INDIVIDUAL DIFFERENCES IN CORTICAL GLUTAMATE BASED ON LOCOMOTOR ACTIVITY IN A NOVEL ENVIRONMENT

by

Kristin Aileen McCarthy

A THESIS

Presented to the Department of Behavioral Neuroscience

and the Oregon Health & Science University

School of Medicine

In partial fulfillment of the requirements for the degree of

Master's of Science

June 2007

School of Medicine

Oregon Health & Science University

Certificate of Approval

This is to certify that the Master's Thesis of

Kristin Aileen McCarthy

"Individual Differences in Cortical Glutamate Based on Locomotor Activity in a Novel Environment"

Has been approved by:

[redacted]

Charles K. Meshul, Ph.D., Advisor
Department of Behavioral Neuroscience
Thesis Examination Committee Chair

[redacted]

John C. Crabbe, Ph.D.
Department of Behavioral Neuroscience
Thesis Examination Committee Member

[redacted]

Gregory P. Mark, Ph.D.
Department of Behavioral Neuroscience
Thesis Examination Committee Member

[redacted]

S. Paul Berger, M.D.
Department of Psychiatry
Thesis Examination Committee Member

TABLE OF CONTENTS

ABBREVIATIONS

CORT	Corticosterone (plasma)
CPP	Conditioned Place Preference
EPM	Elevated Plus Maze
EPN	Entopeduncular Nucleus
FR	Fixed Ratio Schedule of Reinforcement
GPe	External Globus Pallidus
HACT	Horizontal Activity Counts
HPA	Hypothalamic-Pituitary-Adrenal Axis
HR	High Responder
L-PDC	L-*trans*-pyrrolidine-2,4-dicarboxylate
LR	Low Responder
mPFC	Medial Prefrontal Cortex
NMDA	N-methyl-D-aspartate
PCP	Phencyclidine
PFC	Prefrontal Cortex
PR	Progressive Ratio Schedule of Reinforcement
SNc	Substantia Nigra Pars Compacta
SNr	Substantia Nigra Pars Reticulata
STR	Striatum
VEH	Vehicle
VM/VL	Ventromedial/ventrolateral Thalamus
VP	Ventral Pallidum

ACKNOWLEDGEMENTS

To my mentor, Dr. Charles Meshul, thank you for your indelible support, patience, understanding, wisdom, friendship and sagacious appreciation for life and its many adventures. You are an admirable scientist and your enthusiasm for research is contagious.

To my labmate, Jeffrey Wiedemann, you have taught me that there is no such thing as a mistake, only lessons learned and that life is full of possibilities. Thank you for your friendship, patience, and invaluable microdialysis assistance. It was an absolute joy working with you and I wish you the best of luck in all of your future endeavors.

To my labmates, Cindy Moore and Mojgan Keyghobadi, thank you not only for the technical support with electron microscopy and immunolabeling, but for your moral support and friendship.

To my committee members, Drs. John Crabbe, Gregory Mark, Robert Hitzemann and S. Paul Berger, thank you for your insightful comments beginning with the 2nd year research paper. They have helped shape my writing from a novice research paper to a Masters thesis. During this study, I have learned that a single data set can be analyzed at least 36 different ways, probe recoveries are essential, mice are not just small rats, environmental effects are extremely difficult to isolate, and the more I learn the less I know.

To my parents, John and Mecki, you have taught me how love, fortitude, perseverance, perfectionism, stubbornness and a little bit of Catholic guilt thrown into the mix can shape a person's vision of the world. Thank you for your unconditional love and support that has allowed me to make my own mistakes and grow.

To my siblings, Monika and Brian, thank you for being my personal cheerleaders.

To Katie Gililland, Lauren Milner, Ethan Beckley, and Dustin Johnsen, thank you for making graduate school some of the best years of my life. May the remainder of your graduate school careers be productive, enlightening, fun, and may they last no more than six years.

To Drs. Deborah Finn and Tamara Phillips, thank you for your sage advice, personal guidance, and assistance with the behavioral experiments. This study would not have come to fruition without your support.

Funding for this study was provided by the Dept. of Veterans Affairs Merit Review Program to CKM, NIH/NIDA Biological Bases of Drug Seeking Behavior Training Grant T32-DA007262, and the N.L. Tartar Trust Fellowship to KAM.

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Locomotor activity in a novel environment has been used to predict the propensity to self-administer drugs of abuse, such as cocaine, amphetamine, nicotine, ethanol, and opiates. However, the majority of studies employed outbred rats, which led to difficulty in interpreting genetic and environmental effects. In this thesis, inbred mice were used to assess the environmental influences on locomotor activity in a novel environment. Male C57BL/6J mice were screened for their locomotor response in a novel environment, automated activity chambers, for 30 min. Based on a median split, mice exhibiting a heightened locomotor response were termed high responders (HR) and mice having a lower locomotor response were termed low responders (LR). The activity phenotype of HRs and LRs was correlated with three other phenotypes, which to our knowledge, have not been studied in HRs and LRs. These three experiments were conducted as a means to explain individual differences in activity measured in a novel environment.

In experiment 1, the relation between locomotor activity and nerve terminal glutamate immunolabeling was examined. Although glutamate has been shown to be differentially regulated in the striatum of HRs and LRs, differences in nerve terminal glutamate immunolabeling and extracellular glutamate have not been investigated in the medial prefrontal cortex (mPFC). Because nerve terminal glutamate immunolabeling is a measure of presynaptic stores of glutamate, HRs and LRs were administered a selective postsynaptic N-methyl-D-aspartate (NMDA) receptor antagonist, phencyclidine (PCP; 2.5 mg/kg, *i.p.*) daily for 10 consecutive days. PCP has been shown to increase extracellular glutamate, most likely by stimulating glutamate release. We hypothesized that LRs would have greater nerve terminal glutamate immunolabeling than HRs and that

HRs would have less nerve terminal glutamate immunolabeling than LRs following repeated PCP administration. Results revealed a greater number of gold-labeled glutamate particles in vehicle-treated HRs than LRs. PCP significantly reduced the number of gold-labeled glutamate particles in HRs compared to drug-naïve HRs, but did not appear to affect nerve terminal glutamate immunolabeling in LRs compared to drug-naïve LRs.

In experiment 2, the relation between locomotor activity in a novel environment and extracellular glutamate was investigated. Because nerve terminal glutamate immunolabeling and extracellular glutamate have been shown to be inversely related, we can determine if this relationship also applies in inbred mice differentially responsive in a novel environment. We hypothesized that HRs would have lower extracellular cortical glutamate than LRs. Results showed that HRs had significantly less extracellular glutamate than LRs, an effect that was in support of our predictions. As in experiment 1, HRs and LRs were also repeatedly administered PCP in experiment 2. We hypothesized that PCP would increase extracellular glutamate in HRs and LRs alike compared to their respective vehicle-treated controls, and that PCP-treated HRs would exhibit greater extracellular glutamate compared to PCP-treated LRs. Repeated PCP resulted in greater extracellular glutamate in HRs compared to vehicle-treated HRs, but lower extracellular glutamate in LRs compared to vehicle-treated LRs. Lastly, PCP- and vehicle-treated HRs and LRs were administered a PCP challenge (2.5 mg/kg) in order to test if HRs and LRs also differ in their acute glutamatergic response. We hypothesized that vehicle-treated HRs would experience a greater increase in extracellular glutamate than vehicle-treated LRs. Although not statistically significant, acute PCP tended to increase extracellular

glutamate in HRs, but tended to decrease extracellular glutamate in LRs, an effect similar to repeated PCP administration.

Finally, the goal of experiment 3 was to test if individual differences in locomotor activity in a novel environment were actually due to variations in stress response elicited by the novel environment. Plasma corticosterone (CORT) was measured for 2 h following exposure to the novel environment. We hypothesized that HRs experienced greater stress reactivity—indicated by increased CORT—in the novel environment than LRs, which augmented locomotion within the activity chamber and ultimately allowed separation of high and low activity phenotypes. Results revealed no differences in CORT between HRs and LRs suggesting that variation in stress reactivity did not facilitate the separation of HRs and LRs in a novel environment. Overall, the results from experiments 1 and 2 suggested that mPFC glutamate was differentially regulated in HRs and LRs, which may explain differences in locomotion in a novel environment via indirect activation of basal ganglia circuitry. Furthermore, the results from experiment 3 suggested that HRs and LRs had similar CORT levels following exposure to a novel environment and that these similar patterns of stress reactivity could not explain why HRs exhibited greater locomotor activity in a novel environment than LRs.

CHAPTER I: GENERAL INTRODUCTION

Locomotor activity is a complex and easily measurable behavioral trait that is commonly used as an indication of a behavioral, genetic, or environmental change that has taken place within the animal. For instance, behavioral sensitization is a drug-induced phenotype characterized by a marked increase in locomotor response to a drug challenge following repeated drug exposure. In the case of methamphetamine-induced behavioral sensitization[1], rats are administered repeated methamphetamine injections (ranging from consecutive, to every other day, to every third day of drug administration), followed by a period without drug exposure (generally 2-28 days of withdrawal), and are lastly administered a challenge dose of methamphetamine as a test of behavioral sensitization. In this example, locomotor activity denotes the presence of a behavioral alteration within the animal.

This phenotypic response has also been associated with neural plasticity occurring within the reward and limbic circuitry. For example, in brain extracts from outbred Sprague-Dawley rats, marked increases of N-methyl-D-aspartate (NMDA) channel receptor 2ε subunit and phosphorylated cAMP response element binding protein (pCREB) expressions in the frontal cortex (McDaid et al., 2006; Yamamoto et al., 2006), decreased pCREB protein expression in the nucleus accumbens (NAc), and increased ΔFosB protein expression in the NAc and ventral pallidum (VP) were observed following behavioral sensitization to methamphetamine (McDaid et al., 2006). These neuroadaptations illustrate the extent to which a complex trait such as behavioral sensitization can alter both plasma membrane delineated protein signaling and

[1] Behavioral sensitization is not limited to the stimulant effects of methamphetamine, but can also extend to many other drugs such as cocaine, nicotine and morphine (Bartoletti et al., 2007; Harrod et al., 2004; Nakamura et al., 2006).

intracellular protein signaling cascades. There is no doubt that the alterations associated with behavioral sensitization are regulated by multiple genes, yet these specific changes manifest themselves as an easily measurable trait: heightened locomotor response.

Locomotor activity has been used to investigate pleiotropy, or the ability of the same allele(s) to modulate more than one distinct phenotype. For instance, the locomotor response to a low dose of ethanol (the selection trait) has been used to elucidate the genes underlying acute ethanol sensitivity in mice selectively bred for either their stimulant (FAST-1, FAST-2) or depressant (SLOW-1, SLOW-2) response to ethanol. This selection trait was subsequently used to investigate the underlying genetic correlatives in response to acute morphine (Holstein & Phillips, 2006). In FAST selected mice, greater locomotor response to morphine may indicate that the same alleles responsible for the locomotor stimulating effects of ethanol (the selection trait) also contribute to the locomotor stimulant effect to morphine (Holstein et al., 2005). The use of high or low locomotor response as the selection criterion within selected mouse lines can thus aid in identification of candidate genes that contribute to phenotypic variation.

Piazza and colleagues (1989) reported another use of locomotor activity phenotypes: their ability to predict a separate phenotype. Without the use of selected lines, Piazza et al. (1989) found significant positive phenotypic correlations when locomotor activity was regressed onto the rate of drug self-administration in outbred rats. This initial study introduced the idea that outbred rats expressing heightened levels of activity when placed in a novel environment (that is, an environment to which the subjects have had no previous exposure) exhibited increased nose pokes in the active hole during subsequent amphetamine self-administration. Outbred rats displaying individual

differences in locomotor response in a novel environment, such as a square activity chamber (Deminiere et al., 1992; Nadal et al., 2002) or circular corridor (Piazza et al., 1989), were divided into high and low activity groups based on a median split of total locomotor counts during a given test duration. Rats above the median locomotor counts were termed "high responders" (HR) whereas rats below the median were termed "low responders" (LR). Piazza et al. (1989) was the first to suggest that locomotor activity in a novel environment could *predict* acquisition rates of amphetamine self-administration in these rats.

This initial finding of locomotor activity correlated with self-administration behavior of an addictive substance such as amphetamine ignited a flurry of research, which inevitably pushed the boundaries of Piazza's suppositions. Does the correlation between activity in a novel environment and acquisition of self-administration extend to other drugs of abuse (e.g., cocaine, methamphetamine, nicotine)? Does locomotor activity in a novel environment predict other behaviors (e.g., conditioned place preference)? Finally, is locomotor activity in a novel environment phenotypically correlated with neurochemical markers of addiction (e.g., increased dopaminergic firing rates in the nucleus accumbens)? The next few paragraphs will review studies that seek to answer these three questions. The outcomes of these studies will direct us to the over-arching theme—and aim of the current thesis—of parsing the genetic from the environment determinants underlying individual differences in locomotor activity in a novel environment.

High and Low Locomotor Response in a Novel Environment

Does increased locomotor activity in a novel environment also predict greater rates of self-administration acquisition of drugs besides amphetamine? Several studies revealed that the phenotypic correlation between high locomotor response in a novel environment and higher rates of acquisition during self-administration held true for cocaine (Marinelli & White, 2000), methamphetamine (Bevins & Peterson, 2004), nicotine (Suto et al., 2001), morphine (Ambrosio et al., 1995) and ethanol (Nadal et al., 2002). These results suggested that common neurochemical mechanisms facilitated self-administration behavior of these drugs.

However, there are limitations to the prediction, as it depends on pre-exposure to the drug, dose of drug, and extent of training (Marinelli, 2005; Mitchell et al., 2005). For instance, heightened locomotor activity in a novel environment predicted greater number of active nose pokes during amphetamine self-administration only for rats that had no previous exposure to amphetamine prior to self-administration testing (Piazza et al., 1989). Once HR and LR rats were behaviorally sensitized to the stimulant effects of amphetamine by receiving amphetamine (1.5 mg/kg, *i.p.*) once every three days, the correlation between heightened locomotor activity and faster acquisition of amphetamine self-administration disappeared across the five days of testing (2 h per day) (Piazza et al., 1989). This suggested that the specious ability of locomotor activity in a novel environment to predict rates of drug self-administration might infer "vulnerability" or "susceptibility" to acquire drug-taking behavior *only* in animals that have not had any previous experience with the drug.

In a second example of the effects of drug pre-exposure, Pierre and Vezina (1997) found that HR and LR rats pre-exposed to amphetamine (nine daily injections, 1.5 mg/kg,

i.p.) prior to a two-lever continuous reinforcement operant task for amphetamine displayed greater number of active versus inactive lever presses, but only the HR rats continued to exhibit increased active versus inactive lever presses for the duration of the ten day study. Furthermore, saline pre-exposed HRs differentiated between active versus inactive lever during amphetamine self-administration whereas saline pre-exposed LRs did not (Pierre & Vezina, 1997). Saline pre-exposed HRs initially had higher rates of responding on the active lever similar to that observed in amphetamine pre-exposed HRs, but the number of lever presses slowly diminished over the course of the study to the point where rate of lever pressing was indistinguishable from saline pre-exposed LRs (Pierre & Vezina, 1997). These data suggested that locomotor activity in a novel environment may be predictive of an animal's "susceptibility" to acquire self-administration (similar to the conclusions of Piazza et al. (1989)), but less predictive of the total intake and maintenance of self-administration.

Hooks et al. (1992c) found varying results on the locomotor sensitizing effects of amphetamine between amphetamine pre-exposed HR and LR rats depending on the dose of drug used in the experiment. The authors observed that pre-exposed HRs exhibited significantly greater number of line crossings than LRs following systemic injections of 0.5 and 1 mg/kg amphetamine, but not for 1.5 mg/kg amphetamine (Hooks et al., 1992c). These data suggested that the predictive power of locomotor activity in a novel environment may only apply when low doses are used to investigate sensitization differences between HR and LR rats.

The extent of training has also been shown to reduce the predictive power of locomotor activity in a novel environment on acquisition of drug self-administration.

Mitchell et al. (2005) reported that in outbred rats characterized as HRs and LRs and subsequently trained to lever press for a cocaine reinforcement, trained HRs no longer exhibited higher rates of cocaine self-administration compared to trained LRs. However, untrained HRs continued to exhibit greater rates of responding for cocaine reinforcement than untrained LRs (Mitchell et al., 2005), an effect consistent with previous findings (Marinelli & White, 2000). Mitchell et al. (2005) posited that untrained HRs exhibited greater rates of responding for cocaine because they received more reinforced pairings than LRs simply by chance due to the fact that HRs encountered the active lever more often during the given test period. This hypothesis was corroborated by similar levels of cocaine or amphetamine-reinforced lever presses during self-administration between LRs and HRs once LRs were provided with sufficient exposure to the testing apparatus prior to the task (10 days vs. 5 days of testing) (Mitchell et al., 2005; Vezina et al., 1999). Therefore, locomotor response may predict rates of self-administration in *untrained* animals, but it does not necessarily predict drug intake in *trained* animals.

Suto et al. (2001) investigated the relation between locomotion in a novel environment and acquisition of nicotine self-administration in Sprague-Dawley rats. Motor behavior in a novel environment was assessed in square activity chambers for 2 h. Following behavioral testing, animals were placed in similarly constructed boxes and set up for nicotine self-administration. Active lever presses resulted in an infusion of nicotine (0.03 mg/kg/infusion). Rats were tested for 15 consecutive days for 2 h sessions in which a fixed ratio of reinforcement (FR) increased from FR1, FR2, FR3 and FR5 schedules. Priming infusions (0.03 mg/kg, *i.v.*) were administered before the first 12 sessions. A progressive ratio (PR) schedule of reinforcement was executed during the last three

i.p.) prior to a two-lever continuous reinforcement operant task for amphetamine displayed greater number of active versus inactive lever presses, but only the HR rats continued to exhibit increased active versus inactive lever presses for the duration of the ten day study. Furthermore, saline pre-exposed HRs differentiated between active versus inactive lever during amphetamine self-administration whereas saline pre-exposed LRs did not (Pierre & Vezina, 1997). Saline pre-exposed HRs initially had higher rates of responding on the active lever similar to that observed in amphetamine pre-exposed HRs, but the number of lever presses slowly diminished over the course of the study to the point where rate of lever pressing was indistinguishable from saline pre-exposed LRs (Pierre & Vezina, 1997). These data suggested that locomotor activity in a novel environment may be predictive of an animal's "susceptibility" to acquire self-administration (similar to the conclusions of Piazza et al. (1989)), but less predictive of the total intake and maintenance of self-administration.

Hooks et al. (1992c) found varying results on the locomotor sensitizing effects of amphetamine between amphetamine pre-exposed HR and LR rats depending on the dose of drug used in the experiment. The authors observed that pre-exposed HRs exhibited significantly greater number of line crossings than LRs following systemic injections of 0.5 and 1 mg/kg amphetamine, but not for 1.5 mg/kg amphetamine (Hooks et al., 1992c). These data suggested that the predictive power of locomotor activity in a novel environment may only apply when low doses are used to investigate sensitization differences between HR and LR rats.

The extent of training has also been shown to reduce the predictive power of locomotor activity in a novel environment on acquisition of drug self-administration.

Mitchell et al. (2005) reported that in outbred rats characterized as HRs and LRs and subsequently trained to lever press for a cocaine reinforcement, trained HRs no longer exhibited higher rates of cocaine self-administration compared to trained LRs. However, untrained HRs continued to exhibit greater rates of responding for cocaine reinforcement than untrained LRs (Mitchell et al., 2005), an effect consistent with previous findings (Marinelli & White, 2000). Mitchell et al. (2005) posited that untrained HRs exhibited greater rates of responding for cocaine because they received more reinforced pairings than LRs simply by chance due to the fact that HRs encountered the active lever more often during the given test period. This hypothesis was corroborated by similar levels of cocaine or amphetamine-reinforced lever presses during self-administration between LRs and HRs once LRs were provided with sufficient exposure to the testing apparatus prior to the task (10 days vs. 5 days of testing) (Mitchell et al., 2005; Vezina et al., 1999). Therefore, locomotor response may predict rates of self-administration in *untrained* animals, but it does not necessarily predict drug intake in *trained* animals.

Suto et al. (2001) investigated the relation between locomotion in a novel environment and acquisition of nicotine self-administration in Sprague-Dawley rats. Motor behavior in a novel environment was assessed in square activity chambers for 2 h. Following behavioral testing, animals were placed in similarly constructed boxes and set up for nicotine self-administration. Active lever presses resulted in an infusion of nicotine (0.03 mg/kg/infusion). Rats were tested for 15 consecutive days for 2 h sessions in which a fixed ratio of reinforcement (FR) increased from FR1, FR2, FR3 and FR5 schedules. Priming infusions (0.03 mg/kg, *i.v.*) were administered before the first 12 sessions. A progressive ratio (PR) schedule of reinforcement was executed during the last three

sessions in order to compare break point averages, or the amount of effort a subject is willing to perform to obtain a reinforcer. Break points can thus provide an indication of the drug's reinforcing efficacy between HRs and LRs.

Significant positive correlations were found for novelty-induced behavior over 2 h and number of active lever presses during nicotine self-administration (Spearman's rank correlation coefficient, $r = 0.591$) as well as locomotor behavior and average break points during the PR schedule of reinforcement ($r= 0.509$) (Suto et al., 2001). However, partitioning novelty-induced behavior into 30 or 60 min bins from the total 120 min test did not produce positive correlations with self-administration behavior, except for the 61-90 min bin. Greater activity during this 61-90 min bin predicted greater numbers of active lever presses during self-administration ($r_S = 0.582$, $p< 0.01$) and higher break point averages ($r_S = 0.528$, $p< 0.05$) (Suto et al., 2001). Although HRs and LRs had similar number of locomotor activity counts for the first hour of the 2 h test session, activity clearly diverged after 60 min. At this point, HRs continued to display greater activity than LRs for the remainder of the test session whereas locomotor activity steadily decreased in the LRs (Suto et al., 2001). This observation suggested that HRs not only had greater locomotor reactivity, but also blunted habituation to the novel environment as compared with LRs, an observation consistent with previous reports (Marinelli, 2005).

In contrast to the findings of Suto et al. (2001), Nadal et al. (2002) discovered that only locomotor activity during the first 10 min of a 2 h exposure to novelty was positively correlated with subsequent ethanol operant conditioning, an effect also seen during cocaine self-administration (Grimm & See, 1997). A possible explanation for these varying results might be that Suto and colleagues maintained rats on a reverse

light:dark cycle in which activity was assessed during the dark cycle whereas Nadal and colleagues tested rats during the light cycle. Testing during the dark phase under dim illumination (i.e., red light) has been shown to lead to increased locomotor activity in C57BL/6J mice compared to testing under lit conditions (Hossain et al., 2004; Valentinuzzi et al., 2000). Because rodents are naturally nocturnal animals, it is expected that ambulations would increase during their normal "active" phase (Whishaw et al., 1999). However, the time of activity testing was unlikely to account for much of the discrepancy among results because HRs continued to display significantly greater locomotor activity in the novel environment than LRs, regardless of whether the rats were tested in the light or dark phase (Nadal et al., 2002; Suto et al., 2001).

