Alterations in responsiveness and mRNA expression of alpha-1 adrenergic receptors in neonatal ventral hippocampus lesioned rats

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ABBREVIATIONS

DA, Dopamine

AMPH, amphetamine

NE, norepinephrine

AR, adrenergic receptor

PCP, phencyclidine

NMDA, N-methyl-D-aspartate

nVH, neonatal ventral hippocampus

VH, ventral hippocampus

VTA, ventral tegmental area

PFC, prefrontal cortex

mPFC, medial prefrontal cortex

NAcc, nucleus accumbens

mRNA, messenger ribonucleic acid

PD, postnatal day

PPI, prepulse inhibition

PP, prepulse intensity

ANOVA, analysis of variance

ABSTRACT

Neonatal ventral hippocampus (nVH) lesion in rats is a widely accepted animal model of schizophrenia due to the exclusively post-pubertal emergence of many schizophrenia-like abnormalities. Previous studies have shown increased ligand binding of α-1 adrenergic receptors (AR) in the frontal cortex of post-pubertal nVH lesioned rats, compared to sham-operated control rats. Also, pretreatment with α-1 AR antagonist prazosin reversed amphetamine-induced hyperlocomotion in controls, but failed to do so in lesioned animals. In order to investigate the hypothesis that nVH lesions may lead to hyperactivity of α-1 AR, we studied locomotor behavior and prepulse inhibition (PPI) in post-pubertal (PD56-90) sham and lesioned animals in response to different doses of α-1 AR agonist cirazoline. Results showed that 0.6 mg/kg cirazoline significantly increased locomotor activity in both groups of animals, without any differential effect on nVH lesioned animals. However, lesioned animals showed a substantial decrease in PPI in response to an intermediate dose of 0.45 mg/kg cirazoline that did not significantly reduce PPI in control animals. This deficit was blocked by pretreatment with prazosin, demonstrating that the effect of cirazoline was through activation of α-1 ARs. Together, these results suggest that nVH lesioned animals show a hyperactive α-1 AR system that may regulate at least some behaviors that are abnormal in these animals. In situ hybridization using digoxigenin-labeled, subtype-specific oligonucleotide probes for α-1 AR mRNA revealed a significantly higher number of cells labeled for α-1A AR mRNA, a lower number of cells labeled for α-1D AR mRNA, and no difference in the number of cells labeled for α-1B AR mRNA in the medial prefrontal cortex of nVH lesioned rats. These results suggest that alterations at the level of transcription of genes encoding for some α-1 AR subtypes may be responsible for the abnormal adrenergic system in nVH lesioned animals.

RÉSUMÉ

La lésion ventrale néonatale de l'hippocampe (nVH) chez les rats est largement admise comme modèle animal de la schizophrénie. Des études antérieures ont montré une augmentation de liaison aux récepteurs adrénergiques (AR) α-1 dans le cortex frontal des rats post-pubères ayant des lésions au niveau du nVH (nVHL) comparativement aux rats contrôles. De plus, l'hyperlocomotion normalement induite chez les rats contrôles par l'amphétamine est abolit par le prétraitement de ces rats avec le prazosin, un antagoniste du AR α-1, alors que ce dernier est sans effet sur les rats avec lésions.. Afin d'investiguer l'hypothèse que des lésions au niveau du nVH peuvent mener à l'hyperactivité du AR α-1, nous avons étudié les comportements locomoteurs et la «prepulse inhibition» (PPI) chez les rats contrôles (PD56-90) et les nVHL, en réponse à différentes doses de l'agoniste adrénergique α-1 cirazoline. Les résultats montrent qu'une dose de 0.6 mg/kg de cirazoline augmente significativement l'activité motrice dans les deux groupes d'animaux, sans effet différentiel chez les nVHL. Cependant, les nVHL présentent une diminution substantielle du PPI suite à l'administration de 0.45 mg/kg de cirazoline, laquelle dose ne réduit pas de manière significative la PPI chez les contrôles. Ce déficit a été bloqué par le prétraitement avec le prazosin, démontrant que les effets de la cirazoline sont médiés via l'activation du AR α-1. Ensemble, ces résultats suggèrent que les nVHL ont une hyperactivité du système adrénergique α-1, laquelle peut mener à certains comportements anormaux chez ces animaux. Nous avons utilisé des sondes marquées avec digoxigenin pour faire de l'hybridation ***in situ.*** Les sondes étaient spécifiques pour différents sous-types d'oligonucléotides d'ARNm de AR α-1**.** Les resultats ont indiqué un nombre sensiblement plus élevé de cellules marquées par l'ARNm α-1A, un nombre inférieur de cellules marquées par l'ARNm α-1D , et aucune différence dans le nombre de cellules marquées par l'ARNm α-1B des AR dans le cortex préfrontal médial de nVHL. Ces résultats suggèrent que des changements au niveau de la transcription des gènes codant pour certains sous-types de α-1 AR sont responsables d'anomalies du système adrénergique chez les animaux avec des lésions du nVH.

