td>
<td>8070.03</td>
<td></td>
</tr>
<tr>
<td>MCPG (2.5)+saline-AMPH</td>
<td>G5</td>
<td>24906.19</td>
<td></td>
</tr>
<tr>
<td>MCPG (2.5)+AMPH-AMPH</td>
<td>G6</td>
<td>27972.65</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MCPG Pretreatment</th>
<th>Homogeneous Subset</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Set 1</td>
<td>Set 2</td>
</tr>
<tr>
<td>MCPG (25)+saline-Saline</td>
<td>G7</td>
<td>9731.50</td>
<td></td>
</tr>
<tr>
<td>MCPG (25)+Saline-AMPH*</td>
<td>G8</td>
<td>32033.41</td>
<td></td>
</tr>
<tr>
<td>MCPG (25)+AMPH-AMPH</td>
<td>G9</td>
<td>41693.01</td>
<td></td>
</tr>
</tbody>
</table>

* 1 missing data

AMPH, M = 25299.80); however, the difference was not significant. It is important to note that upon reviewing the distance traveled averaged across all animals in each treatment group, the standard deviations were very large. This may be a contributing factor to the absence of statistical significance between groups, though the patterns are consistent with the expected sensitized
response of amphetamine pretreated and amphetamine
callenged rats compared to rats exposed to an acute
exposure of amphetamine on the test day (see Figure 2).
Similarly, for rats pretreated with low MCPG (2.5nM) or
high MCPG (25nM), higher levels of locomotor activity were
observed for those rats pretreated with AMPH and challenged
with AMPH compared to those acutely exposed to AMPH on test
day (G6 vs. G5 and G9 vs. G8); however again, the
differences were not significant.

It was also predicted that developing rats, in
contrast to what is seen in adult rats, would express
behavioral sensitization when challenged with amphetamine
following pretreatment with amphetamine in combination with
either a high or low dose of MCPG. Interestingly, results
indicate that the expression of behavioral sensitization in
rat pups pretreated with amphetamine and challenged with
amphetamine was, to a certain degree, influenced by the
given amount of MCPG. [Main effect of MCPG: F(2,27) =
8.56, p = .001]. As indicated in Table 7, a low dose
(2.5nM) of MCPG reduced the level of locomotor activity
(when compared to no MCPG); however, the effect was not
significant. Surprisingly, instead of blocking the
expression of behavioral sensitization, a high dose (25nM)
Figure 2. Locomotor Activity on Test Day. Mean (±SEM) distance traveled of rats receiving a challenge injection of 0.5 mg/kg amphetamine after one drug abstinence day. During the pretreatment phase, rats received daily injections of MCPG (0.0, 2.5, or 25.0 nM, i.c.) followed, 15 min later, by an injection of 2.0 mg/kg amphetamine or saline. Behavioral testing lasted 120 min and occurred immediately after injections.
Table 7: Tukey HSD Tests for Rats Receiving a Challenge Injection of Amphetamine Following Pretreatment with Amphetamine and MCPG

<table>
<thead>
<tr>
<th>MCPG Pretreatment</th>
<th>Homogeneous Subset</th>
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<tbody>
<tr>
<td></td>
<td>Set 1</td>
<td>Set 2</td>
</tr>
<tr>
<td>MCPG (2.5)+AMPH-AMPH</td>
<td>G6</td>
<td>27972.65</td>
</tr>
<tr>
<td>MCPG (0)+AMPH-AMPH</td>
<td>G3</td>
<td>29783.61</td>
</tr>
<tr>
<td>MCPG (25)+AMPH-AMPH</td>
<td>G9</td>
<td>41693.01</td>
</tr>
</tbody>
</table>

of MCPG significantly increased the locomotor response to a challenge injection of amphetamine.