The second question posed was whether locomotor activity in a novel environment could predict other measures of drug reinforcement aside from self-administration. Greater novelty-induced activity was *not* associated with amphetamine, morphine, or cocaine conditioned place preference (CPP) (Erb & Parker, 1994; Gong et al., 1996; Xigeng et al., 2003). This is in contrast to Nadal et al. (2005) who found a positive correlation between morphine (5 mg/kg, *s.c.*) CPP and locomotor activity (Pearson's correlation coefficient, $r = 0.50$) during the last 15 min of a 30 min activity period measured in a white circular corridor novel environment (80 cm diameter x 34 cm high; inner cylinder 52 cm x 34 cm). It is important to note that greater ambulations during CPP was associated with higher CPP score, suggesting that HRs might have been predisposed to develop morphine CPP compared to LRs (Nadal et al., 2005). It was not specified whether CPP activity correlated with locomotor activity in the circular corridor. Moreover, despite lack of CPP for amphetamine, morphine or cocaine, phenotypic

correlation between higher locomotor activity in a novel environment and greater acquisition of self-administration for these drugs has been reported (Grimm & See, 1997; Nadal et al., 2002; Piazza et al., 1989; Piazza et al., 1990).

Finally, outbred HR rats were found to have increased sensitivity to the locomotor stimulating effects of 0.5 mg/kg amphetamine (Hooks et al., 1992c; Hooks et al., 1991a), 10 mg/kg cocaine (Hooks et al., 1991a), 0.5 mg/kg scopolamine (Hooks et al., 1991a), and 5 mg/kg morphine (Nadal et al., 2005) compared to LRs. In contrast to Hooks et al. (1992c, 1991a), Sprague-Dawley rats characterized as high and low novelty *seekers* did not differ in their locomotor stimulant response to 1 or 3 mg/kg amphetamine (Klebaur & Bardo, 1999). In those studies, animals received a total of four drug injections.

One reason for the inconsistent results between Hooks et al. (1992c, 1991a) and Kelbaur and Bardo (1999) could be the type of novel environment used to distinguish high and low activity animals. For example, Klebaur et al. (1999) used a free-choice novel environment termed a "playground maze," which was a modified version of the object recognition task. In the playground maze, eight distinct shapes are placed equidistant from each other and secured to a black circular plywood platform (100 cm diameter) elevated 55 cm off the floor. Animals were exposed to the playground maze for 3 min on three consecutive days. On the fourth day, one of the objects was removed and replaced with a novel object. The animals were then placed back in the playground maze and the amount of time that the animal spent exploring the novel object was measured. Based on a median split of the data, those animals that spent greater time exploring the novel object were termed high novelty seekers and those that spent less time were termed low novelty seekers. In comparison, Hooks et al. (1992c, 1991a) characterized high and

low activity animals based on their locomotor activity in an inescapable novel environment (i.e., plexiglas automated activity chamber, 39 cm long x 25 cm wide x 24 cm high) over a 2 h period. These results suggested that the type of novel environment used to characterize outbred rats can considerably alter the outcome of a study and the interpretation of its results.

Overview of Anatomical Connections between Limbic and Basal Ganglia Circuitry

It can be thought that integration of limbic and basal ganglia circuitry act to control the expression of drug-related motivational behaviors measured by changes in voluntary motor movements (Kalivas & Volkow, 2005). Figure 1 illustrates the interconnections between limbic and basal ganglia (motor-associated) pathways. The ventral tegmental area (VTA), ventral striatum or NAc, and the prefrontal cortex (PFC) comprise part of the limbic system referred to as the reward pathway, which is activated by drugs of abuse such as cocaine and amphetamine (Kalivas & Volkow, 2005; Koob & Bloom, 1988; Koob & Le Moal, 2001; Wise & Rompre, 1989). Expression of locomotor activity requires activation of the basal ganglia pathway, which is comprised of cortical, striatal, midbrain and thalamic areas (Smith et al., 1998).

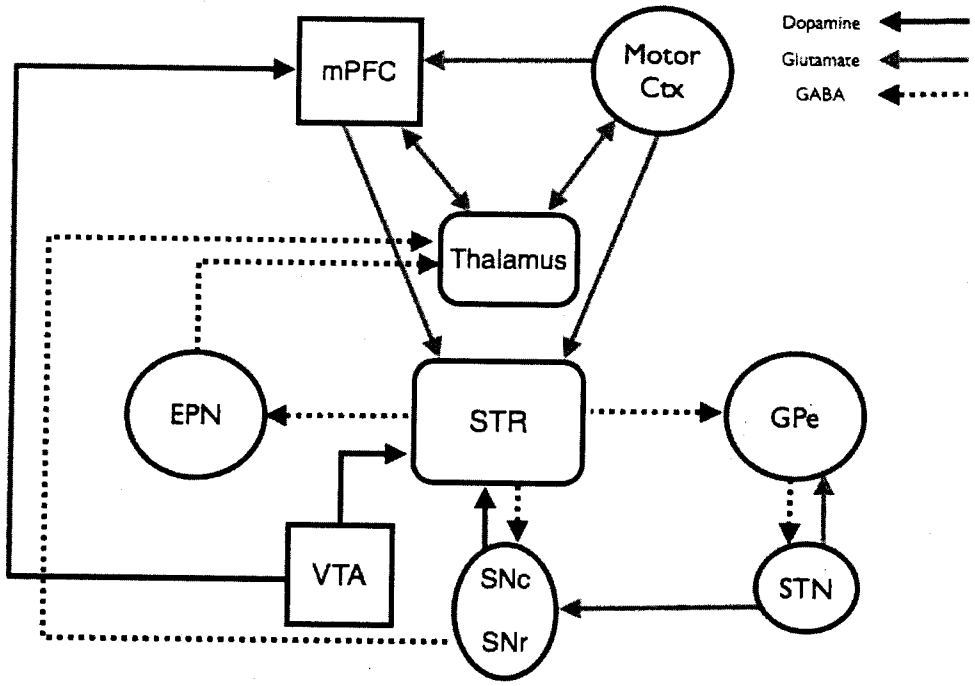


Figure 1. Anatomical connections between limbic and basal ganglia circuitry. Limbic regions are depicted as squares. Basal ganglia (motor) regions are depicted as circles. Substructures (not delineated) of the STR and thalamus are part of both limbic and basal ganglia pathways. Crossed connections omitted for clarity. mPFC=medial prefrontal cortex; Ctx=cortex; EPN=entopeduncular nucleus; STR=striatum; GPe=external globus Pallidus; STN=subthalamic nucleus; SNc=substantia nigra pars compacta; SNr=substantia nigra pars reticulata; VTA=ventral tegmental area.

The VTA sends dopaminergic projections to the ventral striatum (NAc) and the prelimbic/medial areas of the rat prefrontal cortex (mPFC) (Carr & Sesack, 2000; Pierce & Kalivas, 1997). The mPFC is connected to the substantia nigra pars reticulata (SNr) through direct glutamatergic projections to the NAc, which then projects GABA to the SNr (Maurice et al., 1999). There is also evidence of direct mPFC afferents to the SNr, but these connections are thought to be sparse (Frankle et al., 2006).The major outputs of the STR are the entopeduncular nucleus (EPN) in the rat (the internal globus pallidus is the primate counterpart) and the external globus pallidus (GPe) (Albin et al., 1989;

Maurice et al., 1999; Smith et al., 1998). The EPN communicates directly with the thalamus whereas the GPe communicates with the thalamus indirectly via GABA output to the subthalamic nucleus (STN), which in turn sends glutamatergic afferents to the SNr (and also back to the GPe) (Albin et al., 1989; Gerfen, 1992). Lastly, the SNr provides inhibitory input to the thalamus (Gerfen, 1992). Within the thalamus, the mediodorsal nucleus sends glutamatergic afferents to the medial prefrontal cortex (Conde et al., 1990) and the ventromedial/ventrolateral nucleus sends glutamatergic afferents to the motor cortex (Albin et al., 1989; Gerfen, 1992).

Output pathways from the STR include direct and indirect pathways. The direct pathway constitutes STR-EPN-thalamus-cortical connections whereas the indirect pathway is comprised of STR-GPe-STN-SNr-thalamus-cortical connections (Albin et al., 1989; Smith et al., 1998). The STN constitutes the only excitatory nucleus within in the basal ganglia; therefore, decreased inhibitory tone on the STN would lead to greater activation of the SNr, which then inhibits the thalamus (Galvan et al., 2006). Lastly, mPFC and motor cortex have been shown to communicate via excitatory corticocortical projections in non-human primates, which illustrates an additional avenue for information processing and integration between limbic- and motor-associated pathways (Bates & Goldman-Rakic, 1993; Goldman-Rakic et al., 1992).

Neurochemical Differences Between HRs and LRs

The third question posed was whether locomotor activity in a novel environment was associated with neurochemical differences between HRs and LRs. Structures that comprise limbic and basal ganglia pathways, particular striatal and midbrain areas, have been found to be differentially regulated in HR and LR rats (see Figure 1). Marinelli and

White (2000) provided evidence that there was a functional difference between HR and LR rats that may explain their behavior in a novel environment. Under ketamine-induced anesthesia, it was shown *in vivo* that HRs had higher basal dopaminergic firing rates and bursting activity in the VTA than LRs. Because the VTA innervates the NAc (Lynd-Balta & Haber, 1994), it is expected that differences in dopamine neurotransmission also exist within this region. Consistent with this hypothesis, it has been shown that HR rats have higher basal (Hooks et al., 1992a; Piazza et al., 1991b) and amphetamine-stimulated extracellular dopamine (Wolf et al., 2000) and prolonged dopamine release following cocaine or stress administration in the NAc compared to LR rats (Hooks et al., 1992a; Rouge-Pont et al., 1993).

In parallel to limbic circuitry, greater dopaminergic firing rates and bursting activity were measured in the substantia nigra pars compacta (SNc), a core dopaminergic output structure within basal ganglia circuitry (Albin et al., 1989), of HRs than LRs (Marinelli & White, 2000). Moreover, HRs were further shown to be more resistant to the inhibitory effects of the dopamine D2 receptor class agonist, quinpirole, as illustrated by a rightward shift in the dose-response curve of SNc (Marinelli & White, 2000). These data suggested that functional differences in basal ganglia circuitry may also contribute to phenotypic differences between high and low activity rats. Similar to results found in the ventral striatum of the limbic system, greater dopamine neurotransmission was measured in the dorsal striatum of HRs than LRs (Piazza et al., 1991b), which receive dopaminergic afferents from the SNc (Albin et al., 1989). These data suggested that increased dopamine activity within the midbrain may also lead to augmented neuronal activity within the dorsal striatum.

The dorsal striatum also receives glutamatergic afferents from the mPFC and motor cortex (Pierce & Kalivas, 1997), therefore our laboratory was interested in determining the presence of glutamatergic differences within this region. We found that HR mice had lower extracellular glutamate and tended to have higher density of nerve terminal glutamate immunolabeling (25%, NS) compared to LR mice (Shakil et al., 2005). This suggested that HRs and LRs not only display variations in dopaminergic neurotransmission, but also glutamatergic transmission.

Why Study the Medial Prefrontal Cortex?

Studies have shown that HRs exhibited greater dopamine neurotransmission in midbrain regions of the brain, the VTA and SNc, and also in the ventral and dorsal striatum, terminal fields of the VTA and SNc, respectively, compared to LRs (Marinelli & White, 2000; Piazza et al., 1991b). Moreover, VTA and NAc dopaminergic activity were correlated with cocaine and amphetamine self-administration acquisition rates (Hooks et al., 1992a; Piazza et al., 1989). Taken together these data suggested that functional differences in critical areas of the reward circuitry may underlie motivational differences exemplified by greater rate of acquisition and higher intake of drug during self-administration whereas functional differences in primary areas of basal ganglia circuitry may underlie the motor-related phenotypic variations of outbred rats. However, research focusing on the neurochemical differences within the frontal cortex, which is also innervated by the midbrain, has been largely ignored despite its role in reward circuitry (Kalivas & Volkow, 2005; Pierce & Kalivas, 1997).

The mPFC supplies rich excitatory input to the VTA, which sends reciprocal dopaminergic projections to the mPFC (Pierce & Kalivas, 1997). Functionally, these

glutamatergic afferents from the mPFC to the VTA have been shown to be important for the induction of behavioral sensitization (Kalivas & Volkow, 2005; Vezina & Queen, 2000). HR rats that display greater sensitivity to the locomotor stimulating effects of psychostimulants such as amphetamine support the idea that glutamatergic differences within the mPFC may be important for the enhanced expression of behavioral sensitization in HRs compared to LRs (Hooks et al., 1992b; Hooks et al., 1992c; Hooks et al., 1991b; Marinelli & White, 2000; Piazza et al., 1989).

Lesions of the PFC have been shown to prevent the induction of behavioral sensitization in rats by removing the principal source of glutamate in the VTA (Christie et al., 1985; Wolf et al., 1995). Additionally, intra-VTA administration of a NMDA receptor competitive antagonist, AP-5, blocked the induction of behavioral sensitization in Hooded Listar rats (Vezina & Queen, 2000) whereas local application of a glutamate uptake inhibitor produced an augmented locomotor response to a later systemic challenge of amphetamine following sensitization in Sprague-Dawley rats (Aked et al., 2005). Together these studies point to the mPFC as an area of interest in investigating potential glutamatergic differences between HRs and LRs. Neurochemical differences within this region may help explain the behavioral phenotypes of HRs and LRs within a novel environment.

Genetically Heterogeneous versus Homogeneous Animal Model

Although past research was able to draw restricted conclusions about the predictive values of the locomotor response in a novel environment, the investigators are faced with an obstacle regarding interpretation of genetic versus environmental effects on the measured phenotypes (e.g., number of active lever presses, locomotor sensitivity to a

psychostimulant). Using an outbred stock (i.e., genetically heterogeneous) to study phenotypic correlations creates difficulty in separating genetic from environmental effects on the dependent variable. Are similarities in allelic phenotypes between outbred HR and LR rats driving the expression of the measured behavioral phenotype? More importantly, what underlying mechanism(s) allows the initial separation of high and low activity groups? To investigate this issue in the current study, mice that were identical on all alleles at a given gene (i.e., inbred) were divided into HRs and LRs based on locomotor activity in a novel environment. To our knowledge, this method of employing inbred mice has not been used before and provides an opportunity to eliminate allelic influences on the dependent variable. In doing so, one can say concretely that any differences observed between HRs and LRs must be exclusively environmental.

Goals and Hypotheses

The overarching goal of this thesis was to elucidate further the neurochemical and environmental factors driving locomotor behavior in a novel environment. In order to accomplish this goal, the activity phenotype of HRs and LRs was correlated with three other phenotypes, which to our knowledge have not been studied in HRs and LRs. The aims of the first two experiments were to examine the relation between locomotion in a novel environment and indices of glutamate regulation in the mPFC between HRs and LRs as a means to explain locomotor activity in a novel environment. The aim of the last experiment was to test if individual differences in locomotor activity in a novel environment of inbred mice could be explained by variations in stress response elicited by the novel environment.

In experiment 1, the relation between locomotor activity and nerve terminal glutamate immunolabeling was examined using immunogold electron microscopy. HR inbred mice have been shown to have lower extracellular glutamate in the striatum than LRs, suggesting that glutamate was regulated differently between activity groups (Shakil et al., 2005). However, differences in glutamate have not been investigated in the mPFC of HR and LR inbred mice. Understanding glutamatergic differences in the mPFC of HR and LR inbred mice may help explain individual differences in locomotion in a novel environment. We hypothesized that LRs would have greater nerve terminal glutamate immunolabeling than HRs.

Because nerve terminal glutamate immunolabeling is a measure of presynaptic stores of glutamate, HRs and LRs were administered a selective noncompetitive postsynaptic NMDA receptor antagonist, phencyclidine (PCP), daily for 10 consecutive days. PCP has been shown to increase extracellular glutamate, most likely by stimulating glutamate release (Abekawa et al., 2006; Moghaddam & Adams, 1998). Moreover, an increase in extracellular glutamate was associated with a decrease in nerve terminal glutamate immunolabeling (Meshul et al., 1999; Touchon et al., 2004). Therefore, we predicted that HRs would have less nerve terminal glutamate immunolabeling than LRs following repeated PCP administration.

In experiment 2, the relation between locomotor activity in a novel environment and extracellular glutamate was investigated using *in vivo* microdialysis. Because nerve terminal glutamate immunolabeling and extracellular glutamate have been shown to be inversely related (Meshul et al., 1999; Touchon et al., 2004), we can determine if this relationship also applies in inbred mice differentially responsive in a novel environment.

The results from *in vivo* microdialysis, which measures extracellular levels of neurotransmitters, combined with nerve terminal glutamate immunolabeling would provide a better picture of glutamatergic differences between HRs and LRs. Based on previous results from our laboratory which showed that HR inbred mice had significantly lower extracellular striatal glutamate than LR inbred mice—an effect that may be attributed to reduced glutamatergic neurotransmission from the mPFC (see Figure 1)—we hypothesized that HRs would have lower extracellular glutamate than LRs in the mPFC (Shakil et al., 2005).

As in experiment 1, HRs and LRs were also repeatedly administered PCP in experiment 2. We hypothesized that PCP would increase extracellular glutamate in HRs and LRs alike compared to their respective vehicle-treated controls, and that PCP-treated HRs would exhibit greater extracellular glutamate compared to PCP-treated LRs. Lastly, PCP- and vehicle-treated HRs and LRs were administered a PCP challenge (2.5 mg/kg) in order to test if HRs and LRs also differ in their acute glutamatergic response. We predicted that vehicle-treated HRs would experience a greater increase in extracellular glutamate than vehicle-treated LRs.

In experiment 3, the relation between locomotor activity in a novel environment and plasma corticosterone was investigated. It has been shown that greater plasma levels of corticosterone were found in HR outbred rats compared to LRs following novelty (Piazza et al., 1989) and stress (Deminiere et al., 1992), suggesting that enhanced HPA activity may facilitate higher locomotor activity in a novel environment. Therefore, novelty-induced stress and the stress response following an acute restraint were examined in the current study. We predicted that HRs experienced greater stress-reactivity—

indicated by higher plasma corticosterone—in the novel environment than LRs. The results from experiment 3 may provide an indication of *a priori* differences among inbred mice possibly stemming from yet undetermined environmental effects. Overall, the grand aim of the current thesis sought to eliminate potential genetic effects underlying locomotor activity in a novel environment by using inbred mice.

CHAPTER II: IMMUNOGOLD ELECTRON MICROSCOPY

Experiment 1. To determine differences in nerve terminal glutamate immunolabeling in high and low responding mice following repeated vehicle or phencyclidine administration.

Introduction

Previous research in our laboratory examined differences in nerve terminal glutamate immunolabeling within the striatum of twenty C57BL/6J mice characterized as HRs and LRs. LRs were found to have approximately 25% less nerve terminal glutamate immunolabeling in the striatum than HRs ($p= 0.25$) (Shakil et al., 2005). Shakil et al. (2005) also found significantly greater extracellular glutamate in the LRs compared to HRs. The results from this study outlined the inverse relationship regarding lower nerve terminal glutamate immunolabeling and higher extracellular glutamate; this inverse relationship has been observed previously in the striatum of Sprague-Dawley rats (Meshul et al., 1999). Based on the direction of the results from Shakil and colleagues and corroborated by the fact that they used the same strain of animals and same methods to discern HRs from LRs as in the current experiment, we hypothesized that LRs would have less nerve terminal glutamate immunolabeling than HRs within the mPFC.

A working model of limbic and basal ganglia circuitry for LRs is presented in Figure 2 and serves to explain the hypothesized effects of lower nerve terminal glutamate immunolabeling in the mPFC on locomotor activity in a novel environment. Because lower nerve terminal glutamate immunolabeling has been associated with increased extracellular glutamate (Meshul et al., 1999; Touchon et al., 2004), it is hypothesized that greater extracellular glutamate in the mPFC of LRs would augment excitatory

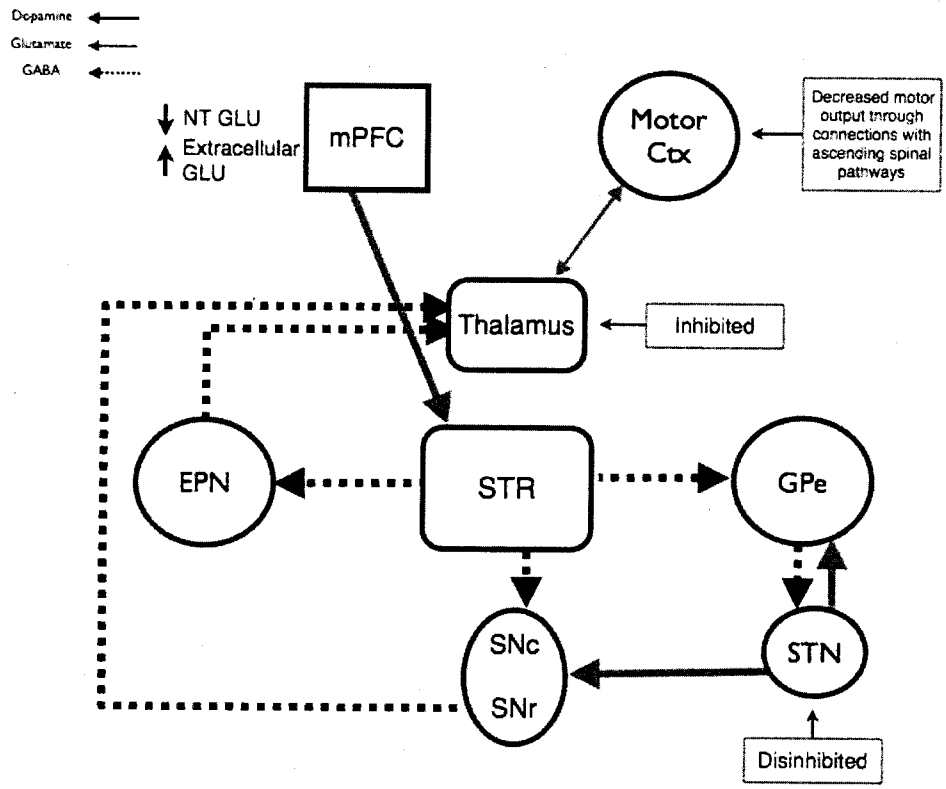


Figure 2. Working model of LR circuitry. We hypothesized that LRs would have lower nerve terminal glutamate immunolabeling (NT GLU) in the mPFC than HRs. Lower NT GLU has been associated with greater extracellular glutamate (Meshul et al., 1999; Touchon et al., 2004). Cross connections have been omitted for clarity. See text for details. mPFC=medial prefrontal cortex; EPN=entopeduncular nucleus; STR=striatum; GPe=external globus pallidus; STN=subthalamic nucleus; SNc=substantia nigra pars compacta; SNr=substantia nigra pars reticulata.

neurotransmission to the STR. Greater glutamatergic tone in the STR would increase GABA neurotransmission to both the EPN and GPe, inhibiting the major output structures of the STR. The inhibition of the GPe would disinhibit the STN thus allowing the STN to activate the SNr. In turn, activation of the SNr and EPN, two structures which project GABA to the motor thalamus, would lead to net inhibition of the motor thalamus. Therefore, greater thalamocortical inhibition of the motor pathway may explain decreased locomotor activity in a novel environment.