I. INTRODUCTION:

1. Schizophrenia:

The term 'schizophrenia', derived from the Greek words ***schizo*** (split) and ***phren*** (mind), was coined by Swiss psychiatrist Eugen Bleuler to describe a mental illness characterized by, but not limited to, a loss of contact with reality[1]. This debilitating illness affects 1% of the general population, with symptoms emerging during late adolescence to early adulthood, and, on average, at an earlier age for males than for females[2]. The symptoms that usually lead to diagnosis are the 'positive symptoms' of schizophrenia that include delusions, hallucinations, and bizarre behavior that cannot be justified by mood disorders[3]. However, in addition to positive symptoms, patients may show 'negative symptoms' that include avolition, alogia, anhedonia, and blunted affect, as well as cognitive deficits that include impairments in attention, learning and memory, and sensorimotor gating. The combination of symptoms exhibited by each patient diagnosed with schizophrenia varies, making the categorization of these various symptoms into one disorder debatable.

2. History and Etiology of Schizophrenia:

Early theories suggested that the positive symptoms that we now associate with schizophrenia were a result of possession of the body by evil spirits. However, German physician Emile Kraepelin later identified this illness as an organic disorder of the mind and named it 'dementia praecox'[4], which was later changed to 'schizophrenia' by Eugen Bleuler[1]. Although we have a much better understanding of this disorder since then, the etiology of schizophrenia is still largely unknown. Family studies have shown

that the risk of developing schizophrenia is higher in family members of patients with schizophrenia in comparison to the general population, with the risk being higher in first degree relatives and decreasing in more distant relatives[5]. While this familial aggregation suggests a genetic predisposition to schizophrenia, twin studies have revealed that genetic inheritance alone may be insufficient to explain the etiology of this disorder. Monozygotic twins, who share all their genes, have a concordance rate of 30-50% for schizophrenia, while dizygotic twins, who share half a set of their genes, have a concordance rate of approximately 10%, similar to that of non-twin siblings[6]. A more thorough analysis into the etiology of schizophrenia reveals that a complex interaction of multiple genes and environmental factors, some of which are discussed later, increases an individual's vulnerability to the disorder[7, 8].

3. Neurochemical Aberrations in Schizophrenia:

Similar to its etiology, the neuropathologies of schizophrenia have also been controversial. However, over the years, converging evidence have implicated an abnormality of the neurotransmitter system in the brain in schizophrenia. In particular, dopamine, a neurotransmitter that plays a role in voluntary movements and motivation, has been shown to be disrupted in schizophrenia.

3.1. Dopamine Hypothesis:

The 'dopamine hypothesis' of schizophrenia developed following the discovery that conventional anti-psychotic drugs, which can ameliorate positive symptoms in schizophrenia patients, are efficient D2 dopamine receptor blockers[9, 10]. Also, drugs

such as amphetamine (AMPH) and cocaine, which increase DA release and/or inhibit its reuptake, trigger psychosis in normal individuals and amplify symptoms in schizophrenia patients[11]. In addition, positron emission tomography (PET) studies have shown an exaggerated increase in dopamine release and its catabolism in the striatum of schizophrenia patients in comparison to normal individuals following AMPH treatment[12, 13], suggesting a hyperactive dopamine system in schizophrenia. Later extensions to the dopamine hypothesis incorporated negative and cognitive symptoms of schizophrenia. Studies of the prefrontal cortex (PFC) in schizophrenia patients have shown a reduced dopaminergic tone in this brain region, supporting the hypothesis that negative and cognitive symptoms may be due to a hypodopaminergic state in regions of the prefrontal cortex[14, 15].

Despite the intial popularity of the dopamine hypothesis, conventional antipsychotic drugs, such as haloperidol, failed to improve negative and cognitive symptoms in patients[16, 17]. Also, atypical antipsychotic drugs such as clozapine, which have lower affinity for D2 dopamine receptors and higher affinity for other neurotransmitter receptors such as D4 dopamine receptors[18], adrenergic[19], serotonergic[20] and cholinergic[21] receptors, were more efficient at ameliorating positive, negative and cognitive symptoms of schizophrenia[22, 23], forcing scientists to consider additional explanations to the neurobiology of this disorder.