Histology

A representative photomicrograph of a thionin-stained coronal section with bilateral cannulae tracts is shown in Figure 3. Schematic illustrations of the locations of microinjection sites of all the rats included in the data analyses are shown in Figures 4, 5 and 6.
Figure 3. Representative Image of a Thionin-stained Section Indicating the Cannulae Tracts Directed 1 mm Above the Lateral Ventricles.
Figure 4. Approximate Locations of the Microinjection Sites in the Lateral Ventricles of Rats Included in the Data Analyses. Representative line drawing from Paxinos and Watson (1994) at -0.40 mm from Bregma.
Figure 5. Approximate Locations of the Microinjection Sites in the Lateral Ventricles of Rats Included in the Data Analyses. Representative line drawing from Paxinos and Watson (1994) at -0.80 mm from Bregma.
Figure 6. Approximate Locations of the Microinjection Sites in the Lateral Ventricles of Rats Included in the Data Analyses. Representative line drawing from Paxinos and Watson (1994) at -0.92 mm from Bregma.
The behavioral response to repeated amphetamine differs between developing and adult rats. While adult animals readily develop psychostimulant-induced sensitization, in developing animals sensitization is only weakly detected (Tirelli & Ferrara, 1997; Zavala et al., 2000). Two neurotransmitter systems (dopamine and glutamate) have been found to be important in the mediation of behavioral sensitization in adult rats (Pierce & Kalivas, 1997; Wolf, 1998). Moreover in adult rats, these systems interact as mGlu receptors regulate amphetamine-induced locomotor activity and the development of behavioral sensitization by influencing dopaminergic neurotransmission (Attarian & Amalric, 1997; Kim & Vezina, 1997, 1998b). In contrast to adult rats, the neurosystems responsible for behavioral sensitization in the developing brain are much less understood. Moreover, much of the developmental studies on behavioral sensitization have focused on dopamine systems with very little work done on glutamate systems. Since mGlu receptors have been found to undergo dynamic changes during early postnatal development,
it is possible that this change in receptor expression and function is important in the developmental differences seen in behavioral sensitization. Therefore, in the present study, the role of mGluR5 in short-term behavioral sensitization of preweanling rats was examined. Specifically, the mGluR5 antagonist, MCPG, was given to developing rats to block mGlu5 receptors prior to each repeated amphetamine treatment. It was expected that if mGlu5 receptors are functionally immature during the preweanling period, then blocking these receptors with an antagonist would have no consistent effect on the development of behavioral sensitization. The persistence of behavioral sensitization to develop despite the inhibition of mGlu5 receptors would provide evidence for the contrast between developing and adult animals and the functional interaction between the metabotropic glutamate and dopamine systems in adult behavioral sensitization, which may be absent in developing animals.

Role of mGluR5 in the Development of Amphetamine-induced Behavioral Sensitization

During the pretreatment phase, amphetamine administration produced progressively enhanced locomotor
activity (see Figure 1). This amphetamine-induced behavioral effect is consistent with previous reports on the stimulatory effects of a psychostimulant (Kalivas & Stewart, 1991; Pierce & Kalivas, 1997; Self & Nestler, 1995). Interestingly, pretreatment with a low dose of the mGluR5 antagonist, MCPG (2.5nM), significantly attenuated locomotor activity of the amphetamine-treated rats; while a high dose of MCPG (25nM) did not significantly attenuate these amphetamine-treated rats. MCPG also attenuated locomotor activity of the saline-treated rats; however, the influence was not statistically significant. (See middle and bottom panel of Figure 1.) This set of findings concerning amphetamine-induced behaviors observed in preweanling rats is in contrast with previous studies of adult rats in which MCPG has been shown to consistently block amphetamine-induced locomotor activity while not producing locomotor effects when injected with saline alone (Kim & Vezina, 1998b).

The decrease in basal locomotor activity following MCPG treatment suggests that an interruption of mGlu activity in developing rats leads to attenuation of behavioral activity while a more mature system may not respond similarly. Glutamate stimulates phosphatidyl
inositol hydrolysis and mobilizes intracellular calcium through the mediation of mGluRs, in particular mGluR1 and mGluR5, and this activity has been found to be markedly enhanced in the developing brain relative to the adult brain (Romano et al., 1996). Intracellular calcium mobilization stimulates neurotransmitter release, which regulates cell signaling and motor activity (Grady, Bohm, & Bunnett, 1997). Results from the present study suggest that blocking mGlu5 receptors in the developing system affects mGluR-mediated activity, possibly to the extent of attenuating motor activity.

Role of mGluR5 in the Expression of Amphetamine-induced Behavioral Sensitization

Consistent with previous research, although not statistically significant, rats pre-exposed to amphetamine and challenged with amphetamine exhibit patterns of higher locomotor activity than rats pre-exposed to saline and challenged with amphetamine. This, to a certain degree, demonstrates the expression of behavioral sensitization in which repeated psychostimulant exposure enhances the motor stimulatory response to a later drug challenge (Pierce & Kalivas, 1997; Vanderschuren & Kalivas, 2000).
MCPG pretreatment at the higher dose (25nM) significantly increased the locomotor response to a challenge injection of amphetamine (see Figure 2). This suggests that repeated treatment with MCPG potentiates the locomotor effects of a single dose of amphetamine when administered in the absence of the antagonist. Results from the test day resemble those from the pretreatment phase in that MCPG failed to consistently block behavioral sensitization. In addition, to a certain degree, the effect of MCPG on locomotor activity is independent from the effects of amphetamine-induced locomotor activity. Together these results suggest that MCPG does not consistently affect amphetamine-induced sensitization in preweanling pups.