In order to characterize further differences in glutamate between HRs and LRs, phencyclidine (PCP), a non-competitive NMDA receptor antagonist, was repeatedly administered in the current study (Jentsch & Roth, 1999; Olney et al., 1989). PCP also has reported effects on nicotinic (α7) receptors (Hashimoto et al., 2006), sigma opioid receptors (Ault & Werling, 1999; Hashimoto et al., 2007), α-1 noradrenergic receptors (Bakshi & Geyer, 1997), 5-HT_2 receptors (Kapur & Seeman, 2002; Winter et al., 2005) and partial activity on D_1 receptors in mPFC of rats (Abekawa et al., 2006) and partial activity on D_2 receptors (Kapur & Seeman, 2002). These studies used non-clinical doses of PCP and its reported effects were still less potent than its effects on the NMDA receptor, suggesting that sub-anesthetic doses of PCP (e.g., 2.5 mg/kg) reduce the risk of cross-reactivity to other neurotransmitter systems (Morris et al., 2005).

Increases in extracellular glutamate in the PFC have been demonstrated following acute systemic (B. W. Adams & Moghaddam, 2001; Moghaddam et al., 1997) and intra-PFC PCP administration (Jentsch et al., 1998) in adult rats. Furthermore, acute PCP has been associated with increased locomotion via the direct glutamatergic afferents from the prefrontal cortex to the NAc (Takahata & Moghaddam, 2003) and lesioning the PFC blocked this locomotor stimulant response to PCP (Jentsch et al., 1998). PCP has also been linked to behavioral sensitization (Hanania et al., 1999; Johnson et al., 1998).

The NAc contains rich sources of dopamine (Lynd-Balta & Haber, 1994), which may mediate motor behavior indirectly through basal ganglia activation. However, intra-NAc application of a non-NMDA antagonist did not attenuate dopamine release in the NAc, but prevented PCP-induced locomotion, thus, suggesting that the PCP-induced locomotion was dopamine-independent (Takahata & Moghaddam, 2003). This

neurochemical evidence concomitant with known glutamatergic projections from mPFC to motor cortex and NAc core (Sesack et al., 2003) serves to further implicate variation in glutamatergic neurotransimission within the mPFC as a source of behavioral differences observed in HRs and LRs.

We hypothesized that PCP-treated HRs would have lower nerve terminal glutamate immunolabeling compared to PCP-treated LRs. In line with this hypothesis, we also predicted that PCP-treated HRs would have lower nerve terminal glutamate immunolabeling compared to vehicle-treated HRs and similarly for PCP- and vehicle-treated LRs.

Materials and Methods

Subjects

Male C57BL/6J mice arrived from The Jackson Laboratories (Davis, CA) at 6 weeks of age and were allowed to habituate for 2-3 weeks before use. Animals were housed in a temperature-controlled facility (21±1°C). Four animals were housed in standard polycarbonate shoebox cages (internal dimensions: 28.5 cm long × 17.5 cm wide × 12 cm high) containing ¼ inch Bed-o-cobs™. The housing facility was on a 12:12 light dark cycle (lights on at 6:00 AM).

Weekly cage changing occurred on Wednesdays. All animal cages belonging to our laboratory were changed within 1 h of each; however because our mice were housed in a large room containing mice from different laboratories it took 6 h to change all of the mouse cages located in room. The same animal technician changed the cages and handled the animals. Locomotor behavior was measured at least 24 h *before* the cages were changed. Since injection schedules were 10 days long, animals received an injection on

the same day that cages were changed (usually 1-2 h after cage changes were completed). Water and food (Purina 5001™; Animal Specialties, Inc., Hubbard, OR) were available *ad libitum*. Animal weights were recorded daily as an indication of health. All animal experiments followed the "Principles of laboratory animal care" and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1978). All procedures were approved by the Portland VA Medical Center Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Apparatus

Animals were transported all at once in their home cages from the housing facility to an adjacent testing room on a mobile cart and allowed to habituate to the new room for 1 h prior to testing. Behavioral testing was conducted during the light cycle (9:00AM-1:00PM) (Marinelli & White, 2000; Piazza et al., 1989; Piazza et al., 1990; Shakil et al., 2005). Animals remained in their home cages with water and food available at all times until time of testing.

Locomotor activity was measured in twelve Accuscan™ activity monitors (Columbus, OH). Animals were not habituated to the activity chamber because we were interested in the initial locomotor response in a novel environment. Each test chamber consisted of a 40 x 40 x 30 cm (length x width x height) clear acrylic box enclosed within the activity monitor. Each activity monitor was outfitted with eight evenly spaced photocell beams located 2 cm above the chamber floor. The monitors recorded the number of photocell beam breaks and Accuscan™ software translated these data as total

horizontal activity counts (1 beam break = 1 activity count). The monitors were each housed inside black acrylic chambers. These black chambers were lined with foam to reduce extraneous noise. Each chamber was illuminated by an 8 W fluorescent light bulb (mean lux = 78) during testing. Each chamber also contained a fan that provided ventilation and a consistent level of low background noise during testing.

Locomotor Response in a Novel Environment

These activity chambers served as the novel environment. Horizontal activity (number of beam breaks) of twenty C57BL/6J male mice was monitored for 30 min. Although previous studies used a 2 h activity testing duration, the greatest horizontal activity counts (HACT) were obtained during the first 30 min (Nowak et al., 2000; Shakil et al., 2005). It is also thought that this initial activity response reflects the reactivity in a novel environment opposed to the habituation response, which would occur following prolonged exposure to the apparatus (>1 h) (Marinelli, 2005). Provided by the Accuscan™ software output, HACT during the 30 min period was calculated, and from that the median HACT was obtained. Animals above the median were termed high responders (HR) and animals below the median were termed low responders (LR). HACT passed Shapiro-Wilk and Kolmogorov-Smirnov tests of normality ($p > 0.200$, $\alpha=0.1$). HACT was chosen because of its consistent use in behavioral studies as an indicator of response to or interest in novelty exhibited by the animal (Bevins & Peterson, 2004; Cain et al., 2004; Deminiere et al., 1992; Hooks et al., 1992c; Hooks et al., 1991b; Marinelli et al., 1997; Marinelli & White, 2000; Marquez et al., 2006; Nadal et al., 2002; Nowak et al., 2000; Piazza et al., 1989; Piazza et al., 1990; Piazza & Le Moal, 1996; Shakil et al., 2005).

Drugs

PCP was a generous gift provided by Dr. Aaron Janowsky. PCP was diluted in physiological saline for injection (0.9% NaCl). Injection volume was adjusted using individual body weights in order to administer the desired dose. HR and LR mice were randomly chosen to receive either 2.5 mg/kg PCP or vehicle (VEH, 0.9% NaCl) for 10 consecutive days (1:00-3:00PM) with 24 h between each injection. All injections were intraperitoneal. Repeated exposures to this moderate dose of PCP was shown to elicit glutamate-related neurochemical deficits in the mPFC, but did not impair motor ability during the Morris Water Maze task (Podhorna & Didriksen, 2005). In addition, we wanted to avoid neurotoxic effects resulting from higher doses, at least in rats (5-10 mg/kg) (Olney et al., 1989; C. Z. Wang & Johnson, 2005). The breakdown of treatment was as follows: LR/VEH (n = 5), LR/PCP (n = 6), HR/VEH (n = 4), HR/PCP (n = 5).

Immunogold Electron Microscopy

Twenty-four hours following the end of drug or vehicle administration, animals were deeply anesthetized using 0.1 mL/10 g mouse cocktail (50 mg/kg ketamine, 12 mg/kg xylazine), the chest cavity was opened and the mice were perfused transcardially using a peristaltic pump (Cole Palmer, Vernon Hills, IL) with 3 mL of 1000 units/mL of heparin in 0.1 M HEPES buffer (pH 7.3) followed immediately by 40 mL of 2.5% glutaraldehyde/0.5% paraformaldehyde in 0.1 M HEPES, pH 7.3, containing 0.1% picric acid. After the perfusion, the entire brain was removed and placed in cold (4°C) fixative overnight. Following a coronal cut, the medial prefrontal cortex (mPFC) was *bilaterally* dissected according to Paxinos and Franklin (2001) mouse brain atlas; approximately a 1

mm^2 section of tissue was excised spanning +2.80 mm to +2.20 mm according to Bregma (Figure 3). The tissue was processed as previously described (Meshul et al., 1994).

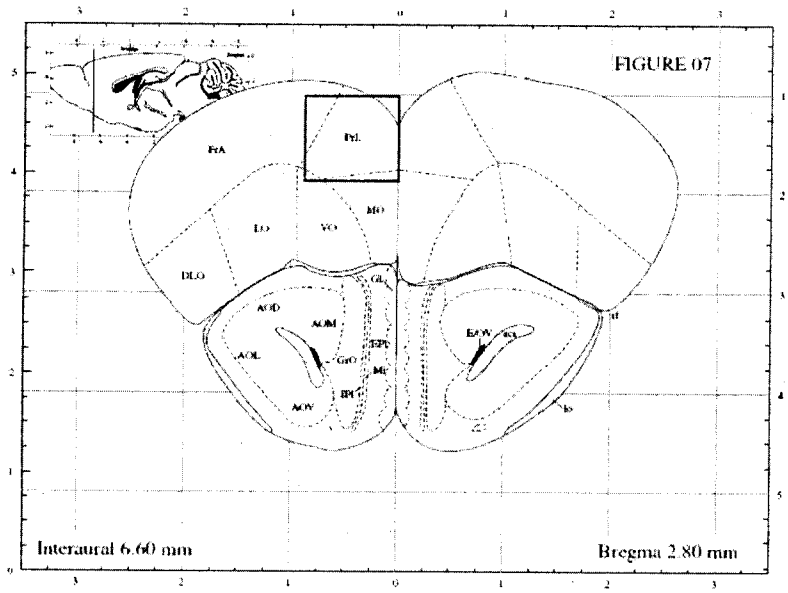


Figure 3. Diagram illustrating the region excised for quantitative immuno-gold electron microscopy. Tissue (approximately 1 mm^2) was taken from the prelimbic cortex (PrL) also referred to as the medial prefrontal cortex. Adapted from the mouse atlas by Paxinos and Franklin (2001), plate 7 (AP +2.80 mm from bregma).

All tissue from each of the treatment groups was cut and processed on the same day in order to reduce variation. Post-embedding immunogold electron microscopy was performed according to the method of Phend et al. (1992), as modified by Meshul et al. (1994). Briefly, the brains were rinsed several times in 0.1 M Hepes buffer pH 7.3 and placed in a Petri dish filled with 0.1 M Hepes buffer 24 h after perfusion. The mPFC were dissected and placed in 1:1 osmium tetroxide (1%): potassium ferricynide (1.5%) solution for 30 min at room temperature. Next, the tissue samples were washed several times in deionized water and stained *en block* with aqueous 0.5% uranyl acetate for 30

min at room temperature. The samples were then dehydrated in graded series of ethanol (twice in 50% for 5 min, twice in 70% for 5 min, three times in 95% for 5 min, three times in 100% for 10 min) and cleared in 100% propylene oxide for 10 min at room temperature. Finally, tissue samples were placed in glass vials containing 1:1 EPON-SPURR: propylene oxide solution overnight. At least 8 h later, tissue samples were transferred to 100% resin in a clean glass vial for 2-3 h, then transferred to embedding molds and allowed to harden at 60°C for 24 h. Once the resin hardened, the tissue was trimmed and thick sectioned at 1 μm. Thin sections were cut on a Leica Ultracut R ultramicrotome and stained with toluidine blue and viewed with a light microscope to ensure proper orientation and identification of cortical layer II. Cortical layer II differed from layer I in that layer II contained a greater number of cell bodies compared to the paucity observed in layer I. We focused on Layer II in the mPFC because glutamatergic neurons in this layer project to the NAc core and dorsal striatum, two areas implicated in motor behavior and sensitization to psychostimulants (Johnson et al., 1998; McKee & Meshul, 2005).

Thin sections (80 nm) of tissue were cut and collected on 200 mesh nickel grids that had been previously coated with a Coat-Quick 'G' pen (Kiyota International, Elk Grove, IL) and air dried for 3-4 h before immunocytochemistry. All incubations and rinses were carried out at room temperature, with grids submerged, tissue side up, in drops of solution (approximately 50 μL) on silicon grid pads or parafilm. All rinse and diluent solutions were filtered (0.22 μm) before use. The glutamate antibody (nonaffinity-purified, rabbit polyclonal; Sigma Chemical, St. Louis, MO), as previously characterized by Hepler et al. (1988), was diluted 1:200,000 in TRIS-buffered saline containing 0.05 M

TRIS, pH 7.6, 0.9% NaCl, and 0.1% Triton X-100 (TBST 7.6). Aspartate (1 mM) was added to the glutamate antibody mixture 24 h prior to incubation with the thin-sectioned tissue to prevent any cross-reactivity with aspartate within the tissue. Following primary glutamate antibody incubation overnight, samples were washed several times in TBST 7.6 and TBST 8.2 (Tris-buffered saline pH 7.6 and 8.2, respectively, containing 0.1% Triton X-100) and incubated for 90 min in goat anti-rabbit IgG (Sigma) (1:400,000) conjugated to 10 nm gold, which was diluted 1:50 in TBST 8.2. Afterward, the samples were rinsed twice in TBST 7.6 for 5 min. Once dry, sections were viewed and photographed using a JEOL 1200 EX transmission electron microscope. The specificity of the immunolabeling for the glutamate antibody was previously established by incubating the antibody overnight with 3 mM glutamate (Meshul et al., 1994). This mixture was then applied to the sections as detailed above, with the final results showing a lack of tissue immunolabeling.

Photographs (10/animal) were taken randomly at a final magnification of x40,000 throughout cortical layer II in the mPFC using a digital camera (AMT, Peabody, MA). The images were directly captured and stored on the computer. The glutamate immunolabeling technique was carried out for all of the treatment groups on the same day in order to reduce day-to-day variance. Approximately 25-45 observations were made per animal and a mean for each animal was calculated for variables of interest and used in statistical analyses.

The number of gold particles per nerve terminal associated with an asymmetrical synaptic contact was counted and the area of the nerve terminal was determined using Image Pro Plus software (Media Cybernetics, Silver Springs, MD, v3.01). Asymmetrical

synaptic contacts were identified by an accumulation of synaptic vesicles within the presynaptic nerve terminal and a prominent postsynaptic density that was wider than the width of the synaptic cleft. The gold particles contacting the synaptic vesicles within the nerve terminal were counted and considered part of the vesicular or neurotransmitter pool as previously established (Meshul et al., 1999). When nerve terminals contained mitochondria, gold particles associated with this organelle were not included as part of the final particle measurement regarding vesicular glutamate, but instead they were included as the part of the metabolic pool contained in nerve terminals. Table 1 depicts the number of synapses analyzed per animal for each treatment group. Outliers more than two standard deviations from the mean were eliminated. Outliers constituted less than 5% of the total number of synapses analyzed per group.

Table 1. The number of synapses analyzed for gold-labeled glutamate immunolabeling per animal in each group.

	LR/VEH ID	**# Syn**	**LR/PCP ID**	**# Syn**	**HR/VEH ID**	**# Syn**	**HR/PCP ID**	**# Syn**
	1.1	36	2.1	39	1.3	34	1.2	33
	2.4	45	2.2	39	3.2	31	1.4	35
	4.1	31	2.3	24	5.3	30	3.1	26
	4.3	36	4.2	30	5.4	33	3.3	28
	5.1	37	4.4	45			3.4	41
			5.2	31				
TOTAL		**185**		**208**		**128**		**163**

Syn = synapses, LR = low responder, HR = high responder, PCP = phencyclidine, VEH = vehicle

Data Analysis

A two-factor analysis of covariance (ANCOVA) was performed on individual means in order to elucidate the effects of activity (high vs. low) and drug (vehicle vs.

PCP) on glutamate immunoreactivity when corrected for nerve terminal size (the covariate). The mean nerve terminal size (μm) for each animal was calculated from the size of the total number of synapses analyzed per individual. Any significant 2 x 2 interactions were further characterized using Tukey's HSD. Pearson's correlation was used to examine the relation between locomotor activity in a novel environment and nerve terminal glutamate immunolabeling. Significance level was set at $\alpha = 0.05$.

Results

Behavioral Testing

The median HACT for all animals over a 30 min period was 10123 activity counts (Figure 4A). The total mean HACT for HRs was 11442 ± 306 activity counts and the total mean HACT for LRs was 1599 ± 28 counts. The HRs had significantly higher activity counts than the LRs ($p< 0.001$).

Immunogold Electron Microscopy

Medial Prefrontal Cortex

Figure 5 illustrates nerve terminals (NT) making asymmetrical synaptic contacts with dendritic spines (Sp) from the HR/VEH, HR/PCP, LR/VEH, and LR/PCP groups. Arrowheads designate prominent postsynaptic densities which are indicative of active synapses. The arrow points to a gold-labeled glutamate particle. Particles associated with the mitochondria (mit), as seen in D, were not included in the analyses.

Results revealed a significant main effect of drug ($F_{(1,20)} = 6.39$, $p= 0.02$), but not activity, on the number of gold-labeled glutamate particles within a nerve terminal when corrected for size of the presynaptic terminal. In addition, there was a significant activity x drug interaction ($F_{(1,20)} = 7.15$, $p= 0.02$) (Figure 4B). Thus, PCP-treated HRs had fewer

glutamate immunogold particles compared to vehicle-treated HRs (HR/PCP vs. HR/VEH, p= 0.002), but there were no differences between PCP- and vehicle-treated LRs (LR/PCP vs. LR/VEH). Furthermore, vehicle-treated HRs had 38% higher number of glutamate immunogold particles compared to vehicle-treated LRs (HR/VEH vs. LR/VEH, p= 0.01) and 48% more than PCP-treated LRs (HR/VEH vs. LR/PCP, p= 0.03). Lastly, Pearson's product moment correlation did not reveal a significant relation between nerve terminal glutamate immunolabeling and locomotor response in a novel environment ($r_{(18)} = 0.156$; Figure 4C).

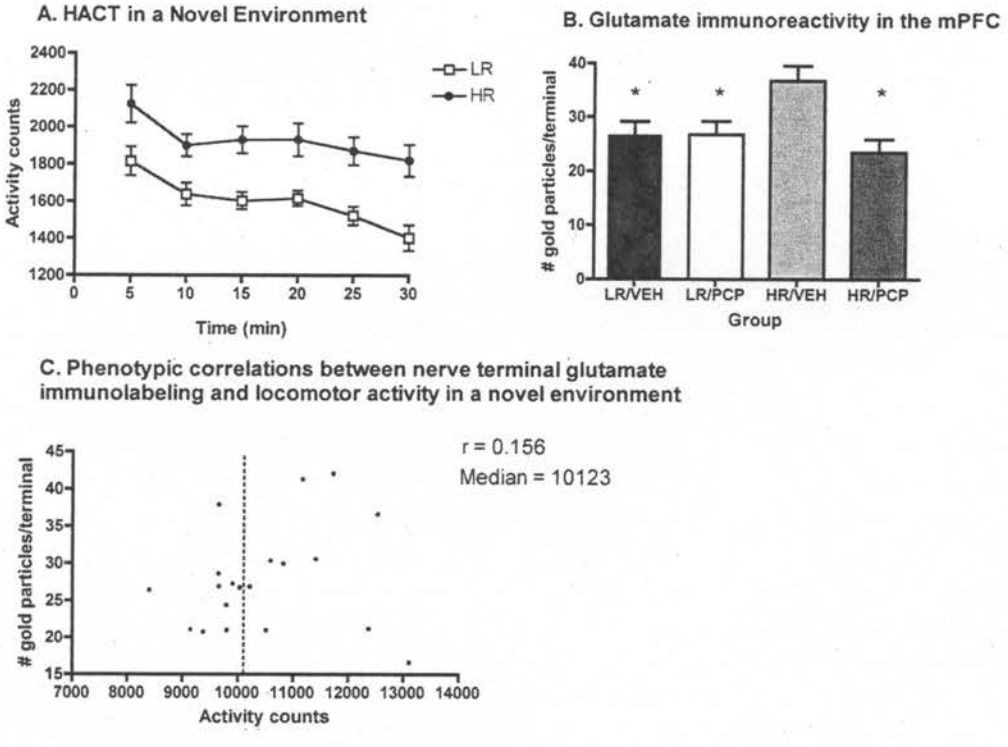


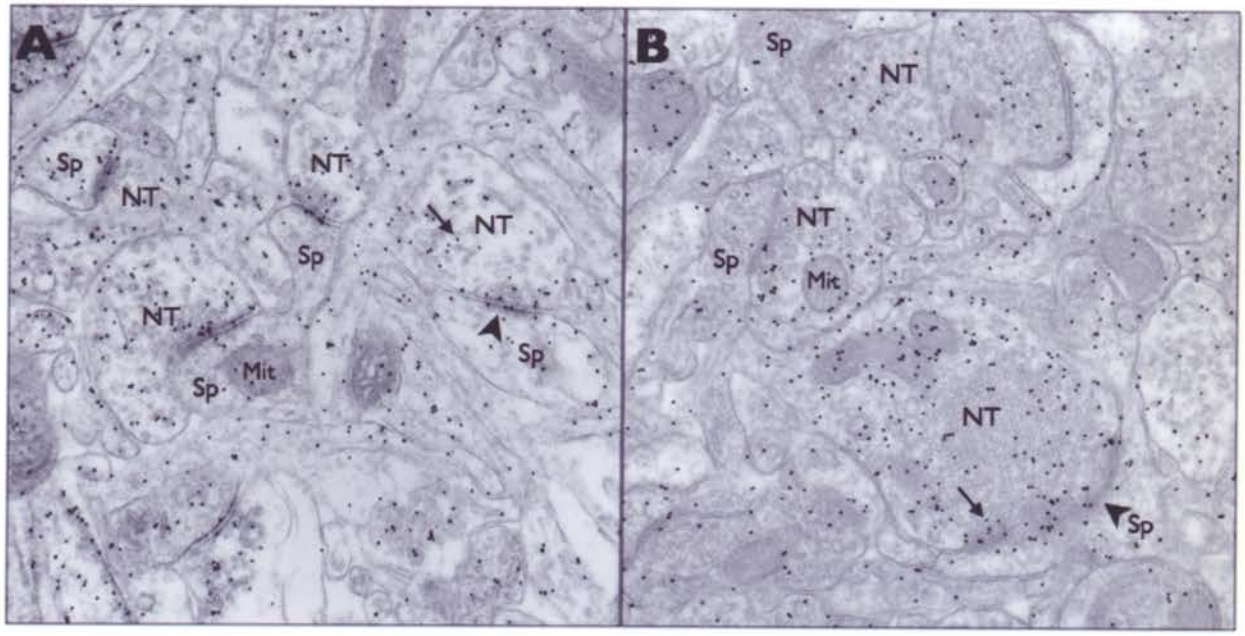
Figure 4. Nerve terminal glutamate immunolabeling in HR and LR mice. A. Horizontal locomotor activity (HACT) in a novel environment (Accuscan™ activity monitors) was measured in 20 male C57BL/6J mice for 30 min. Mice with a locomotor response above the median activity count were termed high responders (HR) and mice with a locomotor response below were termed low responders (LR). HRs had significantly higher activity counts than LRs ($p< 0.001$). Values represent mean ± S.E.M. B. Fewer glutamate particles

were associated with LRs compared to HRs (HR/VEH vs. LR/VEH). PCP reduced the number of gold-labeled glutamate particles in mice that have greater behavioral response to novelty (HR/VEH vs. HR/PCP), but there were no significant differences in the number of gold-labeled particles in LR mice rats after PCP treatment (LR/VEH vs. LR/PCP). Each bar represents the mean # particles per terminal ± S.E.M ($n = 4$-6 for all groups). Significance was determined by a two-factor ANOVA with Tukey's HSD post-hoc to compare differences between groups. *$p < 0.05$ compared to HR/VEH. C. There is no significant relation between the number of gold-labeled glutamate particles per nerve terminal and locomotor activity in a novel environment (Pearson's correlation coefficient, $r = 0.156$). The dotted line designates the median locomotor count, which is used to divide HRs and LRs (median = 10123 counts).