3.2. Glutamate Hypothesis:

The 'glutamate hypothesis' of schizophrenia came about because glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonists, such as phencyclidine (PCP) and ketamine, produce a more extensive range of schizophrenia symptoms (i.e. positive, negative and cognitive symptoms) in normal individuals and exacerbate those in schizophrenia patients, suggesting a hypoactive glutamatergic system in schizophrenia[24-27]. Glutamate receptor antagonists also increase dopamine release in the striatum, while reducing it in the frontal cortex[28]. Furthermore, atypical antipsychotic drugs that have better success at treating all categories of schizophrenia symptoms not only block dopamine receptors, but also appear to enhance NMDA receptor activity[29]. Some studies have shown decreased expression of NMDA and non-NMDA receptors in the hippocampus of post-mortem schizophrenia brains[30-32], while other studies have shown increased glutamate uptake and decreased glutamate release in the frontal cortex[33].

4. Neurodevelopmental Hypothesis of Schizophrenia:

Kraeplin and other neuroscientists in the 20th century suggested that schizophrenia may be a result of insults that cause cerebral maldevelopment[4]. However, this theory did not receive much attention until Daniel Weinberger reinforced 'the neurodevelopmental hypothesis of schizophrenia'[34] proposing that early prenatal or postnatal insults, involving a combination of genetic and environmental factors, can disrupt the normal sequence of brain development such that symptoms of this aberration may be undetectable until later in life, when late maturing neural systems develop.

Support for a neurodevelopmental basis of this disorder comes from post-mortem studies of brains of schizophrenia patients that show abnormalities in neuronal migration, size and organization[35, 36] - all events that occur early in life before any clinical symptoms emerge. In addition, some robust neuronal changes are seen in schizophrenia brains which include increased ventricular volume, decreased brain weight, and decreased hippocampal volume[37-40], which are found in first-episode schizophrenia patients and, in some studies, are found to stay consistent throughout the course of their illness[41, 42]. Histological studies reveal an absence of gliosis that is characteristic of neurodegeneration[43, 44], suggesting that the loss of volume seen in schizophrenia brains may not be due to neurodegeneration but a pre-existing dysplasia. However, other studies have suggested that the morphological changes seen in schizophrenia brains may be indicative of neurodegeneration as they have shown progressive brain volume reductions and ventricular enlargements that are accelerated in comparison to that seen during normal aging[45-48]. It is important to note that with a heterogenous disorder such as schizophrenia, neurodevelopmental or neurodegenerative abnormalities need not be exclusive to explain the etiology of the disorder. It is possible that these two factors act synergistically to produce the abnormalities that lead to the diagnosis of schizophrenia.

5. Genetic Factors Associated with Schizophrenia:

Over the years, linkage studies have indicated numerous genes as being associated with schizophrenia, some of which have been replicated in independent studies. Most of these candidate genes are involved in neurodevelopment or normal

synaptic function. For example, neuregulin-1 (NRG-1) has been linked to schizophrenia via fine-mapping across chromosome 8p21-p22 in different populations[49, 50]. It has been suggested that polymorphisms in the neuregulin gene (NRG-1) may increase vulnerability to the disorder by disrupting one of more of its functions, e.g. NMDA receptor regulation, axon guidance, myelination or glial cell growth and development. Another susceptibility gene for schizophrenia that has shown strong association in various populations is the gene encoding dystrobrevin-binding protein, dysbindin (DTNBP-1)[51-54]. Dysbindin is a protein located at presynaptic terminals and postsynaptic densities in the brain. Presynaptic dysbindin is reduced in glutamatergic neurons of the hippocampus of schizophrenia brains[55], suggesting that a variation of DTNBP1 may increase risk of developing schizophrenia through dysregulation of glutamatergic trafficking or release. A gene located on chromosome 22q11 that encodes catechol-O-methyltransferase (COMT), an enzyme that is involved in the clearance of synaptic dopamine, has also been associated with schizophrenia[56-58]. A functional polymorphism in the alleles alters enzyme activity such that one or two valine alleles increase enzyme activity, decreasing dopamine levels in critical brain regions such as the frontal cortex, and perhaps increasing risk for schizophrenia. Other studies have shown a (1:11) chromosomal translocation, which disrupts the DISC-1 gene, segregates with schizophrenia and other affective disorders[59-61]. DISC-1 protein can be detected in many areas of the cerebral cortex, the hippocampus, lateral septum and hypothalamus, and appears to have a role in neural migration and maturation during development, as well as in synaptic transmission and plasticity in adulthood[62, 63]. In addition, a decrease in the transcription of genes encoding proteins involved in synaptic function has also

been seen in schizophrenia brains[64]. These results support the hypothesis that schizophrenia may be a disease of the synapse where normal synaptic transmission is impaired early in life leading to abnormal synaptic formation, with symptoms of this aberration surfacing later in life.