Mechanisms of mGluR5-mediated Locomotor Activity and Behavioral Sensitization

The role of mGluR5 in modifying behavioral sensitization has been suggested to involve the actions of the released dopamine on dopamine D₁ receptors that are located on glutamate projections, and when activated, lead to an increase of extracellular glutamate (Kalivas & Duffy, 1995; Vezina, 1996). Glutamate activates mGluRs,
initiating long-term intracellular changes such as protein synthesis, possibly one of the neuroadaptations resulting in behavioral sensitization (Vezina & Kim, 1999). Previous studies in adult rats have demonstrated that mGlu5 receptors contribute to the mediating role of the glutamate system in the development of behavioral sensitization. In particular, MCPG administration has been found to block amphetamine-induced locomotion, but have no effect on basal locomotor activity (Kim & Vezina, 1998).

In the current study of preweanling rats, amphetamine-induced behavioral sensitization appears to develop independent of the mGluR5 system, while repeated MCPG treatment during the induction phase slightly decreased basal locomotor activity. These behavioral effects of blocking mGlu5 receptors have been demonstrated in a study by Zhu et al. (2004) using 2-methyl-6-(phenylethynyl)pyridine (MPEP) and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP), both potent, selective mGlu5 receptor antagonists. In their study, MPEP and MTEP administration both significantly reduced spontaneous exploratory activity by approximately 78% (Zhu et al., 2004). This suggests that blocking mGlu5 receptors has
potentially inhibitory effects on the central nervous system, as demonstrated in the current study.

The dose–dependent effects of MCPG pretreatment on locomotor activity following an amphetamine challenge have been previously demonstrated in adult rats. Different doses of MCPG, ranging from 0.25nM to 25.0nM, have been administered to adult rats prior to amphetamine exposure. Results have shown that while 25.0nM of MCPG completely blocked amphetamine-induced locomotor activity, 0.25nM of MCPG potentiated locomotor activity (Kim & Vezina, 1998a; Kim & Vezina, 1998b). The different behavioral response to varying doses of MCPG in adult rats suggest the possibility that blocking mGlu5 receptors has the potential to both attenuate and potentiate amphetamine-stimulated locomotor activity.

Considering previous research findings together with the results from the current study, the functional interactions between the dopamine and glutamate systems and the mediating role of mGlu5 receptors appear to involve complex mechanisms. The enhanced locomotor response to an amphetamine challenge following MCPG pretreatment was unexpected and this heightened activity may possibly be the result of compensatory changes in the dopamine and
glutamate systems (Grace, 1995; Kim & Vezina, 1998c; Robelet, Melon, Guillet, Salin, & Kerkerian-Le Goff, 2004). Repeated treatment with an mGluR5 antagonist, especially at a high dose, may possibly increase synaptic levels of glutamate by blocking the regulatory role of mGluRs on glutamate transmission and release (Kim & Vezina, 1998c). It has been demonstrated that repeated psychostimulant administration leading to behavioral sensitization is also correlated with increased levels of extracellular glutamate (Pierce, Bell, Duffy, & Kalivas, 1996). Taken together, the increased locomotor activity produced by an amphetamine challenge in MCPG pretreated rats, as shown in the present study, may be mediated by increased levels of synaptic glutamate.

Conclusion

The current study has demonstrated that behavioral sensitization can be induced and expressed in preweanling rats, though this sensitization is not as consistently blocked by an mGluR5 antagonist as previously shown in adult studies. Basal locomotor activity was attenuated by MCPG administration, suggesting an overall inhibitory effect of blocking mGlu5 receptors. Upon amphetamine
challenge, repeated MCPG pretreatment resulted in an enhancement of the locomotor stimulatory response to the psychostimulant. This may be an indication of the susceptibility of the developing neuronal system to modifications in neurotransmission. The immediate action of an mGluR5 antagonist may be the interruption of dopamine and glutamate-regulated neuronal functioning, resulting in attenuated locomotor activity. In contrast, prolonged exposure to high concentrations of the antagonist may interact with the neuroadaptations resulting from repeated amphetamine exposure, possibly leading to stimulatory effects upon an amphetamine challenge injection. Further research in developing rats is needed to more fully understand the mediating role of metabotropic glutamate receptors in the process of behavioral sensitization, and to determine the specific neuronal mechanisms responsible for these locomotor effects.
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