Figure 5.

LR/VEH

HR/VEH



LR/PCP

HR/PCP

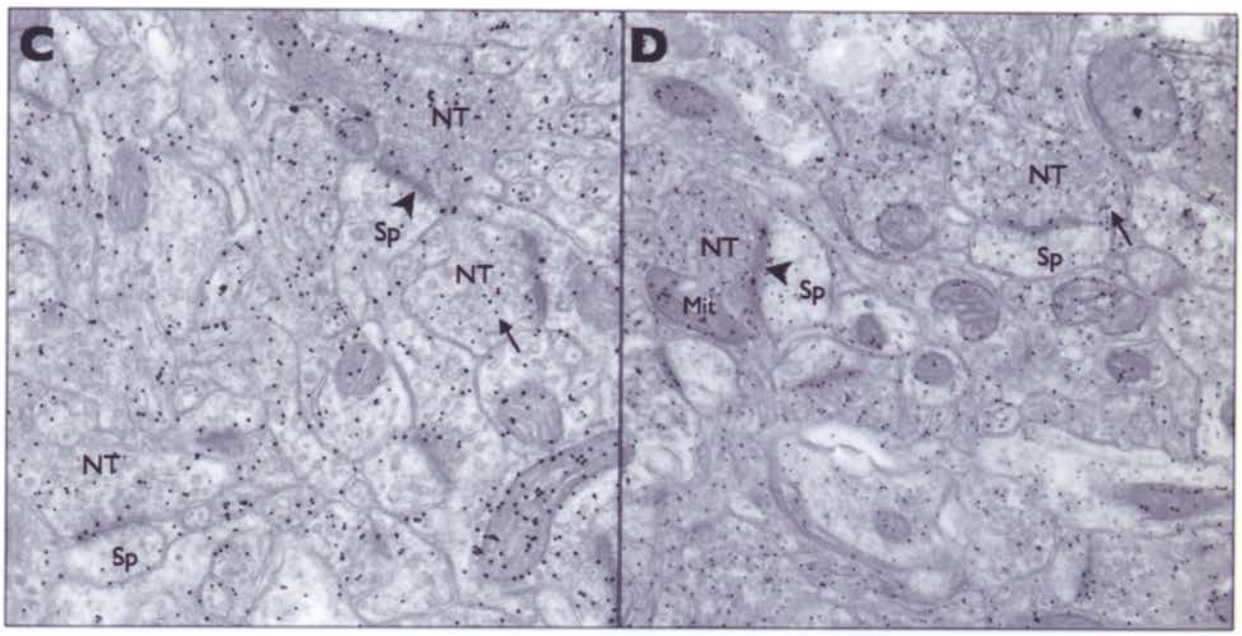


Figure 5. Representative electron micrographs are shown for each group. A presynaptic nerve terminal (NT) makes an asymmetrical excitatory synaptic contact onto a dendritic spine (Sp) indicated by the prominent postsynaptic density (black arrowhead). Gold-labeled particles, noting the localization of the glutamate antibody, appear as black dots (black arrow) and are associated with the synaptic vesicles. Gold-labeled particles are also shown associated with mitochondrial (metabolic) stores (Mit). Only gold mitochondrial-associated gold particles were excluded from analyses. Mice exhibiting a high (HR) or low response (LR) in a novel environment were administered either phencyclidine (PCP 2.5 mg/kg, *i.p.*) or vehicle (0.9% saline) daily for ten days. Note the difference in glutamate immunoreactivity between HR groups (HR/VEH vs. HR/PCP), but the lack of differences between LR groups (LR/VEH vs. LR/PCP). LR=low responder; HR=high responder; VEH=vehicle; PCP=phencyclidine.

Nerve Terminal Mitochondria

Glutamate immunoreactivity within the mitochondrial (or metabolic) pool was also analyzed. Two-way ANOVA did not reveal any main effects of drug ($F_{(1, 20)} = 1.31$) or activity ($F_{(1, 20)} = 0.92$) or a significant drug x activity interaction ($F_{(1, 20)} = 0.17$) on the number of gold-labeled glutamate particles (data not shown). These results suggest that neither activity nor drug is affecting the metabolic pool of glutamate within the nerve terminals forming an asymmetrical (excitatory) synaptic contact within the mPFC. Therefore, any differences seen in nerve terminal glutamate between groups are likely to be due to changes in vesicular (non-mitochondrial) pool of glutamate.

Discussion

In support of our hypothesis, we found that drug-naïve animals exhibiting a lower locomotor response in a novel environment had significantly fewer glutamate immunogold particles within nerve terminals making an asymmetrical (excitatory) contact compared to those with a heightened locomotor activity (LR/VEH vs. HR/VEH). These results could not be explained by changes in metabolic (mitochondrial-associated) stores of glutamate.

Contrary to our hypothesis, the number of glutamate immunogold particles with nerve terminals was similar between PCP-treated HRs and LRs. In support of our

hypothesis, PCP-treated HRs had significantly fewer numbers of glutamate immunogold particles associated with the presynaptic terminal than VEH-treated HRs, an effect that may be explained by PCP-induced glutamate release (B. Adams & Moghaddam, 1998; B. W. Adams & Moghaddam, 2001; Moghaddam & Adams, 1998). Contrary to our hypothesis, repeated PCP appeared to have no effect in LRs such that the numbers of glutamate immunogold particles were similar in PCP- and vehicle-treated LRs. Therefore, we surmised that repeated PCP reduced cortical glutamate immunolabeling selectively in HRs only (HR/VEH vs. HR/PCP) and not LRs (LR/VEH vs. LR/PCP). Together, these data suggested that HRs were somehow more sensitive to the post-synaptic effects of PCP than LRs.

Two possible explanations for why repeated PCP produced a significant effect in HRs but not LRs compared to their vehicle-controls have been proposed. First, repeated NMDA receptor antagonism by PCP has been associated with increased incidence of NR2 subunit mutation in the PFC of rats (Lindahl & Keifer, 2004); this subunit is a necessary component required for proper functioning of the NMDA receptor (Morris et al., 2005). NMDA receptor subunit alteration has been linked with metabolic hypofunction as a consequence of declined receptor function (Cochran et al., 2003) Therefore, NMDA receptor alteration due to repeated systemic PCP administration may lead to reduced NMDA-mediated synaptic neurotransmission; a consequence of which might be increased extracellular glutamate. Under these circumstances, the source of extracellular glutamate is most likely neuronal and occurs as a compensatory mechanism following functional loss (Moghaddam et al., 1997; Moghaddam & Adams, 1998). Furthermore, this increase in glutamate release would be consistent with the lower nerve

terminal glutamate immunolabeing observed in PCP-treated HRs. It is possible that NMDA receptor hypofunction was a neuroadaptation that was more apparent in HRs than LRs. Although it is difficult to directly compare results obtained in rats with the current results obtained in mice due to differences in PCP pharmacokinetics, the aforementioned studies still provided a framework from which the effects of repeated antagonism for 10 consecutive days could be inferred. This may explain the significant differences seen between PCP- and vehicle-treated HRs, but not between PCP- and vehicle-treated LRs.

Second, PCP has been linked to several neurotoxic effects including selective apoptosis of frontal cortical neurons (C. Wang et al., 2001; C. Z. Wang & Johnson, 2005), impaired corticostriatal neurotransmission (C. Wang et al., 2004) and vacuole formation (Olney et al., 1989) within the cytoplasm of cortical neurons in rats. A general survey of micrographs in the current study did not show any pervasive vacuole formation within the cytoplasm indicative of neurotoxicity in HRs or LRs; however, time-dependent changes may have accounted for some of the differences seen between our study and Wang et al. (2001). In the latter study, TUNEL-staining in rat frontal cortical neurons was evident 24 h after a 10 mg/kg dose of PCP administered on postnatal days 7, 9, and 11. We conducted electron microscopy in adult mice following *ten* days of 2.5 mg/kg PCP, and thus may have missed the appearance of neurodegeneration. It is logical to assume that PCP-induced neurotoxicity may show cumulative effects; however, the plasma half-life of PCP is approximately 4 h suggesting that our dosing regime would not lead to additive neurotoxic effects as a result of consistent NMDA receptor antagonism (Shelnutt et al., 1999). Furthermore, our results were consistent with Olney et al. (1989), who investigated PCP-induced vacuole formation ultimately leading to neuronal death.

The authors reported that repeated exposure to a low dose of PCP (0.3 mg/kg) did not induce any obvious morphological changes in mPFC neurons suggesting that the differences in nerve terminal glutamate immunolabeling in the current study were not due to neurotoxicity. Additionally, these results may also suggest development of short-term tolerance to the deleterious effects of PCP in HRs and LRs.

CHAPTER 3: *IN VIVO* MICRODIALYSIS

Experiment 2a. To determine differences in extracellular glutamate following phencyclidine challenge between vehicle- and phencyclidine-treated high and low responders.

Introduction

Baseline Extracellular Glutamate (following repeated PCP)

Following immunogold electron microscopy, it was reported that drug-naïve HRs had a significantly greater number of gold labeled glutamate particles associated with nerve terminals making an asymmetrical (excitatory) contact onto dendritic spines compared to drug-naïve LRs. It was suggested that the HRs were more responsive to repeated postsynaptic antagonism as evidenced by decreased glutamate immunoreactivity (lower number of gold-labeled glutamate particles) within the presynaptic terminal compared to vehicle controls, whereas LRs repeatedly administered PCP showed no difference in glutamate immunoreactivity compared to vehicle controls.

In experiment 2a, we sought to determine if HRs and LRs would also differ in extracellular glutamate within the mPFC following repeated vehicle or PCP administration. Shakil et al. (2005) has shown that C57BL/6J mice divided into high and low activity groups markedly differed in extracellular glutamate within the striatum. Specifically, HRs exhibited significantly lower extracellular glutamate compared to LRs. As seen in Figure 2, we hypothesized that lower extracellular glutamate in the STR may be associated with increased locomotor activity. Based on the results from Shakil et al. (2005) and the results from experiment 1 (drug-naïve animals) concomitant with evidence of an inverse relationship between nerve terminal glutamate immunolabeling

and extracellular glutamate (Meshul et al., 1999; Touchon et al., 2004), we hypothesized that drig-naïve HRs would have lower extracellular glutamate in the mPFC than drug-naïve LRs.

The effects of repeated postsynaptic NMDA receptor antagonism on extracellular glutamate in HRs and LRs were also investigated. Although repeated antagonism did not result in changes in nerve terminal glutamate immunolabeling in the LRs (see experiment 1), changes in extracellular glutamate may still be apparent. Non-neuronal sources of glutamate also contribute to the extracellular pool of glutamate, such as the cystine-glutamate antiporter (Baker et al., 2002). The cystine-glutamate antiporter is located on glia and releases glutamate into the extracellular space in exchange for cystine, thereby contributing to glutamatergic dynamics within the synaptic cleft (Baker et al., 2002). In fact, McKee and Meshul (2005) have shown how extracellular glutamate changes independently of nerve terminal glutamate immunolabeling following 1, 2, 3, or 14 days after acute cocaine exposure in Sprague-Dawley rats. Because repeated antagonism was shown to significantly reduce glutamate immunoreactivity in HRs most likely through PCP-induced neurotransmitter release, we hypothesized that PCP-treated HRs would exhibit greater extracellular glutamate than vehicle-treated HRs. Similarly, we predicted that PCP-treated LRs would also exhibit greater extracellular glutamate than vehicle-treated LRs, albeit to a lesser extent than would be seen among HRs.

Extracellular Glutamate Following PCP Challenge

The acute glutamatergic response to PCP was investigated in order to see if vehicle-treated HRs and LRs responded similarly to a single injection of PCP (2.5 mg/kg) and to see if this response matched the glutamatergic response to repeated PCP

administration. Because it has been shown that acute PCP (5 mg/kg, *i.p.*) elicits significant elevations in mPFC extracellular glutamate in rats by stimulating release mechanisms (B. Adams & Moghaddam, 1998; Moghaddam & Adams, 1998), we hypothesized that both vehicle-treated HRs and LRs would experience an increase in extracellular glutamate compared to baseline. In addition, we observed greater stores of presynaptic glutamate indicated by higher nerve terminal glutamate immunolabeling in HRs than LRs (see Figure 4B, HR/VEH vs. LR/VEH), therefore, we hypothesized that vehicle-treated HRs would exhibit the greatest increase in extracellular glutamate compared to vehicle-treated LRs following a challenge dose of PCP. Furthermore, we hypothesized that HRs repeatedly administered PCP would exhibit increases in extracellular glutamate following a PCP challenge. It was also predicted that LRs repeatedly administered PCP would show elevated extracellular glutamate following a PCP challenge. However, we hypothesized that this increase would be greater in HRs than LRs because the HRs were presumably more sensitive to the effects of PCP as seen in experiment 1 (see Figure 4B, see HR/PCP vs LR/PCP).

Materials and Methods

Subjects

Forty C57BL/6J mice were generously provided by Dr. Donna VanWinkle. These animals were originally purchased from The Jackson Laboratory (Davis, CA) and were not used for studies in the VanWinkle laboratory. Animals were approximately 8-10 weeks old at the time we received them from Dr. VanWinkle. Animals were housed in the same room as we use for our animals; therefore, the animals inherited from Dr. VanWinkle did not need to be transferred to a new location. Animal housing was

identical to that as in experiment 1. Detailed information can be found under experiment 1 (p 26).

Apparatus

See experiment 1 (p 27).

Locomotor Response in a Novel Environment

See experiment 1 (p 28).

Drugs

PCP was a generous gift from Dr. Aaron Janowsky. It was prepared for injection as described in experiment 1. HRs and LRs were randomly chosen to receive PCP (2.5 mg/kg) or vehicle (0.9% NaCl) once a day for 10 consecutive days, with 24 h between each injection. All injections were intraperitoneal. The breakdown of treatment was as follows: LR/VEH (n = 5), LR/PCP (n = 7), HR/VEH (n = 3), HR/PCP (n = 6). This breakdown reflects the final group membership following probe placement verification. Initial group sizes were LR/VEH (n = 10), LR/PCP (n = 11), HR/VEH (n = 9), HR/PCP (n = 10).

In vivo *microdialysis*

Under isoflurane-induced anesthesia, mice were stereotaxically (Cartesian Research, Inc., OR) implanted with a stainless steel guide cannula (8 mm long, 21 gauge) in the left mPFC and allowed to recover for 3 days before receiving the first PCP injection. The coordinates relative to bregma were: +0.4 mm medial/lateral, +2.2 mm anterior/posterior and -1.0 mm dorsal/ventral.

Four to six hours after the tenth PCP administration and one day before the start of the dialysis procedure, dialysis probes (1 mm cellulose tip) were slowly inserted into

the mPFC while the animals were under isoflurane-induced anesthesia. Dialysis probes were prepared as described by Robinson and Wishaw (1988) with modifications (Meshul et al., 1999). The probe was secured to the guide cannula and head mount using epoxy. The artificial cerebrospinal fluid (aCSF; 140 mM NaCl, 3.4 mM KCl, 1.5 mM CaCl2, 1.0 mM $MgCl_2$, 1.4 mM NaH_2PO_4, and 4.85 mM $NaHPO_4$, pH 7.4) flowed through the probe overnight at a rate of 0.2 µl/min. The following morning, the pump speed was increased to 2.0 µl/min for 30 min prior to the start of sample collection. A total of 16 samples were collected at 15-min intervals. Following the collection of the first 4 baseline samples, PCP (2.5 mg/kg, *i.p.*) was administered to all animals and collection of the remaining 12 samples resumed. At the conclusion of the experiment, the animals were perfused with glutaraldehyde fixative. Coronal sections (100 µm) were cut with a vibratome and stained with thionin in order to verify proper probe placement (Figure 6). Only animals

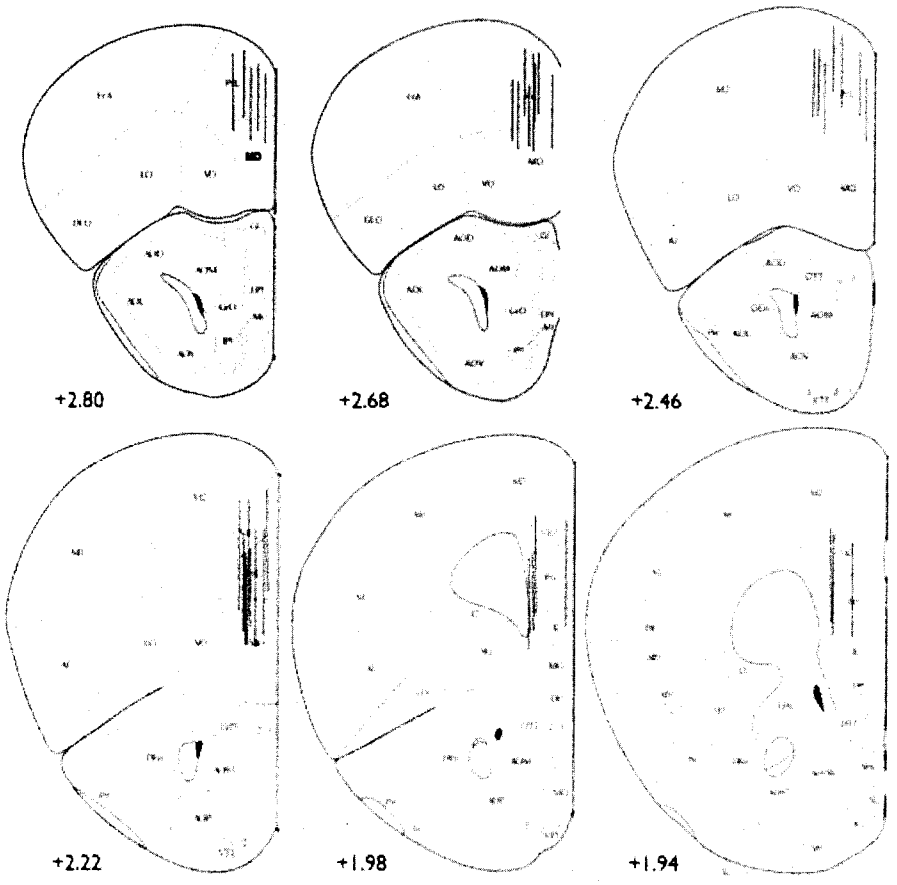


Figure 6. *In vivo* microdialysis probe placements for experiments 2a and 2b. Representations of the probe placements for all of the mice used in the microdialysis experiments 2a and 2b (n = 35). The probes spanned the prelimbic cortex (PrL) and medial orbital cortex and ranged from 2.68 mm to 1.94 mm anterior to bregma (Paxinos & Franklin, 2001). Probe placements correspond to the region excised for electron microscopy.

with correctly placed probes were included in the statistical analyses of microdialysis data. Glutamate concentration in dialysate samples was determined using a Hewlett Packard HPLC 1090 interfaced with a Hewlett Packard 1046A Programmable Fluorescence Detector as previously detailed (Shakil et al., 2005).

In vitro probe recoveries were performed for all probes. The percent probe recovery was consistently 4%; therefore, any differences in extracellular glutamate detected between groups were not due to varying probe kinetics.

Data Analysis

Two-factor ANOVA with *a priori* planned comparisons was used to examine the effects of activity (high vs. low) and drug (PCP vs. vehicle) on baseline extracellular glutamate (collapsed across 0-60 min). Pearson's correlation was calculated on locomotor activity counts in a novel environment and baseline extracellular glutamate. Repeated measures ANOVA was performed in order to compare the effects of activity and drug on mean extracellular glutamate pre- and post-PCP challenge. Significance was set at $\alpha = 0.05$ for all tests.

Results

Behavioral Testing

The median HACT was 9951 activity counts. The mean total HACT for HRs was 11596 ± 263 activity counts and the mean total HACT for LRs was 8708 ± 189 activity counts. HRs had significantly higher HACT than LRs ($p < 0.0001$) (Figure 7A).

Baseline Extracellular Glutamate (following repeated PCP)

It was hypothesized that HRs and LRs would differ in extracellular glutamate and that variation in glutamatergic neurotransmission may explain the divergent locomotor

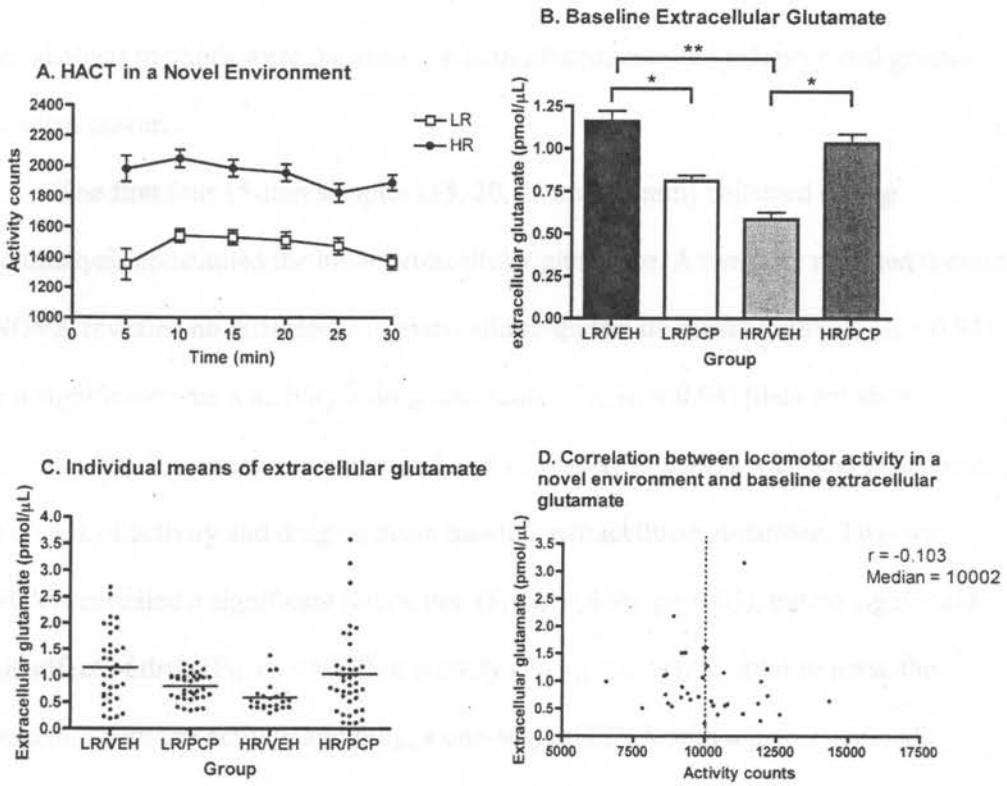


Figure 7. Repeated PCP has differential effects on extracellular glutamate in HR and LR mice. A. Horizontal locomotor activity (HACT) in a novel environment (Accuscan™ activity monitors) was measured in 40 male C57BL/6J mice for 30 min. Mice with a locomotor response above the median activity count were termed high responders (HR) and mice with a locomotor response below were termed low responders (LR). HRs had significantly higher activity counts than LRs ($p < 0.001$). Values represent mean ± S.E.M. B. *In vivo* microdialysis revealed differences in extracellular glutamate in the medial prefrontal cortex (mPFC) of HR and LR mice. HR and LR mice received either 2.5 mg/kg phencyclidine (PCP) or vehicle (VEH; 0.9% saline) daily for 10 days. Glutamate was assessed by HPLC and results were analyzed using two-way ANOVA with planned contrasts. Vehicle-treated LRs had significantly greater extracellular glutamate than vehicle-treated HRs (LR/VEH vs. HR/VEH). Repeated PCP appeared to reduce extracellular glutamate in LRs, but increase extracellular glutamate in HRs. Data are expressed as mean ± S.E.M. (n = 6-10 for all groups). **$p < 0.05$, *$p < 0.1$. C. Individual data points depicting extracellular glutamate following either repeated PCP or VEH are illustrated. Note the increased variance in HRs following repeated PCP compared to vehicle controls and how HR/PCP resembled LR/VEH. D. There is no relation between extracellular glutamate and locomotor activity in a novel environment following a 30 min exposure (Pearson's correlation coefficient, $r_{(33)} = -0.103$). The dotted line designates the median locomotor count, which divides HRs and LRs (median = 10002 counts).

response to novelty. Individuals from experiments 2a and 2b were pooled because microdialysis methods were the same for both experiments and this provided greater statistical power.

The first four 15-min samples (15, 30, 45 and 60 min) collected during microdialysis constituted the basal extracellular glutamate. A two-way repeated measures ANOVA revealed no differences in extracellular glutamate across time ($F_{(3, 75)} = 0.94$), nor a significant time x activity x drug interaction ($F_{(3, 75)} = 0.64$) (data not shown). Therefore, the four times were averaged and a two-way ANOVA was used to determine the effects of activity and drug on mean baseline extracellular glutamate. Two-way ANOVA revealed a significant interaction ($F_{(1, 31)} = 4.96$, $p= 0.03$), but no significant main effect of drug ($F_{(1, 31)} = 0.12$) or activity ($F_{(1, 31)} = 0.49$). In order to parse the interaction between activity and drug, a one-way ANOVA with *a priori* planned comparisons was performed. Results revealed that vehicle-treated LRs had significantly greater basal extracellular glutamate than vehicle-treated HRs (LR/VEH vs. HR/VEH) ($p= 0.02$) (Figure 7B). This effect was as predicted. The direction of this effect between vehicle-treated HRs and LRs was opposite to that observed in nerve terminal glutamate immunolabeling in experiment (see Figure 4B; LR/VEH vs. HR/VEH), a finding that was consistent with previous reports from our laboratory (Meshul et al., 1999; Touchon et al., 2004). In support of our hypothesis, PCP-treated HRs tended to have higher basal extracellular glutamate than vehicle-treated HRs (HR/PCP vs. HR/VEH) ($p= 0.11$). In contrast to our hypothesis, PCP-treated LRs tended to have *lower* basal extracellular glutamate than vehicle-treated LRs (LR/PCP vs. LR/VEH) ($p= 0.10$) (Figure 7B). Furthermore, PCP-treated HRs and LRs had very similar levels of extracellular

glutamate. Individual mean extracellular glutamate values per group are illustrated in Figure 7C.

The relationship between basal extracellular glutamate and locomotor response to novelty was also assessed. Pearson's product moment correlations revealed a very weak relation between these two variables ($r_{(33)} = -0.103$; Figure 7D).

Extracellular Glutamate Following PCP Challenge

Repeated measures ANOVA was performed in order to compare the effects of activity and drug on extracellular glutamate following a PCP challenge. The time x activity x drug interaction showed a trend toward significance ($F_{(1,17)}= 3.04$, $p< 0.1$). These data suggested that extracellular glutamate from vehicle-treated HRs and LRs and PCP-treated HRs and LRs were changing between baseline and post-PCP injection sampling periods (Figure 8). There were no main effects of activity ($F_{(1,17)}= 0.25$) or drug ($F_{(1,17)}= 0.39$). However, vehicle-treated LRs exhibited a slight decrease in extracellular glutamate whereas vehicle-treated HRs showed a slight increase in extracellular glutamate following a PCP challenge. Thus, the hypothesis that vehicle-treated HRs would have greater extracellular glutamate than vehicle-treated LRs following a PCP challenge was not supported statistically, but the data were in the same direction as our predictions (Figure 8, HR/VEH vs. LR/VEH). In contrast, neither PCP-treated HRs nor LRs responded to a PCP challenge since extracellular glutamate levels were similar before and after PCP challenge. This suggested that LRs and HRs that received ten days of repeated PCP might have developed tolerance to the acute effects of PCP.

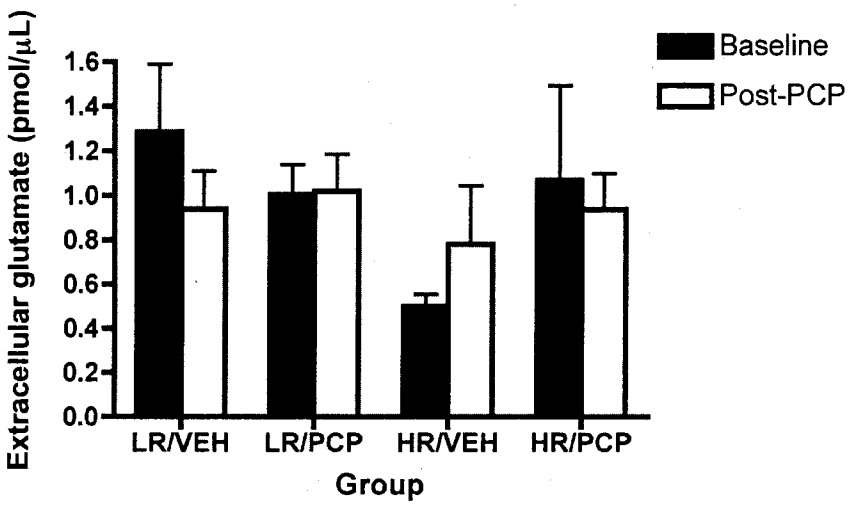


Figure 8. A PCP challenge has differential effects on extracellular glutamate in HR and LR rats. Phencyclidine (PCP) challenge dose (2.5 mg/kg) was administered to all groups in order to examine the acute glutamatergic response during *in vivo* microdialysis. Acute PCP appeared to have no significant effects in vehicle-treated LRs and HRs (LR/VEH vs. HR/VEH). However, a slight nonsignificant decrease in extracellular glutamate was observed in LRs compared to a slight nonsignificant increase in extracellular glutamate in HRs. In contrast to vehicle-treated animals, acute PCP appeared to have no effect on extracellular glutamate in groups that had previously received PCP daily for 10 consecutive days (LR/PCP and HR/PCP). Data are expressed as mean ± S.E.M. (n = 3-7).

Discussion

Baseline Extracellular Glutamate Following Repeated Vehicle and PCP Administration

We found that vehicle-treated HRs had significantly lower extracellular glutamate than vehicle-treated LRs; an effect that was in support of our predictions. A particularly interesting observation was that repeated PCP tended to increase extracellular glutamate in HRs, but tended to decrease extracellular glutamate in LRs. These data suggested that glutamate was differentially regulated in HRs and LRs in response to repeated postsynaptic NMDA receptor antagonism. This interaction of activity and drug on extracellular glutamate and glutamate immunoreactivity in the mPFC corroborated previous studies that revealed increased neurotransmitter regulation in animals characterized as high responders. Specifically, these studies have shown greater

dopamine metabolism in the rat dorsal striatum and NAc, faster firing rates of dopamine cell bodies in the rat VTA and SNc, as well as, higher extracellular glutamate in the mouse dorsal striatum of HRs compared to LRs (Marinelli & White, 2000; Piazza et al., 1991b; Shakil et al., 2005). As a whole, HRs and LRs differed in measures of cortical glutamate, and unlike previous studies performed in outbred rats, our differences must be due to environmental effects.

Moreover, the association regarding lower extracellular glutamate and greater nerve terminal glutamate immunolabeling in vehicle-treated HRs compared to PCP-treated HRs has been demonstrated in our laboratory previously in 6-OHDA lesioned rats (Meshul et al., 1999; Touchon et al., 2004). This suggested that a measure of presynaptic glutamate was able to infer the level of extracellular glutamate and that this relationship was not unique to 6-OHDA lesioned rats in which glutamate was measured in the dorsal striatum. Surprisingly, this inverse relationship was not observed between vehicle- and PCP-treated LRs. Potential alterations in mechanisms underlying presynaptic release and/or postsynaptic feedback activity may account for differences in extracellular glutamate between HRs and LRs. Again, direct measures of release mechanisms, such as quantification of quantal events (packages of neurotransmitters released in an activity-dependent manner and measured by electrophysiology) or quantification of exocytosis using FM 1-43 dye (a membrane dye that is taken up by the presynaptic terminal and released in an activity-dependent manner and measured by confocal microscopy), would be able to clarify differences between HRs and LRs with regards to presynaptic contributions to extracellular glutamate.

In the following paragraphs, we present three potential mechanisms by which PCP may increase extracellular glutamate in the mPFC of HRs (Figure 9). Differential regulation of any of these mesolimbic pathways may explain mPFC glutamatergic differences between HRs and LRs. First, systemic injection of PCP may block NMDA receptors located on GABA interneurons in the VTA. Local GABA modulation has been reported to influence overall activity of VTA dopaminergic neurons (Sesack et al., 2003). Decreased GABA activity may lead to disinhibition of dopaminergic projections from the VTA to the PFC. Spill-over from enhanced dopamine efflux stimulates excitatory presynaptic D_1 receptors stemming from the hippocampal inputs and results in increased extracellular glutamate, an effect that is consistent with previous reports (B. W. Adams & Moghaddam, 2001; Paspalas & Goldman-Rakic, 2005). Second, systemically administered PCP may block NMDA receptors on GABA interneurons in the VTA as previously mentioned, but then disinhibits local dopaminergic projections from the VTA to the NAc (Lynd-Balta & Haber, 1994). This may cause increased dopaminergic efflux in the NAc, which would result in increased GABA output from the NAc to the ventral pallidum (VP) (Groenewegen et al., 1999). The VP would then disinhibit glutamatergic dorsomedial (DM) thalamocortical projections to the mPFC and lead to increased extracellular glutamate (Pierce & Kalivas, 1997). Third, in addition to DM thalamocortical projections to the mPFC, extracellular glutamate could potentially originate from excitatory corticocortical projections (Rotaru et al., 2005; Sesack et al., 2003).

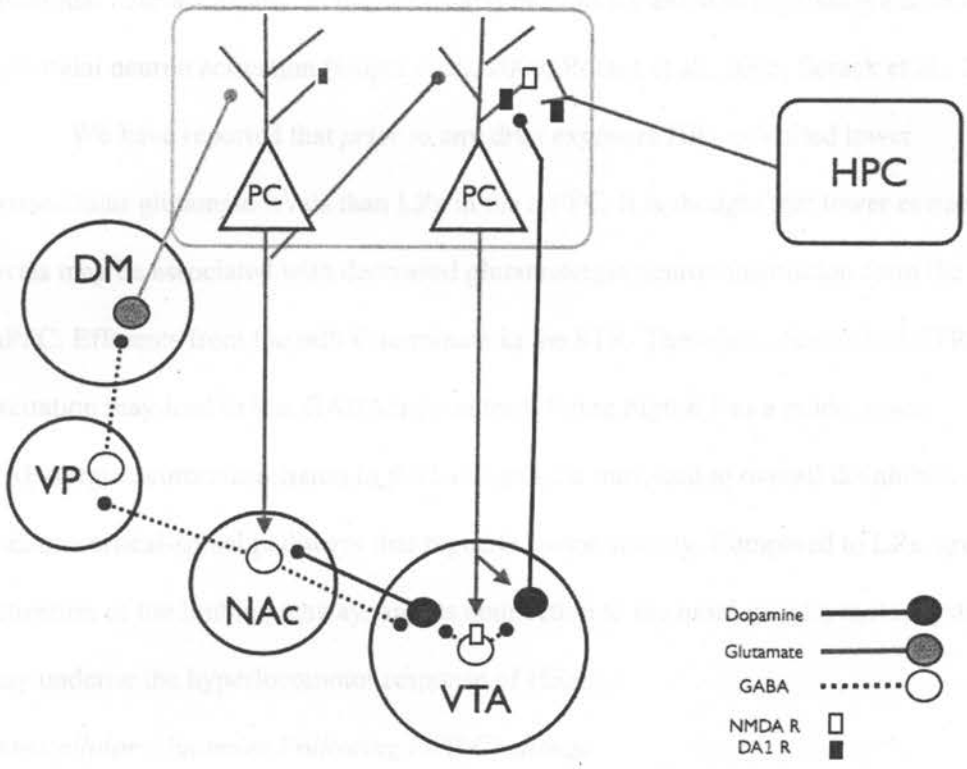


Figure 9. Anatomical connections of limbic circuitry. HRs exhibit increases in extracellular glutamate within the mPFC containing pyramidal projection neurons (PC, large gray area) following repeated systemic administration of the selective N-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP). Systemic PCP may increase extracellular glutamate in the mPFC three ways: (1) PCP blocks postsynaptic NMDA receptors located on GABAergic inhibitory interneurons in the ventral tegmental area (VTA). This causes local disinhibition of dopaminergic (DA) neurons projecting to the mPFC. DA spill-over may activate excitatory dopamine 1 receptors (D_1) located presynaptically on glutamate afferents (stemming from the hippocampus, HPC). As a result of D_1 stimulation, glutamate is released and increases in extracellular glutamate are detected by microdialysis. (2) PCP blocks NMDA receptors on GABA inhibitory interneurons in the VTA and disinhibits local DA projections from the VTA to the nucleus accumbens (NAc). This causes increased DA efflux in the NAc, which results in increased GABA output from the NAc to the ventral pallidum (VP). The VP then disinhibits glutamatergic dorsomedial thalamocortical (DM) projections to the mPFC and leads to increased extracellular glutamate. (3) Extracellular glutamate may originate from excitatory corticocortical projections. Differential regulation of any one of these pathways may account for decreased extracellular glutamate following repeated PCP in LRs. HR=high responder; mPFC=medial prefrontal cortex

Similar effects on extracellular glutamate in the mPFC were seen following administration of MK-801 and ketamine, chemical analogs of PCP (Moghaddam et al., 1997). Also within the PFC, GABA interneurons provided direct inhibitory input onto

pyramidal neurons located in the cortex and most likely aided in regulating excitatory pyramidal neuron activation (Gupta et al., 2000; Rotaru et al., 2005; Sesack et al., 2003).

We have reported that *prior* to any drug exposure HRs exhibited lower extracellular glutamate levels than LRs in the mPFC. It is thought that lower extracellular levels may be associated with decreased glutamatergic neurotransmission form the mPFC. Efferents from the mPFC terminate in the STR. Therefore, diminished STR excitation may lead to less GABAergic output. Using Figure 1 as a guide, lower GABAergic neurotransmission in the basal ganglia may lead to overall disinhibition of thalamocortical-spinal pathways that regulate motor activity. Compared to LRs, greater activation of the limbic pathway, and its connection to the basal ganglia motor system, may underlie the hyperlocomotor response of HRs.

Extracellular Glutamate Following PCP Challenge

We found that a single injection of PCP (2.5 mg/kg) increased extracellular glutamate in vehicle-treated HRs compared to baseline. This effect was consistent with previous reports showing a single injection of PCP (5 mg/kg, *i.p.*) resulted in elevated extracellular glutamate compared to vehicle in rats (B. Adams & Moghaddam, 1998; Moghaddam & Adams, 1998). Surprisingly, a single injection of PCP tended to decrease extracellular glutamate in vehicle-treated LRs. This effect appears to be the first evidence that shows acute PCP eliciting a reduction in extracellular glutamate compared to baseline.

It is unclear what may be driving variation in glutamatergic responses between HRs and LRs. However, LR/VEH had greater basal extracellular glutamate relative to HR/VEH and the addition of PCP-induced elevations of glutamate may have generated

spill-over which activated a negative feedback mechanism via stimulation of group II metabotropic glutamate autoreceptors (mGLUR2/3). Therefore, we posited that the cumulative effects of greater basal extracellular glutamate and PCP administration may explain the lower extracellular glutamate measured in LRs following acute (and repeated) PCP.

We were limited in our sensitivity to detect time-dependent changes because we only collected dialysate samples every 15 min. Therefore, we may have missed the possible initial PCP-induced rise in extracellular glutamate—a mechanism that may have occurred in a shorter time frame than 15 min—but captured the activation of the negative feedback loop, which would be marked by lower extracellular glutamate in LRs. Taking the possibility that the a negative feedback mechanism was stimulated in response to glutamate overspill, then the higher baseline extracellular glutamate plus PCP-induced glutamate efflux may explain how a single injection of PCP tended to decrease extracellular glutamate in LRs but tended to increase extracellular glutamate in HRs. Collectively, these opposing actions led to similar levels in extracellular glutamate between HRs and LRs (see Figure 8, white bars). It was also interesting to find that the effects from a single PCP injection mirrored the effects of repeated PCP administration in HRs and LRs. Overall, our findings suggested that a PCP challenge resulted in very similar levels of extracellular glutamate across groups irrespective of whether these levels occurred following an increase or decrease from baseline.

Differences in non-NMDA receptor activity may explain the lack of change in extracellular glutamate following acute PCP in repeatedly PCP-treated LRs and HRs. Moghaddam and Adams (1998) have shown that a mGLUR2/3 agonist abolished the

PCP-induced increases in extracellular glutamate as measured in rats. Therefore, repeated PCP administrations may act to briefly increase extracellular glutamate in both HRs and LRs, but over time, compensatory mechanisms such as upregulation of mGLUR2/3 activity may mask PCP-induced increases in extracellular glutamate. In order to test the contribution of mGLUR2/3 activity on extracellular glutamate in LRs and HRs, one could infuse mGLUR2/3 agonist directly through the probe and examine mPFC extracellular glutamate in HRs and LRs. If neuronal sources of glutamate contribute to the majority of extracellular glutamate, then by inhibiting presynaptic release via mGLUR2/3 activation, one should observe a marked decrease in extracellular glutamate. Intra-mPFC mGLUR2/3 agonist may lead to different degrees of decreased extracellular glutamate between LRs and HRs suggesting that non-neuronal sources may also account for changes in extracellular glutamate measured by *in vivo* microdialysis.

The source of basal extracellular glutamate measured by *in vivo* microdialysis is very controversial. Basal extracellular glutamate can come from a variety of sources including calcium-dependent neuronal vesicular pool, the calcium-independent, but neuronal, cystine/glutamate antiporter, or the non-neuronal (glial) pool (Baker et al., 2002; Timmerman & Westerink, 1997). The cystine/glutamate antiporter removes cystine located in the synaptic cleft and releases glutamate into the extracellular space (Baker et al., 2002). Therefore, increasing extracellular levels of cystine acts to increase activity of the antiporter and consequently increases extracellular glutamate; however, removing extracellular cystine has not been shown to decrease extracellular glutamate (Melendez et al., 2005). Overall, results regarding the contribution of cystine/glutamate antiporter to basal extracellular glutamate remain unclear. In experiment 2b, we sought to tease out

non-neuronal versus neuronal sources of extracellular glutamate as a means to explain the differences seen in extracellular glutamate among LRs and HRs.

Experiment 2b. To determine differences in extracellular glutamate following local administration of glutamate transport substrate inhibitor between high and low responders.

Introduction

In order to investigate the contribution of neuronal sources of glutamate to the extracellular glutamate pool of HRs and LRs, L-*trans*-pyrrolidine-2,4-dicarboxylate (L-PDC), a glial-associated glutamate transport substrate inhibitor, was infused through the microdialysis probes and applied directly to the mPFC. L-PDC was chosen based on its ability to significantly elevate striatal extracellular glutamate *in vivo* in rats and stimulate exocytotic-dependent transmitter release in rat cortical neuron-glia co-cultures, effects that were shown to be partly Ca^{2+}-dependent and TTX-sensitive (Rawls & McGinty, 1998; Volterra *et al.*, 1996). Volume application of K+ was not favored due to its nonspecific effect in stimulating multiple terminals within the region of interest and potentially eliciting unwanted feedback mechanisms. In addition, pilot studies revealed that 4 mM L-PDC elevated extracellular glutamate by 300% compared to pre-L-PDC levels following 4 mM infusion, suggesting that this concentration was effective in stimulating glutamate release.

We hypothesized that the HR/VEH group would respond more to L-PDC illustrated by an increase in extracellular glutamate compared to all other groups and that LR/VEH would also experience an increase in extracellular glutamate. This hypothesis was predicated on the results from experiment 1 in which immunogold electron microscopy revealed a higher reserve of glutamate stored presynaptically in HR/VEH mice, and thus, more neurotransmitter would be available for presynaptic release.

Furthermore, we hypothesized that the animals repeatedly administered PCP (LR/PCP and HR/PCP groups) would exhibit marked increases in extracellular glutamate, but less than that of HR/VEH and LR/VEH groups. Finally, because L-PDC blocks reuptake of glutamate by glial transporters and elevates extraneuronal levels of glutamate, we hypothesized that a considerable increase in extracellular glutamate in all groups compared to basal extracellular glutamate would be observed.

Materials and Methods

Subjects

Twenty male C57BL/6J mice were ordered from The Jackson Laboratory (Davis, CA) and arrived at 6 weeks of age. Animals were 8-10 weeks of age at time of behavioral testing. Animal housing conditions were the same for experiment 1 and 2a and are detailed in experiment 1 Materials and Methods (p 26).

Apparatus

See experiment 1 (p 27).

Locomotor Activity in a Novel Environment

See experiment 1 (p 28).

Drugs

PCP was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in physiological saline for injection (0.9% NaCl). HRs and LRs were randomly assigned to receive PCP (2.5 mg/kg) or vehicle (0.9% saline) once a day for 10 consecutive days (1:00-3:00) with 24 h between each injection. All injections were intraperitoneal. The breakdown of treatment is as follows: LR/VEH (n = 4), LR/PCP (n = 4), HR/VEH (n = 3), HR/PCP (n = 3). L-PDC was purchased from TOCRIS (Ellisville, MO) and dissolved

in aCSF (pH 7.4). L-PDC (4 mM) was made ahead of time and stored at -20°C until use. On the day of dialysis, L-PDC was thawed at room temperature and syringes used for dialysis were filled with the 4mM L-PDC solution. Only animals with correctly placed probes were included in the statistical analyses of microdialysis data.

In vivo *microdialysis*

Methods in cannulae surgery, PCP administration, and probe placement verification, and HPLC analyses in experiment 2b were identical to experiment 2a. However, there were some specific changes made to the protocol. First, unlike experiment 2a, probes were inserted the *same day* as dialysate collection. We began inserting the probes the same morning of microdialysis because it increased survival rate and decreased gliosis and tissue damage surrounding the area of the probe.

In the morning, animals were briefly anesthetized for 5-10 min with isoflurane while probes were carefully inserted through the guide cannulae. The probes were affixed using epoxy and allowed to dry (~3 min) before isoflurane was stopped. Animals were then placed into the microdialysis cages and allowed to recover for 3 h before dialysate collection. Pilot studies revealed no significant differences in extracellular glutamate between animals that had the probe inserted the night before and animals that had the probes inserted the same day of microdialysis.

Following baseline sample collection (0-60 min), syringes containing aCSF were detached from the microdialysis tubing and syringes containing L-PDC + aCSF (4 mM) were reattached within 3 min. L-PDC was perfused through the microdialysis probe at a rate of 2.0 μL/min. Samples were collected every 15 min for the next 2 h.

In vitro probe recoveries were performed for all probes. The percent probe recovery was consistently 4%; therefore, any differences in extracellular glutamate detected between groups were not due to varying probe kinetics.

Data Analysis

Repeated measures ANOVA was used to examine the effect of drug and activity across time following intra-mPFC perfusion of L-PDC in vehicle- and PCP-treated HRs and LRs. Significance level was set at $\alpha = 0.05$ for all tests.

Results

Behavioral Testing

The median HACT for all animals was 10161 activity counts. The mean HACT for HRs was 11559 ± 328 activity counts and the mean HACT for LRs was 9571 ± 353 activity counts. The HRs had significantly greater activity counts than LRs ($p< 0.001$) (Figure 10A).

Extracellular Glutamate Following L-PDC Challenge

Repeated measures ANOVA revealed a main effect of time ($F_{(5,50)} = 4.08$, $p= 0.004$) suggesting that extracellular glutamate was changing over time following direct application of L-PDC (Figure 10B). However, there was only a trend toward a time x activity interaction ($F_{(5,50)} = 1.87$, $p= 0.12$) and time x drug interaction ($F_{(5,50)} = 1.59$, $p= 0.18$). This suggested that HRs had greater extracellular glutamate than the LRs across time and that vehicle-treated animals had greater extracellular glutamate than PCP-treated animals across time. Examining differences at single time points, within-subject contrasts revealed a significant main effect of time ($F_{(1,10)} = 9.55$, $p= 0.01$) at 135 min. This

suggested that the greatest difference in extracellular glutamate between groups occurred within 1 h after L-PDC perfusion was completed (Figure 10B).

Examining peak extracellular glutamate differences between groups, there was a trend toward a main effect of activity ($F_{(1,10)} = 2.28$, $p = 0.16$) and drug ($F_{(1,10)} = 3.26$, $p = 0.10$), but not activity x drug interaction ($F_{(1,10)} = 0.25$, $p = 0.63$) at 135 min. This suggested that HRs as a whole tended to have higher peak extracellular glutamate (2.15±0.37 pmol/μL) than LRs (1.41±0.32 pmol/μL) and PCP-treated animals as a whole tended to have lower peak extracellular glutamate (1.34±0.35 pmol/μL) than vehicle-treated animals (2.22±0.35 pmol/μL). Figure 10C illustrates mean post-PDC extracellular glutamate as compared to mean baseline values. Overall, these results supported our hypothesis in that HR/VEH group experienced the greatest peak in extracellular glutamate and that vehicle-treated groups exhibited higher extracellular glutamate than PCP-treated groups following perfusion of the glial transport substrate inhibitor, L-PDC.

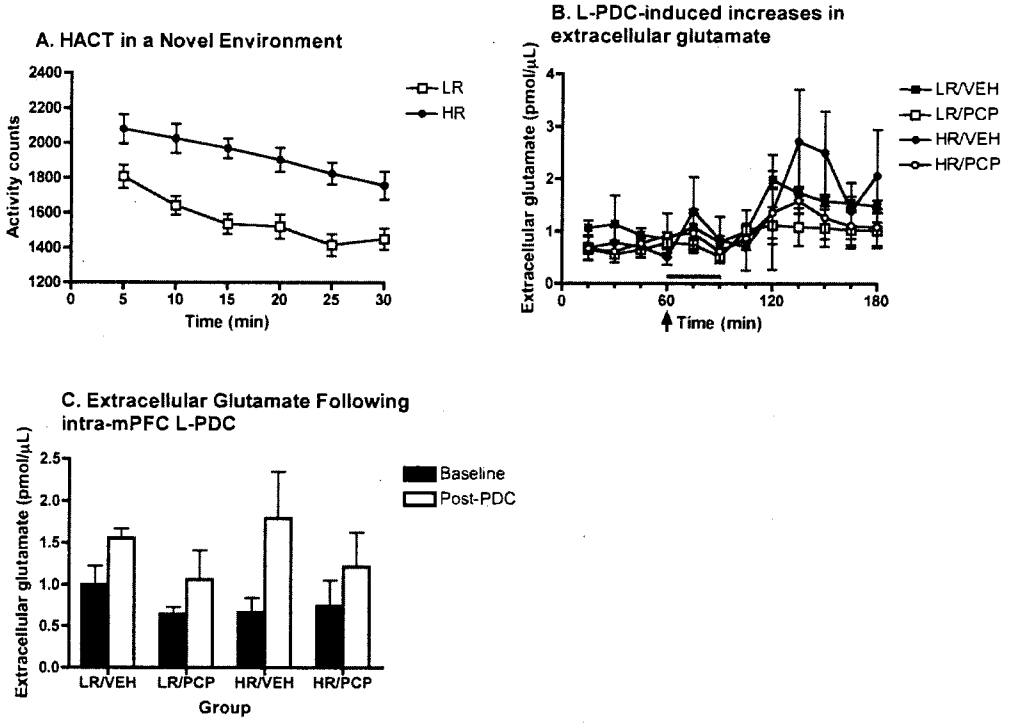


Figure 10. Intra-mPFC L-PDC leads to increased extracellular glutamate in HR and LR mice. A. Horizontal locomotor activity (HACT) in a novel environment (Accuscan™ activity monitors) was measured in 20 male C57BL/6J mice for 30 min. Mice with a locomotor response above the median activity count were termed high responders (HR) and mice with a locomotor response below were termed low responders (LR). HRs had significantly higher activity counts than LRs ($p < 0.001$). Values represent mean ± S.E.M. B. The glial glutamate transporter substrate inhibitor, L-*trans*-pyrrolidine-2,4-dicarboxylate (L-PDC) (4 mM), was perfused through the microdialysis probe after baseline collection at 60 min (arrowhead, gray bar). Vehicle-treated HRs appeared to respond the most to L-PDC evidence by the highest extracellular glutamate at 135 min, approximately 30 min after perfusion of L-PDC was complete. C. Intra-mPFC L-PDC appeared to extracellular glutamate across all treatment groups compared to baseline. Values are expressed as mean ± S.E.M. (n = 3-4 for all groups).

Discussion

L-PDC appeared to increase extracellular glutamate in all groups, with the HR/VEH experiencing the greatest effect (approximately 170% increase from baseline) and the LR/VEH experiencing the smallest effect (approximately 55% increase from baseline). This effect was in the direction of our hypothesis and also corroborated results from previous studies that showed L-PDC-induced increases in extracellular glutamate *in*

vivo and *in vitro* (Rawls & McGinty, 1998; Volterra et al., 1996). Interestingly, LR/PCP and HR/PCP groups appeared to respond similarly to glutamate reuptake blockade, with both groups exhibiting approximately 63% increase in extracellular glutamate. This suggested that repeated PCP may have masked the effects of L-PDC by possibly affecting glutamate transport activity. The interactions between L-PDC and PCP are currently unknown. However, it is possible that the combined effects of local L-PDC administration and systemic PCP—both of which have been shown to increase extracellular glutamate *in vivo* and *in vitro* (Moghaddam & Adams, 1998; Volterra et al., 1996)—actually counteracted each other. Consequently, with concomitant administration of PCP and L-PDC no additional effect of L-PDC on extracellular glutamate would be observed in LR/PCP and HR/PCP animals. The dual effects of PCP and L-PDC provided additional evidence of glutamatergic interactions within HRs and LRs.

CHAPTER IV: STRESS

Experiment 3. To determine the role of stress in differentiating high and low responding mice.

Introduction

The acute stress study was performed in order to determine whether differences in stress reactivity between HRs and LRs before the animals were exposed to a novel environment explained differences in locomotor activity in that novel environment, such that increased stress response was related to increased activity induced by a presumably stressful novel environment.

It was thought that HRs and LRs may have experienced some forms of prenatal or postnatal stress which ultimately led to varying hypothalamic-pituitary-adrenal (HPA) axis activity. Prenatal stress has been associated with increased activity during an open field test (Fride et al., 1986). Specifically, pregnant Sabra dams were exposed to unpredictable flashing lights and noise during each week of gestation. At 6 months of age, the male offspring of the stressed dams were tested for open field activity by measuring the number of lines crossed in a 1 x 1 m black wooden box. Compared to unstressed controls, prenatally stressed rats exhibited prolonged heightened activity in the open field following 1, 2, 4 or 8 consecutive days of 10-min testing sessions (Fride et al., 1986). This suggested that prenatally stressed rats did not habituate to the open field even after 8 days of repeated exposure and that this effect may be explained by over-activation of the HPA axis.

To investigate HPA axis activity, plasma corticosterone (CORT) was measured in prenatally stressed and unstressed rats. Elevated CORT in prenatally stressed rats

compared to unstressed controls was found after 1, 2, 4 and 8 days of open field testing (Fride et al., 1986). It is important to note that pregnant dams were singly housed during the unpredictable stress period. This may have confounded the results because social isolation has been reported to increase CORT in female rats (Belz et al., 2003). In turn, this added stress may have contributed to generally higher CORT in the dams and subsequently the offspring. This implied that the observed increase in CORT may not have been solely due to unpredictable noise and light stress, but to an interaction of two distinct stressors. Overall, the results suggested that prenatal stress resulted in higher CORT levels and this elevation in steroidal hormones may have facilitated activity in an open field.

Vallèe et al. (1997) examined the effects of prenatal and postnatal stress on activity in an open field. Pregnant Sprague-Dawley dams were subjected to 30 min restraint period during the last week of gestation. At 4 months of age, the male offspring of this group were subjected to 30 min of restraint stress. CORT was measured at 30 and 120 min following restraint. The authors found that prenatally stressed rats showed prolonged stress-induced increases in CORT compared to control and postnatally-handled rats with a significant difference found at 120 min. Furthermore, prenatally stressed rats also exhibited the greatest activity (measured in distance traveled in meters) in an open field (1 x 1 m white wooden box) during a 15-min test session. Finally, Piazza et al. (1991) found that HRs had significantly higher CORT than LRs at 120 min after a 2-h exposure to a novel environment. Taken together, we hypothesized that these differences in stress reactivity may account for the initial separation of HR and LR during behavioral testing in a novel environment.

Based on these studies, we hypothesized that HRs would have a greater stress response in the novel environment compared to LRs. Furthermore, it was hypothesized that the stress response following exposure to the novel environment (the activity test) would be positively correlated with the stress response following a 20-min restraint stress. Stress response of HRs and LRs was determined by measuring plasma corticosterone at 0, 20, 40, and 120 min.

Materials and Methods

Subjects

Twenty male C57BL/6J mice were ordered from The Jackson Laboratory (Davis, CA) and arrived at 6 weeks of age. Animals were 8-10 weeks of age at time of behavioral testing. Animal housing conditions were the same for experiment 1 and 2 and are detailed in experiment 1 Materials and Methods (p 26).

Apparatus

See experiment 1 (p 27).

Behavior

Locomotor activity in a novel environment

See experiment 1 (p 28).

Stress response following exposure to a novel environment

CORT was assessed immediately following 30 min of activity in the novel environment (Accuscan™ activity monitors). Testing occurred from 8:00-16:00 h. Testing occurred 48 h prior to the weekly scheduled cage changes. Testing was staggered by 5 min such that four animals were in the activity monitors at any given time. Activity monitors were cleaned with 10% isopropynol between individual runs. All blood samples

were taken under a ventilated hood located in the corner of the testing room. Animals were individually housed during tail blood sampling. Tail blood samples were taken by placing the animal in a clear cylindrical Plexiglas restraint device (9.5 cm length x 2.5 cm diameter) and secured with a rubber stopper. Blood samples (20 μL) were collected in heparized Micohematocrit capillary tubes (Scientific Products, McGraw, IL). Sampling lasted less than 3 min so as not to collect samples stimulated by handling (Hennessy et al., 1979; Hennessy & Levine, 1978). The first tail blood sample taken was termed "0 min." Additional tail blood samples were collected at 20, 40 and 120 min in order to capture the rise and fall of CORT levels. The hematocrit tubes were stored on ice until completion of the study and then centrifuged at 1000 x g for 15 min at 4°C to separate plasma from red blood cells. Plasma was removed and stored at -20°C until assayed.

Restraint stress

Seven days after the activity test, the stress response in the same set of animals was assessed following a 20 min restraint period. Testing occurred from 8:00-16:00 h. Animals were placed in a clear Plexiglas cylindrical restraint device (9.5 cm length x 2.5 cm diameter) secured with a rubber stopper for 20 min. These restraint devices were the same device used for taking blood samples following the activity test. Immediately following removal from the restraint device, animals were placed in a different, but similarly constructed restraint device. Tail blood samples were taken as detailed above. The devices were cleaned with 10% isopropynol between individual runs. The hematocrit tubes were stored on ice until completion of the study and then centrifuged at 1000 x g for 15 min at 4°C to separate plasma from red blood cells. Plasma was removed and stored at -20°C until assayed.

Plasma Corticosterone Radioimmunoassay

Samples from the activity and restraint test were analyzed simultaneously in order to reduce variation. Samples were diluted 1:200 with steroid diluent by taking 5 μL of sample to 1.0 mL and stored at -20°C until assayed. Plasma corticosterone was detected using ImmuChem™ Corticosterone [^{125}I] radioimmunoassay kit from MP Biomedicals (formerly ICN Biomedicals, Orangeburg, NY). Unknown samples were interpolated from least-squares regression based on log transformation of the standard curve (goodness of fit, $R^2 = 0.9976$). Unknown samples were run in singles and standard samples were run in doubles. The minimum detectable limit of the assay was 7.7 ng/mL. The specificity of the assay was high with only 0.34% cross-reactivity to desoxycorticosterone, 0.1% cross-reactivity to testosterone, 0.02% cross-reactivity to progesterone and less than 0.02% cross-reactivity to other endogenous steroids.

Data Analysis

Repeated measures ANOVA was performed on individual means in order to determine the effects of activity (high and low) on CORT following 30 min activity test. A separate repeated measures ANOVA was run on CORT following 20 min restraint test. For both ANOVAs, the within-subjects factor was time (0, 20, 40 and 120 min) and between-subjects factor was activity (HR vs. LR). Pearson's correlation was calculated on CORT following exposure to the activity chamber and acute restraint device. Significance was set at $\alpha = 0.05$ for all tests.

Results

Behavioral Testing

The median HACT for all animals was 10024 activity counts. The mean HACT for HRs was 11599 ± 327 activity counts and the mean HACT for LRs was 8801 ± 202 activity counts. The HRs had significantly higher activity counts than the LRs ($p= 0.0001$) (Figure 11A).

Stress reactivity following exposure to a novel environment

Examining stress reactivity of HRs and LRs to the automated activity chamber, results found a significant main effect of time ($F_{(3,51)} = 10.6$, $p< 0.001$), but not time x activity interaction ($F_{(3,51)} = 0.98$), suggesting that CORT from HR and LR groups are changing similarly across the 120 min sampling period (Figure 11B). Between-subject effects did not reveal a significant main effect of activity ($F_{(1,17)} = 0.16$) suggesting that HRs and LRs had similar CORT levels, and thus similar stress reactivity, following 30 min exposure to the automated activity chambers.

Discriminating the stress response of HRs and LRs to a 20 min restraint test, results indicated a significant main effect of time ($F_{(3,54)} = 12.76$, $p< 0.001$), but not time x activity interaction ($F_{(3,54)} = 0.35$) (Figure 11B). This suggested that a 20 min restraint test altered CORT levels over time and that HRs and LRs reacted similarly to an acute restraint period. Finally, there was no main effect of activity between groups ($F_{(1,18)} = 0.05$). Overall, CORT following a 30 min activity test (23.06 ± 1.12 μg/dL) was greater than CORT following a 20 min restraint (20.46 ± 1.02 μg/dL) when HR and LR groups were combined.

Because there was a significant main effect of time following both the activity and restraint tests and because within-group contrasts revealed a significant difference at

20 min ($F_{(1,18)} = 52.22$, $p < 0.001$), suggested that this time point elicited the highest CORT levels compared to 0, 40 and 120 min time points.

Individual mean CORT following exposure to novel environment and restraint stress are shown in Figure 11C. Overall, the HRs and LRs responded to the activity and restraint stress similarly and there was a moderate relation between stress response following activity and restraint tests (Pearson's correlation coefficient, $r_{(18)} = 0.41$, $p = 0.07$) (Figure 11D).

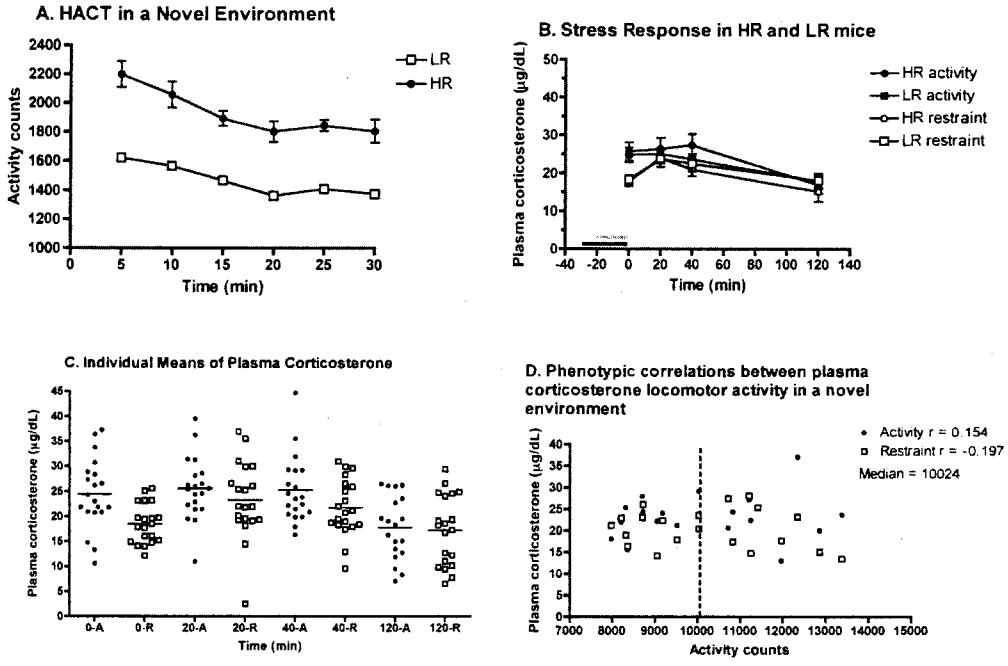


Figure 11. HR and LR mice have similar patterns of stress reactivity. A. Horizontal locomotor activity (HACT) in a novel environment (Accuscan™ activity monitors) was measured in 20 male C57BL/6J mice for 30 min. Mice with a locomotor response above the median activity count were termed high responders (HR) and mice with a locomotor response below the median were termed low responders (LR). HRs had significantly higher activity counts than LRs ($p < 0.001$). Values represent mean ± S.E.M. B. Plasma corticosterone (CORT) levels of HRs and LRs were assessed at 0, 20, 40, and 60 min following a 30 min activity test (black line) and a 20 min restraint test (gray line) in the same set of animals. CORT levels were

higher across activity groups following 30 min activity test than 20 min restraint test. Overall, HRs and LRs had similar stress responses following activity and restraint tests. When HRs and LRs were combined, the highest CORT level following activity and acute restraint was observed at 20 min when compared to 0 min (immediately upon removal from the apparatus), 40 min and 120 min. Values represent mean ± S.E.M. C. Individual mean CORT levels within each activity groups are illustrated for each time point. Closed circles (●) indicate individual CORT levels at each time point (0-A, 20-A, etc.) following 30 min activity test and open squares (□) indicate individual CORT levels at each time point (0-R, 20-R, etc.) following 20 min restraint test. The black horizontal line designates the mean CORT value. Notice the slightly lower CORT levels following the restraint test, suggesting animals are less stressed after 20 min of confinement. D. There is no relation between mean CORT after 30 min activity test (●) and locomotor activity in a novel environment (Pearson's correlation coefficient, $r_{(18)} = 0.154$) or CORT after 20 min restraint test (□) and locomotor activity in a novel environment (Pearson's correlation coefficient, $r_{(18)} = -0.197$). Activity and restraint tests were performed in the same animals. Each point represents mean CORT for each individual.

Discussion

The mechanisms producing HR and LR have not been fully elucidated. In the current study, differences in the amount of stress experienced by each animal may account for the segregation of HRs and LRs. It is possible that some of the mice experienced greater stress, either parentally or postnatally, than others, which may have facilitated locomotion in a novel environment and subsequent separation into high and low activity groups. However, because mice were obtained directly from The Jackson Laboratory at 6 weeks of age, it is impossible to determine whether the animals experienced any forms of stress prior to their arrival (e.g., social hierarchy stress, cage disturbances, and stress during transportation from Davis, CA to Portland, OR). Furthermore, we were unable to make comparisons between littermates because related individuals were not known.

Previous evidence has shown that HRs have greater ACTH and CORT levels following exposure to a novel environment that was deemed as a "stressor" (Marquez et al., 2006) and that this may have promoted self-administration of psychostimulants (Billingslea et al., 2003; Marinelli et al., 1997; Piazza et al., 1991a). Moreover, previous reports have drawn an association between increased stress and increased novelty-

induced locomotor activity (Deminiere et al., 1992; Piazza & Le Moal, 1996; Piazza et al., 1991a). Therefore, it was hypothesized that exposure to a novel environment would stimulate HPA response more in some animals than others and that this difference in stress reactivity could be measured via horizontal locomotion. In the study by Marquez et al. (2006), HRs and LRs did not have significantly different stress hormone levels when assessed after classification of HRs and LRs using a circular corridor, but *prior* to a hole-board or light-dark test (i.e., the "stressor"). Likewise, stress hormone data was collected only after the animals had been divided into HRs and LRs. This suggested that perhaps differences in stress reactivity existed before the locomotor test.

In experiment 3, we found that exposure to a novel environment resulted in moderately stressed animals as indicated by peak plasma CORT (at 20 min, 26.16 ± 2.57 vs. 25.04 ± 1.23 μg/dL, HRs and LRs, respectively). Our observation that HRs had similar CORT responses to LRs following a stressful experience was inconsistent with previous results (Deminiere et al., 1992; Jones et al., 1992; Piazza et al., 1991a). The stress response following two different stressors was also examined in order to see if the HRs and LRs exhibited similar patterns of stress reactivity after novelty exposure and after an acute stressor known to elicit marked increases in CORT (Moghaddam et al., 1994). CORT levels following the activity and acute restraint tests were not correlated. This result was consistent with previous studies using inbred rats, in which no association was found between CORT and acquisition of self-administration of drugs (Dhabhar et al., 1993; George & Goldberg, 1989). However, it is important to note that the same restraint devices were used to take tail vein samples (a total of four times for each animal) and used to immobilize the animals during the 20-min restraint stress. Therefore, it was

possible that the animals became habituated to the restraint device prior to the immobilization test. This may explain the lowered CORT response to an immobilization test compared to physiological response to novelty, even though immobilization is considered a more potent stressor than novelty (Moghaddam, 1993; Moghaddam et al., 1994).

In addition, CORT secretions emit circadian rhythms in accordance with the diurnal cycle, in which the peak occurs near the onset of the dark period in rats (Allen-Rowlands et al., 1980; Girotti et al., 2007). In the current study CORT testing occurred from 8-5PM, ending close to the onset of dark cycle. Therefore, it is possible that CORT samples in the latter group of animals tested may reflect natural fluctuations in CORT secretions. Student's independent t-test revealed that the first 10 animals tested had significantly lower CORT than the second 10 animals tested ($t_{(18)} = -2.96$, $p= 0.008$), although significantly elevated CORT in the latter group of animals was only observed following the acute restraint test and not the activity test ($p= 0.47$). Our evidence of elevated CORT secretions would be in agreement with previous reports showing that HPA activity is upregulated near the onset of the dark period (Allen-Rowlands et al., 1980; Girotti et al., 2007).

It was speculated that HPA axis activity only partially explains behavioral differences since HRs and LRs reacted similarly. A different mechanism, such as anxiety, may be aiding the expression of novelty-induced activity because novel stimuli had the propensity to evoke both exploratory and fear states of behavior (Pellow et al., 1985). In order to test whether anxiety reflects HR and LR phenotypes, this behavior should change in the presence of anxiolytic or anxiogenic drugs. It is possible that HRs are, in fact, less

anxious than LRs and therefore exhibit decreased freezing behavior, which facilitated more exploratory forms of behavior in a novel environment (and ultimately leads to greater activity counts).

CHAPTER V: GENERAL DISCUSSION

In this thesis, we aimed to elucidate the environmental correlations between locomotor activity in a novel environment and nerve terminal glutamate immunolabeling, locomotor activity and extracellular glutamate, and locomotor activity and plasma corticosterone. The first two experiments were conducted in order to further characterize the locomotor behavior of HRs and LRs and expand upon previously published literature by investigating glutamate in the mPFC, a structure that, to our knowledge, has not been investigated. The third experiment was conducted in order to test whether HRs and LRs differed in their stress response following exposure to a potentially stress-inducing novel environment. It was suggested that *a priori* differences in stress reactivity contributed to individual differences in locomotor activity in a novel environment. Most importantly, all three experiments used male mice that had identical genetic makeup. Therefore, any differences observed between high and low activity groups must have been due to environmental factors and not due to allelic differences.

A previous study performed in drug-naïve adult male C57BL/6J mice (the same age and strain of mice used in the current study) subsequently divided into HRs and LRs based upon the number of line crossing in a shoe box-cage novel environment, found a 25% difference in the dorsal STR density of nerve terminal glutamate immunolabeling between HRs and LRs with HRs tending to have a higher density ($p= 0.25$) (Shakil et al., 2005). Moreover, HRs were found to have a significantly lower extracellular glutamate in the dorsal STR than LRs ($p= 0.0001$) (Shakil et al., 2005). The presence of this inverse relationship between the two indices of glutamate regulation is in accordance with the

results from the current study and with previous findings (Meshul et al., 1999; Touchon et al., 2004).

To illustrate, the respective results from experiment 1 and 2 found lower glutamate immunoreactivity associated with the presynaptic terminals in the mPFC and significantly greater extracellular glutamate in drug-naïve LRs relative to HRs. Because lower nerve terminal glutamate immunolabeling has been associated with greater extracellular glutamate, enhanced glutamatergic transmission from the mPFC to the STR may explain higher extracellular glutamate detected within this region by Shakil and colleagues (2005). Collectively, these results suggested that the glutamatergic projections from the mPFC to the dorsal striatum may be important in mediating high or low motor output in a novel environment through the basal ganglia (see Figure 1 and 2). However, due to the complexity of basal ganglia circuitry, it is unlikely that glutamate was acting alone to dictate behavior, but in fact was acting in concert with another neurotransmitter system, GABA.

Along with glutamate, GABA is a major neurotransmitter involved in regulating activation of basal ganglia components. Competition between glutamatergic and GABAergic inputs is likely to have occurred in the GPe where the excitatory efferents from the STN and inhibitory efferents from the STR converge in the GPe (see Figure 2). For instance, if we observed increased extracellular glutamate in the mPFC in LRs and this resulted in increased glutamate transmission to the dorsal STR (an effect observed by Shakil and colleagues(2005)), then increased activation of the STR would inhibit the EPN and GPe. At this point, downstream locomotor behavior may largely depend on the degree of inhibition of the VM/VL thalamus, which contains GABA afferents directly

from the EPN and indirectly from the GPe via the SNr. Thus, converging GABA and glutamate projections control VM/VL thalamus activation. In the case of LRs, greater net inhibition of the VM/VL thalamus may explain lower locomotor activity in a novel environment. Conversely, for HRs less net inhibition (that is, more excitation) of the VM/VL thalamus may explain higher locomotor activity in a novel environment. The ramifications of GABA and glutamate inputs to the GP and VM/VL thalamus are detailed in the following paragraphs.

First, increased dorsal STR GABA transmission to the EPN would inhibit this structure and lead to disinhibition of the VM/VL thalamus and ultimately greater locomotor activity. However, inhibition of the GPe may also disinhibit the STN less, which, in turn, sends reciprocal glutamatergic projections to the GPe, but also has projections to the EPN (not shown in Figure 2). Collectively, the product of increased dorsal STR activity may lead to inhibition of EPN and excitation of the GPe via GABAergic dorsal STR afferents and glutamatergic STN afferents, respectively (Albin et al., 1989). Therefore, the degree of motor thalamic inhibition (and onward to the motor cortex) may largely depend on the strength of opposing forces between inhibition and activation of striopallidal connections (Pierce & Kalivas, 1997).

Second, an additional effect of STN disinhibition may be increased glutamate output to the SNr. The SNr sends GABAergic projections to the VM/VL thalamus (Gerfen, 1992). This critical input forms the second site where glutamate (from the SNr) and GABA (from the GP) exert opposing influencing. The outcome of competing inhibitory and excitatory pathways essentially decides the strength of the glutamatergic output from the VM/VL thalamus to the motor cortex and ultimately helps regulate

locomotor behavior. Thus, the role of GABA in mediating locomotor behavior, particularly at the level of the VM/VL thalamus, of HRs and LRs warrants further investigation.

The preceding paragraphs described potential mechanisms that may help explain locomotor activity in a novel environment of drug-naïve animals; the following paragraphs will examine the consequences of repeated glutamate receptor antagonism. In experiment 2a, the postsynaptic NMDA receptor antagonist PCP was administered randomly to half of the HRs and LRs to challenge the glutamate system. Interestingly, repeated PCP led to a slight increase in extracellular glutamate in HRs, but a slight decrease in LRs. Moreover, extracellular glutamate of PCP-treated HRs and LRs were not significantly different, suggesting that these animals developed tolerance to repeated antagonism of NMDA receptors so that no changes in extracellular glutamate from baseline were observed. In experiment 2b, local administration of the glial transport substrate inhibitor L-PDC resulted in a greater increase in extracellular glutamate among vehicle-treated HRs than vehicle-treated LRs. PCP-treated HRs and LRs did not differ in extracellular glutamate suggesting that PCP masked the effects of L-PDC, an observation that was in opposition to our predictions. Taking into account the converging glutamate and GABA inputs on several structures comprising the basal ganglia (including mPFC as part of extended basal ganglia), the results from experiment 2a and 2b illustrate further the importance of glutamate/GABA balance within basal ganglia circuitry and how this relationship corresponds to the expression of high or low activity phenotypes.

A limitation in the current study was not assessing changes in locomotor activity following repeated PCP and intra-mPFC L-PDC. The direction in change of locomotor

activity could have provided an indication of the shift within the glutamate/GABA balance. For example, repeated PCP may have augmented locomotor activity in LRs (possibly to the level of HRs) suggesting that the motor cortex received greater glutamatergic input from the VM/VL thalamus. L-PDC may also have lead to a similar shift toward greater excitation in the basal ganglia of LRs, which would make sense since both PCP and L-PDC have been shown to increase levels of extracellular glutamate (Abekawa et al., 2006; Rawls & McGinty, 1998). Based on these conclusions from the current study, future studies should investigate the effects of repeated PCP and L-PDC on locomotion in HRs and LRs. Proposed hypotheses may be that repeated PCP and L-PDC would augment locomotion in LRs more than HRs. This hypothesis is predicated on data that indicate lower baseline activity in LRs suggesting lower net inhibition of basal ganglia circuitry. It is also possible that further excitation in the HRs might hit a ceiling effect and therefore no additional increase in activity would be observed.

Finally, the role of GABA in mediating locomotor activity should be investigated within the dorsal STR, GP and VM/VL thalamus of HRs and LRs. It is hypothesized that LRs may have greater extracellular GABA in the GP but less GABA in the STN (although one cannot discount ventropallidal inputs to the STN) than HRs and this may be why LRs exhibit less locomotor activity in a novel environment.

Lastly, the results from experiment 3 suggested that differences in stress reactivity as assessed by CORT between HRs and LRs did not facilitate the separation of HRs and LRs in a novel environment. It is possible that anxiety may underlie the locomotor activity in an unfamiliar environment. In a pilot study conducted in our laboratory, twenty drug-naïve C57BL/6J male mice, which had been previously characterized as HRs

and LRs following a 30 min activity test in Accuscan™ monitors, were tested on the elevated plus maze (EPM) seven days after the activity test. On the morning of testing, animals were transported to an adjacent testing room and allowed to habituate for 1 h prior to testing. Testing occurred from 10:00-14:00 h. The maze comprised of two open (0.5 cm lip) and two enclosed (15.5 cm lip) horizontal perpendicular arms extending from a central platform (5 × 5 cm) elevated 50 cm above the floor (Finn et al., 2000). Animals were tested for a single 5-min run on the EPM (central area lux was 850; overhead fluorescent lights were on). HRs had significantly greater number of open arm entries than LRs (3.4 ± 0.67 vs. 1.7 ± 0.33 entries, respectively, p= 0.04) (unpublished findings). HRs and LRs did not differ in the number of closed arm entries (8.1 ± 1.12 vs 6.8 ± 0.69 entries, respectively, p= 0.41) suggesting that this effect was specific to open arm entries was not due to a general increase in activity. The HRs ranged from 7 to 15 closed arm entries whereas the LRs ranged from 2 to 6 closed arm entries also suggesting that these inbred mice, particularly the HRs, exhibited greater variation in horizontal locomotor activity than LRs when measured in a separate novel environment. Overall, the difference in total open arm entries between HRs and LRs suggested that anxiety-like behavior may underlie locomotor activity in a novel environment.

It has been shown that prenatally stressed rats spent significantly less time in the open arms of the wooden EPM (open arms 50 x 10 cm, closed arms 40 cm high walls, positioned 1 m above the ground; 480 lux in central area) compared to unstressed controls (Vallee et al., 1997). Because prenatal stress has been linked to increased plasma corticosterone, and increased plasma corticosterone has been linked to elevated locomotor activity, it is possible that HRs and LRs may differ in open arm entries.

Therefore, decreased anxiety-like behavior indicated by greater open arm entries (or greater percent time spent in open arms) may be correlated phenotypically with locomotor behavior in a novel environment. These results further point to a role of GABA in mediating locomotor activity differences between HRs and LRs. A future study could test the anxiogenic effects of an allosteric $GABA_A$ receptor antagonist, such as bicuculline, on the number of open arm entries in order to more accurately measure the contribution of GABA receptor activity to the behavioral phenotypes of HRs and LRs. If GABA neurotransmission is playing a larger role in modulating locomotor activity in a novel environment than anticipated, bicuculline would be predicted to decrease the number of open arm entries in HRs.

The same mice tested in the EPM were also exposed to a modified open field apparatus seven days following elevated plus maze. This experiment was performed primarily to test whether HRs and LRs in one novel environment (e.g., Accuscan™ activity monitors) retained their HR and LR phenotypes in a second novel environment (e.g., open field). Animals were transported in their home cages to an adjacent room (the same room that was used for EPM) and allowed to habituate for 1 h prior to testing. Under red light conditions, animals were placed in the center of the open field apparatus and activity was recorded for 15 min. The number of line crossings in the periphery of the open field was later hand-scored. The number of total line crossings during a 15-min test in the open field did not differ between HRs and LRs (395 ± 42 vs. 336 ± 18 line crossings, respectively, $p= 0.21$), nor did the number of total line crossings positively correlate with the total number of closed arm entries in the EPM ($r_P= 0.27$, $p= 0.24$) (unpublished findings). Closed arm entries have been used as a measure of locomotor

activity in the EPM (Pellow et al., 1985). These data implied that HRs and LRs do not exhibit high and low activity levels in different environments. In other words, the high and low activity phenotypes were not a conserved behavior. An individual's locomotor activity in one environment was not the same as its activity in a second environment (i.e., these were state-specific behaviors). Specific attributes of the context (i.e., size, shape, lighting, background noise) in which the testing occurred may have increased or decreased locomotor behavior of HRs and LRs depending on how aversive or anxiety-inducing the environment may have been perceived.

Furthermore, we were unable to investigate *in utero* environmental effects such as intrauterine position, which can subject pups arrayed linearly in two horns of the uterus to varying assortments of sex hormones. For instance, a male pup neighbored by two female pups may encounter greater levels of estrogen within the gestational sac than a male pup surrounded by one female and one male. Male fetuses who were positioned between two females *in utero* have been shown to exhibit more feminization, whereas the reverse was true for female fetuses surrounded by two males or even a male fetus surrounded by two males (Ryan & Vandenbergh, 2002). In the former case, increased masculinization has been linked to heightened aggressive behavior and dominance (Ryan & Vandenbergh, 2002). Ryan and Vandenbergh (2006) also reported that dominant males exhibited greater locomotor activity and higher number of open arm entries in the elevated plus maze, two behaviors which we observed in HRs. Therefore, intrauterine position between two male fetuses may ultimately lead to the development of HRs.

Conclusions

Overall, the results from this thesis left many doors open for exploration. There are several questions left unanswered as to why HRs and LRs exhibit different nerve terminal glutamate immunolabeling and different extracellular glutamate in the mPFC and how these differences relate to locomotor activity in a novel environment. Although significant differences were found between HRs and LRs regarding nerve terminal glutamate immunolabeling and extracellular glutamate in the mPFC, the lack of meaningful correlation between these measures and locomotor activity suggested that perhaps our investigative efforts were misdirected and glutamate was not the answer. However, before discarding all glutamate-related efforts, locomotor activity should be assessed following manipulations to the glutamate system, namely after repeated PCP, to determine the presence (or absence) of behavioral sensitization in HRs and LRs as an indication of possible basal ganglia dysregulation between groups. Locomotor activity should also be measured following intra-mPFC L-PDC administration to determine the effects of concomitant NMDA receptor antagonism *and* glutamate uptake blockade on locomotor activity. The latter two experiments would ideally strengthen the connection between environmental effects on measures of glutamate regulation and locomotor activity in a novel environment. Future studies could also examine the relation between GABA in the dorsal STR and VM/VL thalamus, two important information processors and integrators within the basal ganglia circuitry, and locomotor activity in a novel environment.

In the end, stress could not explain differences in locomotor activity in a novel environment. However, a pilot study in which HRs were found to have greater open arm

entries than LRs during a 5-min trial on the elevated plus maze suggested that perhaps anxiety-like behavior, not stress, is driving locomotor differences in a novel environment.

Lastly, because the mice used in this study were acquired from fellow laboratories (experiment 1 and 2a) and at 6 weeks of age from an outside animal breeding facility (experiment 2b and 3), it was impossible for us to control environmental factors such as maternal care, litter effects/social hierarchy (dominant vs. submissive mice), housing disturbances (e.g., construction noise), or possible environmental effects that may have occurred during transportation from the mouse breeding facility to OHSU.

In conclusion, we sought to determine whether differences in stress reactivity, or environmental influences, accounted for the variation in locomotor activity in a novel environment measured in genetically homogeneous HR and LR mice. Furthermore, we examined whether animals differed in indices of cortical glutamate; these differences could have potentially pointed to neurochemical mechanisms that may have also contributed to behavioral differences. Unlike previous studies using outbred rats characterized as HRs and LRs and asking whether this phenotype could predict other phenotypes such as cocaine self-administration, the current study used inbred animals divided into HRs and LRs and asked what effects the environment may have on glutamate regulation. Because the animals in the current study were identical on all alleles, any differences observed between HRs and LRs must be entirely due to environment. It is clear that environment factors influencing the HR/LR phenotype have not been fully elucidated. Further research is needed to clarify the role of environment on the expression of activity in a given novel environment, whether it is in outbred or inbred rodents.

REFERENCES

Abekawa, T., Ito, K., & Koyama, T. (2006). Role of the simultaneous enhancement of nmda and dopamine d(1) receptor-mediated neurotransmission in the effects of clozapine on phencyclidine-induced acute increases in glutamate levels in the rat medial prefrontal cortex. *Naunyn Schmiedebergs Arch Pharmacol, 374*(3), 177-193.

Adams, B., & Moghaddam, B. (1998). Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci, 18*(14), 5545-5554.

Adams, B. W., & Moghaddam, B. (2001). Effect of clozapine, haloperidol, or m100907 on phencyclidine-activated glutamate efflux in the prefrontal cortex. *Biol Psychiatry, 50*(10), 750-757.

Aked, J., Coizet, V., Clark, D., & Overton, P. G. (2005). Local injection of a glutamate uptake inhibitor into the ventral tegmental area produces sensitization to the behavioural effects of d-amphetamine. *Neuroscience, 134*(2), 361-367.

Albin, R. L., Young, A. B., & Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends Neurosci, 12*(10), 366-375.

Allen-Rowlands, C. F., Allen, J. P., Greer, M. A., & Wilson, M. (1980). Circadian rhythmicity of acth and corticosterone in the rat. *J Endocrinol Invest, 3*(4), 371-377.

Ambrosio, E., Goldberg, S. R., & Elmer, G. I. (1995). Behavior genetic investigation of the relationship between spontaneous locomotor activity and the acquisition of morphine self-administration behavior. *Behav Pharmacol, 6*(3), 229-237.

Ault, D. T., & Werling, L. L. (1999). Phencyclidine and dizocilpine modulate dopamine release from rat nucleus accumbens via sigma receptors. *Eur J Pharmacol, 386*(2-3), 145-153.

Baker, D. A., Xi, Z. X., Shen, H., Swanson, C. J., & Kalivas, P. W. (2002). The origin and neuronal function of in vivo nonsynaptic glutamate. *J Neurosci, 22*(20), 9134-9141.

Bakshi, V. P., & Geyer, M. A. (1997). Phencyclidine-induced deficits in prepulse inhibition of startle are blocked by prazosin, an alpha-1 noradrenergic antagonist. *J Pharmacol Exp Ther, 283*(2), 666-674.

Bartoletti, M., Ricci, F., & Gaiardi, M. (2007). A gaba(b) agonist reverses the behavioral sensitization to morphine in rats. *Psychopharmacology (Berl).*

Bates, J. F., & Goldman-Rakic, P. S. (1993). Prefrontal connections of medial motor areas in the rhesus monkey. *J Comp Neurol, 336*(2), 211-228.

Belz, E. E., Kennell, J. S., Czambel, R. K., Rubin, R. T., & Rhodes, M. E. (2003). Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. *Pharmacol Biochem Behav, 76*(3-4), 481-486.

Bevins, R. A., & Peterson, J. L. (2004). Individual differences in rats' reactivity to novelty and the unconditioned and conditioned locomotor effects of methamphetamine. *Pharmacol Biochem Behav, 79*(1), 65-74.

Billingslea, E. N., Mastropaolo, J., Rosse, R. B., Bellack, A. S., & Deutsch, S. I. (2003). Interaction of stress and strain on glutamatergic neurotransmission: Relevance to schizophrenia. *Pharmacol Biochem Behav, 74*(2), 351-356.

Cain, M. E., Smith, C. M., & Bardo, M. T. (2004). The effect of novelty on amphetamine self-administration in rats classified as high and low responders. *Psychopharmacology (Berl), 176*(2), 129-138.

Carr, D. B., & Sesack, S. R. (2000). Dopamine terminals synapse on callosal projection neurons in the rat prefrontal cortex. *J Comp Neurol, 425*(2), 275-283.

Christie, M. J., Bridge, S., James, L. B., & Beart, P. M. (1985). Excitotoxin lesions suggest an aspartatergic projection from rat medial prefrontal cortex to ventral tegmental area. *Brain Res, 333*(1), 169-172.

Cochran, S. M., Kennedy, M., McKerchar, C. E., Steward, L. J., Pratt, J. A., & Morris, B. J. (2003). Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: Differential modulation by antipsychotic drugs. *Neuropsychopharmacology, 28*(2), 265-275.

Conde, F., Audinat, E., Maire-Lepoivre, E., & Crepel, F. (1990). Afferent connections of the medial frontal cortex of the rat. A study using retrograde transport of fluorescent dyes. I. Thalamic afferents. *Brain Res Bull, 24*(3), 341-354.

Deminiere, J. M., Piazza, P. V., Guegan, G., Abrous, N., Maccari, S., Le Moal, M., et al. (1992). Increased locomotor response to novelty and propensity to intravenous amphetamine self-administration in adult offspring of stressed mothers. *Brain Res, 586*(1), 135-139.

Dhabhar, F. S., McEwen, B. S., & Spencer, R. L. (1993). Stress response, adrenal steroid receptor levels and corticosteroid-binding globulin levels--a comparison between sprague-dawley, fischer 344 and lewis rats. *Brain Res, 616*(1-2), 89-98.

Erb, S. M., & Parker, L. A. (1994). Individual differences in novelty-induced activity do not predict strength of amphetamine-induced place conditioning. *Pharmacol Biochem Behav, 48*(3), 581-586.

Finn, D. A., Gallaher, E. J., & Crabbe, J. C. (2000). Differential change in neuroactive steroid sensitivity during ethanol withdrawal. *J Pharmacol Exp Ther, 292*(1), 394-405.

Frankle, W. G., Laruelle, M., & Haber, S. N. (2006). Prefrontal cortical projections to the midbrain in primates: Evidence for a sparse connection.

Fride, E., Dan, Y., Feldon, J., Halevy, G., & Weinstock, M. (1986). Effects of prenatal stress on vulnerability to stress in prepubertal and adult rats. *Physiol Behav, 37*(5), 681-687.

Galvan, A., Kuwajima, M., & Smith, Y. (2006). Glutamate and gaba receptors and transporters in the basal ganglia: What does their subsynaptic localization reveal about their function? *Neuroscience, 143*(2), 351-375.

George, F. R., & Goldberg, S. R. (1989). Genetic approaches to the analysis of addiction processes. *Trends Pharmacol Sci, 10*(2), 78-83.

Gerfen, C. R. (1992). The neostriatal mosaic: Multiple levels of compartmental organization. *Trends Neurosci, 15*(4), 133-139.

Girotti, M., Weinberg, M. S., & Spencer, R. L. (2007). Differential responses of hpa axis immediate early genes to corticosterone and circadian drive. *Endocrinology*.

Goldman-Rakic, P. S., Bates, J. F., & Chafee, M. V. (1992). The prefrontal cortex and internally generated motor acts. *Curr Opin Neurobiol, 2*(6), 830-835.

Gong, W., Neill, D. B., & Justice, J. B., Jr. (1996). Locomotor response to novelty does not predict cocaine place preference conditioning in rats. *Pharmacol Biochem Behav, 53*(1), 191-196.

Grimm, J. W., & See, R. E. (1997). Cocaine self-administration in ovariectomized rats is predicted by response to novelty, attenuated by 17-beta estradiol, and associated with abnormal vaginal cytology. *Physiol Behav, 61*(5), 755-761.

Groenewegen, H. J., Wright, C. I., Beijer, A. V., & Voorn, P. (1999). Convergence and segregation of ventral striatal inputs and outputs. *Ann N Y Acad Sci, 877*, 49-63.

Gupta, A., Wang, Y., & Markram, H. (2000). Organizing principles for a diversity of gabaergic interneurons and synapses in the neocortex. *Science, 287*(5451), 273-278.

Hanania, T., Hillman, G. R., & Johnson, K. M. (1999). Augmentation of locomotor activity by chronic phencyclidine is associated with an increase in striatal nmda receptor function and an upregulation of the nr1 receptor subunit. *Synapse, 31*(3), 229-239.

Harrod, S. B., Mactutus, C. F., Bennett, K., Hasselrot, U., Wu, G., Welch, M., et al. (2004). Sex differences and repeated intravenous nicotine: Behavioral sensitization and dopamine receptors. *Pharmacol Biochem Behav, 78*(3), 581-592.

Hashimoto, K., Fujita, Y., Ishima, T., Hagiwara, H., & Iyo, M. (2006). Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of tropisetron: Role of alpha7 nicotinic receptors. *Eur J Pharmacol, 553*(1-3), 191-195.

Hashimoto, K., Fujita, Y., & Iyo, M. (2007). Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of fluvoxamine: Role of sigma-1 receptors. *Neuropsychopharmacology, 32*(3), 514-521.

Hennessy, M. B., Heybach, J. P., Vernikos, J., & Levine, S. (1979). Plasma corticosterone concentrations sensitively reflect levels of stimulus intensity in the rat. *Physiol Behav, 22*(5), 821-825.

Hennessy, M. B., & Levine, S. (1978). Sensitive pituitary-adrenal responsiveness to varying intensities of psychological stimulation. *Physiol Behav, 21*(3), 295-297.

Hepler, J. R., Toomim, C. S., McCarthy, K. D., Conti, F., Battaglia, G., Rustioni, A., et al. (1988). Characterization of antisera to glutamate and aspartate. *J Histochem Cytochem, 36*(1), 13-22.

Holstein, S. E., Pastor, R., Meyer, P. J., & Phillips, T. J. (2005). Naloxone does not attenuate the locomotor effects of ethanol in fast, slow, or two heterogeneous stocks of mice. *Psychopharmacology (Berl), 182*(2), 277-289.

Holstein, S. E., & Phillips, T. J. (2006). Gaba(b) receptor stimulation accentuates the locomotor effects of morphine in mice bred for extreme sensitivity to the stimulant effects of ethanol. *Pharmacol Biochem Behav, 85*(4), 697-704.

Hooks, M. S., Colvin, A. C., Juncos, J. L., & Justice, J. B., Jr. (1992a). Individual differences in basal and cocaine-stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. *Brain Res, 587*(2), 306-312.

Hooks, M. S., Jones, G. H., Liem, B. J., & Justice, J. B., Jr. (1992b). Sensitization and individual differences to ip amphetamine, cocaine, or caffeine following repeated intra-cranial amphetamine infusions. *Ann N Y Acad Sci, 654*, 444-447.

Hooks, M. S., Jones, G. H., Neill, D. B., & Justice, J. B., Jr. (1992c). Individual differences in amphetamine sensitization: Dose-dependent effects. *Pharmacol Biochem Behav, 41*(1), 203-210.

Hooks, M. S., Jones, G. H., Smith, A. D., Neill, D. B., & Justice, J. B., Jr. (1991a). Individual differences in locomotor activity and sensitization. *Pharmacol Biochem Behav, 38*(2), 467-470.

Hooks, M. S., Jones, G. H., Smith, A. D., Neill, D. B., & Justice, J. B., Jr. (1991b). Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. *Synapse, 9*(2), 121-128.

Hossain, S. M., Wong, B. K., & Simpson, E. M. (2004). The dark phase improves genetic discrimination for some high throughput mouse behavioral phenotyping. *Genes Brain Behav, 3*(3), 167-177.

Jentsch, J. D., & Roth, R. H. (1999). The neuropsychopharmacology of phencyclidine: From nmda receptor hypofunction to the dopamine hypothesis of schizophrenia. *20*(3), 201.

Jentsch, J. D., Taylor, J. R., & Roth, R. H. (1998). Subchronic phencyclidine administration increases mesolimbic dopaminergic system responsivity and augments stress- and psychostimulant-induced hyperlocomotion. *19*(2), 105.

Johnson, K. M., Phillips, M., Wang, C., & Kevetter, G. A. (1998). Chronic phencyclidine induces behavioral sensitization and apoptotic cell death in the olfactory and piriform cortex. *J Neurosci Res, 52*(6), 709-722.

Jones, G. H., Hernandez, T. D., Kendall, D. A., Marsden, C. A., & Robbins, T. W. (1992). Dopaminergic and serotonergic function following isolation rearing in

rats: Study of behavioural responses and postmortem and in vivo neurochemistry. *Pharmacol Biochem Behav, 43*(1), 17-35.

Kalivas, P. W., & Volkow, N. D. (2005). The neural basis of addiction: A pathology of motivation and choice. *Am J Psychiatry, 162*(8), 1403-1413.

Kapur, S., & Seeman, P. (2002). Nmda receptor antagonists ketamine and pcp have direct effects on the dopamine d(2) and serotonin 5-ht(2)receptors-implications for models of schizophrenia. *Mol Psychiatry, 7*(8), 837-844.

Klebaur, J. E., & Bardo, M. T. (1999). Individual differences in novelty seeking on the playground maze predict amphetamine conditioned place preference. *Pharmacol Biochem Behav, 63*(1), 131-136.

Koob, G. F., & Bloom, F. E. (1988). Cellular and molecular mechanisms of drug dependence. *Science, 242*(4879), 715-723.

Koob, G. F., & Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology, 24*(2), 97-129.

Lindahl, J. S., & Keifer, J. (2004). Glutamate receptor subunits are altered in forebrain and cerebellum in rats chronically exposed to the nmda receptor antagonist phencyclidine. *Neuropsychopharmacology, 29*(11), 2065-2073.

Lynd-Balta, E., & Haber, S. N. (1994). The organization of midbrain projections to the striatum in the primate: Sensorimotor-related striatum versus ventral striatum. *Neuroscience, 59*(3), 625-640.

Marinelli, M. (2005). The many facets of the locomotor response to a novel environment test: Theoretical comment on mitchell, cunningham, and mark (2005). *Behav Neurosci, 119*(4), 1144-1151.

Marinelli, M., Rouge-Pont, F., Deroche, V., Barrot, M., De Jesus-Oliveira, C., Le Moal, M., et al. (1997). Glucocorticoids and behavioral effects of psychostimulants. I: Locomotor response to cocaine depends on basal levels of glucocorticoids. *J Pharmacol Exp Ther, 281*(3), 1392-1400.

Marinelli, M., & White, F. J. (2000). Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. *J Neurosci, 20*(23), 8876-8885.

Marquez, C., Nadal, R., & Armario, A. (2006). Influence of reactivity to novelty and anxiety on hypothalamic-pituitary-adrenal and prolactin responses to two different novel environments in adult male rats. *Behav Brain Res, 168*(1), 13-22.

Maurice, N., Deniau, J. M., Glowinski, J., & Thierry, A. M. (1999). Relationships between the prefrontal cortex and the basal ganglia in the rat: Physiology of the cortico-nigral circuits. *J Neurosci, 19*(11), 4674-4681.

McDaid, J., Graham, M. P., & Napier, T. C. (2006). Methamphetamine-induced sensitization differentially alters pcreb and deltafosb throughout the limbic circuit of the mammalian brain. *Mol Pharmacol, 70*(6), 2064-2074.

McKee, B. L., & Meshul, C. K. (2005). Time-dependent changes in extracellular glutamate in the rat dorsolateral striatum following a single cocaine injection. *Neuroscience, 133*(2), 605-613.

Melendez, R. I., Vuthiganon, J., & Kalivas, P. W. (2005). Regulation of extracellular glutamate in the prefrontal cortex: Focus on the cystine glutamate exchanger and group i metabotropic glutamate receptors. *J Pharmacol Exp Ther, 314*(1), 139-147.

Meshul, C. K., Emre, N., Nakamura, C. M., Allen, C., Donohue, M. K., & Buckman, J. F. (1999). Time-dependent changes in striatal glutamate synapses following a 6-hydroxydopamine lesion. *Neuroscience, 88*(1), 1-16.

Meshul, C. K., Stallbaumer, R. K., Taylor, B., & Janowsky, A. (1994). Haloperidol-induced morphological changes in striatum are associated with glutamate synapses. *Brain Res, 648*(2), 181-195.

Mitchell, J. M., Cunningham, C. L., & Mark, G. P. (2005). Locomotor activity predicts acquisition of self-administration behavior but not cocaine intake. *Behav Neurosci, 119*(2), 464-472.

Moghaddam, B. (1993). Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: Comparison to hippocampus and basal ganglia. *J Neurochem, 60*(5), 1650-1657.

Moghaddam, B., Adams, B., Verma, A., & Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from nmda receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci, 17*(8), 2921-2927.

Moghaddam, B., & Adams, B. W. (1998). Reversal of phencyclidine effects by a group ii metabotropic glutamate receptor agonist in rats. *281*(5381), 1349.

Moghaddam, B., Bolinao, M. L., Stein-Behrens, B., & Sapolsky, R. (1994). Glucocorticoids mediate the stress-induced extracellular accumulation of glutamate. *Brain Res, 655*(1-2), 251-254.

Morris, B. J., Cochran, S. M., & Pratt, J. A. (2005). Pcp: From pharmacology to modelling schizophrenia. *5*(1), 101.

Nadal, R., Armario, A., & Janak, P. H. (2002). Positive relationship between activity in a novel environment and operant ethanol self-administration in rats. *Psychopharmacology (Berl), 162*(3), 333-338.

Nadal, R., Rotllant, D., Marquez, C., & Armario, A. (2005). Perseverance of exploration in novel environments predicts morphine place conditioning in rats. *Behav Brain Res, 165*(1), 72-79.

Nakamura, S., Ago, Y., Hayashi, A., Itoh, S., Kakuda, M., Hashimoto, H., et al. (2006). Modification of cocaine-induced behavioral and neurochemical effects by serotonin1a receptor agonist/antagonist in mice. *Synapse, 60*(7), 479-484.

Nowak, K. L., Ingraham, C. M., McKinzie, D. L., McBride, W. J., Lumeng, L., Li, T. K., et al. (2000). An assessment of novelty-seeking behavior in alcohol-preferring and nonpreferring rats. *Pharmacol Biochem Behav, 66*(1), 113-121.

Olney, J. W., Labruyere, J., & Price, M. T. (1989). Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science, 244*(4910), 1360-1362.

Paspalas, C. D., & Goldman-Rakic, P. S. (2005). Presynaptic d1 dopamine receptors in primate prefrontal cortex: Target-specific expression in the glutamatergic synapse. *J Neurosci, 25*(5), 1260-1267.

Paxinos, G., & Franklin, K. (2001). *The mouse brain in stereotaxic coordinates* (2 ed.). San Diego: Academic Press.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods, 14*(3), 149-167.

Phend, K. D., Weinberg, R. J., & Rustioni, A. (1992). Techniques to optimize post-embedding single and double staining for amino acid neurotransmitters. *J Histochem Cytochem, 40*(7), 1011-1020.

Piazza, P. V., Deminiere, J. M., Le Moal, M., & Simon, H. (1989). Factors that predict individual vulnerability to amphetamine self-administration. *Science, 245*(4925), 1511-1513.

Piazza, P. V., Deminiere, J. M., Maccari, S., Mormede, P., Le Moal, M., & Simon, H. (1990). Individual reactivity to novelty predicts probability of amphetamine self-administration. *Behav Pharmacol, 1*(4), 339-345.

Piazza, P. V., & Le Moal, M. L. (1996). Pathophysiological basis of vulnerability to drug abuse: Role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annu Rev Pharmacol Toxicol, 36*, 359-378.

Piazza, P. V., Maccari, S., Deminiere, J. M., Le Moal, M., Mormede, P., & Simon, H. (1991a). Corticosterone levels determine individual vulnerability to amphetamine self-administration. *Proc Natl Acad Sci U S A, 88*(6), 2088-2092.

Piazza, P. V., Rouge-Pont, F., Deminiere, J. M., Kharoubi, M., Le Moal, M., & Simon, H. (1991b). Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. *Brain Res, 567*(1), 169-174.

Pierce, R. C., & Kalivas, P. W. (1997). A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Res Brain Res Rev, 25*(2), 192-216.

Pierre, P. J., & Vezina, P. (1997). Predisposition to self-administer amphetamine: The contribution of response to novelty and prior exposure to the drug. *Psychopharmacology (Berl), 129*(3), 277-284.

Podhorna, J., & Didriksen, M. (2005). Performance of male c57bl/6j mice and wistar rats in the water maze following various schedules of phencyclidine treatment. *16*(1), 25.

Rawls, S. M., & McGinty, J. F. (1998). Kappa receptor activation attenuates l-trans-pyrrolidine-2,4-dicarboxylic acid-evoked glutamate levels in the striatum. *J Neurochem, 70*(2), 626-634.

Robinson, T. E., & Whishaw, I. Q. (1988). Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-ohda lesion of the substantia nigra: A microdialysis study in freely moving rats. *Brain Res, 450*(1-2), 209-224.

Rotaru, D. C., Barrionuevo, G., & Sesack, S. R. (2005). Mediodorsal thalamic afferents to layer iii of the rat prefrontal cortex: Synaptic relationships to subclasses of interneurons. *J Comp Neurol, 490*(3), 220-238.

Rouge-Pont, F., Piazza, P. V., Kharouby, M., Le Moal, M., & Simon, H. (1993). Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals predisposed to amphetamine self-administration. A microdialysis study. *Brain Res, 602*(1), 169-174.

Ryan, B. C., & Vandenbergh, J. G. (2002). Intrauterine position effects. *Neurosci Biobehav Rev, 26*(6), 665-678.

Ryan, B. C., & Vandenbergh, J. G. (2006). Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. *Horm Behav, 50*(1), 85-93.

Sesack, S. R., Carr, D. B., Omelchenko, N., & Pinto, A. (2003). Anatomical substrates for glutamate-dopamine interactions: Evidence for specificity of connections and extrasynaptic actions. *Ann N Y Acad Sci, 1003*, 36-52.

Shakil, S. S., Holmer, H. K., Moore, C., Abernathy, A. T., Jakowec, M. W., Petzinger, G. M., et al. (2005). High and low responders to novelty show differential effects in striatal glutamate. *Synapse, 58*(3), 200-207.

Shelnutt, S. R., Gunnell, M., & Owens, S. M. (1999). Sexual dimorphism in phencyclidine in vitro metabolism and pharmacokinetics in rats. *J Pharmacol Exp Ther, 290*(3), 1292-1298.

Smith, Y., Bevan, M. D., Shink, E., & Bolam, J. P. (1998). Microcircuitry of the direct and indirect pathways of the basal ganglia. *Neuroscience, 86*(2), 353-387.

Suto, N., Austin, J. D., & Vezina, P. (2001). Locomotor response to novelty predicts a rat's propensity to self-administer nicotine. *Psychopharmacology (Berl), 158*(2), 175-180.

Takahata, R., & Moghaddam, B. (2003). Activation of glutamate neurotransmission in the prefrontal cortex sustains the motoric and dopaminergic effects of phencyclidine. *Neuropsychopharmacology, 28*(6), 1117-1124.

Timmerman, W., & Westerink, B. H. (1997). Brain microdialysis of gaba and glutamate: What does it signify? *Synapse, 27*(3), 242-261.

Touchon, J. C., Moore, C., Frederickson, J., & Meshul, C. K. (2004). Lesion of subthalamic or motor thalamic nucleus in 6-hydroxydopamine-treated rats: Effects on striatal glutamate and apomorphine-induced contralateral rotations. *Synapse, 51*(4), 287-298.

Valentinuzzi, V. S., Buxton, O. M., Chang, A. M., Scarbrough, K., Ferrari, E. A., Takahashi, J. S., et al. (2000). Locomotor response to an open field during c57bl/6j active and inactive phases: Differences dependent on conditions of illumination. *Physiol Behav, 69*(3), 269-275.

Vallee, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., & Maccari, S. (1997). Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. *J Neurosci, 17*(7), 2626-2636.

Vezina, P., Pierre, P. J., & Lorrain, D. S. (1999). The effect of previous exposure to amphetamine on drug-induced locomotion and self-administration of a low dose of the drug. *Psychopharmacology (Berl), 147*(2), 125-134.

Vezina, P., & Queen, A. L. (2000). Induction of locomotor sensitization by amphetamine requires the activation of nmda receptors in the rat ventral tegmental area. *Psychopharmacology (Berl), 151*(2-3), 184-191.

Volterra, A., Bezzi, P., Rizzini, B. L., Trotti, D., Ullensvang, K., Danbolt, N. C., et al. (1996). The competitive transport inhibitor l-trans-pyrrolidine-2, 4-dicarboxylate triggers excitotoxicity in rat cortical neuron-astrocyte co-cultures via glutamate release rather than uptake inhibition. *Eur J Neurosci, 8*(9), 2019-2028.

Wang, C., Anastasio, N., Popov, V., Leday, A., & Johnson, K. M. (2004). Blockade of n-methyl-d-aspartate receptors by phencyclidine causes the loss of corticostriatal neurons. *Neuroscience, 125*(2), 473-483.

Wang, C., McInnis, J., Ross-Sanchez, M., Shinnick-Gallagher, P., Wiley, J. L., & Johnson, K. M. (2001). Long-term behavioral and neurodegenerative effects of perinatal phencyclidine administration: Implications for schizophrenia. *Neuroscience, 107*(4), 535-550.

Wang, C. Z., & Johnson, K. M. (2005). Differential effects of acute and subchronic administration on phencyclidine-induced neurodegeneration in the perinatal rat. *J Neurosci Res, 81*(2), 284-292.

Whishaw, I. Q., Haun, F., & Kolb, B. (1999). Analysis of behavior in laboratory rodents. In U. Windhurst & H. Johansson (Eds.), *Modern techniques in neuroscience research* (pp. 1243-1275). Berlin: Springer-Verlag.

Winter, J. C., Eckler, J. R., Rice, K. C., & Rabin, R. A. (2005). Serotonergic/glutamatergic interactions: Potentiation of phencyclidine-induced stimulus control by citalopram. *Pharmacol Biochem Behav, 81*(3), 694-700.

Wise, R. A., & Rompre, P. P. (1989). Brain dopamine and reward. *Annu Rev Psychol, 40*, 191-225.

Wolf, M. E., Dahlin, S. L., Hu, X. T., Xue, C. J., & White, K. (1995). Effects of lesions of prefrontal cortex, amygdala, or fornix on behavioral sensitization to amphetamine: Comparison with n-methyl-d-aspartate antagonists. *Neuroscience, 69*(2), 417-439.