6. Environmental Factors Associated with Schizophrenia:

Along with genetic variations, many environmental factors have also been suggested to increase vulnerability to schizophrenia. Obstetric complications, which include complications during pregnancy (e.g. bleeding, diabetes and pre-eclempsia), complications during delivery (e.g. asphyxia and uterine atony), and fetal abnormality (e.g. low birth weight and congenital abnormality), have been suggested to increase risk of the offspring to schizophrenia[65-68]. Other environmental insults suggested to increase susceptibility to schizophrenia include maternal infection, maternal malnutrition, increased stress, and adolescent drug abuse[69-72]. However, the effect size of these environmental factors is small, suggesting that these insults may better predict the emergence of schizophrenia or its pathologies in individuals who have a genetic predisposition to the disorder than in those who have a lower genetic risk.

7. Limitations to Human Studies:

Due to the complexity of schizophrenia and a different combination of symptoms in each patient suffering from the disorder, identifying specific neuronal markers and causes of the disease is very difficult. Also, the population of non-medicated patients of schizophrenia is small, and limitations in present experimental

methods restrict the information obtained. Studies on post-mortem brains allow more in-depth analysis of molecular and neuropathological abnormalities in schizophrenia; however, these data are most often confounded by cause and age of death, and antipsychotic medication, further complicating the search for a better understanding of schizophrenia. Therefore, in order to study the basis of schizophrenia, the neuronal aberrations involved in the disease, and effective therapeutics to treat its symptoms and pathologies, animal models of schizophrenia are often necessary.

8. Animal Models of Schizophrenia:

Generating animal models for mental illnesses that are exclusive to humans is complicated. One of the obvious reasons is the inability of animals to represent hallmark features of psychiatric disorders such as hallucinations, delusions, obsessions, or mania. Since neuropathological markers for most mental disorders have yet to be identified, the validity of these animal models have to be based on particular symptoms, which may or not may not be characteristic to the disorder. Furthermore, the strength of these models depends on the efficiency of our experimental methods to capture and analyze behavioral analogies to human symptoms. Other complications include the structural and functional variation of genes between animals and humans, such that manipulation of certain genes may have different effects on the animal brain from the human brain. Despite these drawbacks, animal models for psychiatric illnesses are, for the time being, indispensable.

In order to study schizophrenia using animal models, certain animal behaviors are identified as being analogous to specific symptoms seen in patients. For example, positive symptoms in patients are associated with hyperlocomotion to stress and novelty in animals; supersensitivity of patients to psychostimulants is associated with hyperlocomotion in animals in response to psychostimulants. Negative symptoms seen in patients such as social withdrawal and anhedonia, can be studied in animals by monitoring social interaction with conspecifics and motivation for certain drugs of abuse, respectively. Arguably, the more valid animal correlates of schizophrenia symptoms are cognitive deficits that can be more easily and efficiently studied in both animals and humans because fundamental cognitive processes in both are very similar. For example, attentional deficits in animals can be studied through tasks on latent inhibition, sensorimotor deficits can be studied through pre-pulse inhibition (PPI) tests, and memory deficits can be monitored in animals using delayed non-matching to sample tasks and radial-arm mazes.

Animal models have been generated to better test the various hypotheses of schizophrenia – the oldest models being based on 'the dopamine hypothesis' of the disorder. In this model, animals are tested on their behaviors in response to various dopamine receptor agonists, e.g. amphetamine and apomorphine, as well as dopamine receptor antagonists, e.g. raclorpide [73]. This model was strong for a short while because dopamine receptor agonists simulate many symptoms of schizophrenia such as stimulant-related stereotypies and sensorimotor deficits, while antagonists such as haloperidol and other neuroleptics are able to ameliorate these aberrant behaviors[74].

However, these models are unable to mimic many of the negative symptoms seen in schizophrenia patients, making it an incomplete model of the disorder. With the emergence of 'the glutamate hypothesis' of schizophrenia, animals models were generated involving manipulations to NMDA receptors that showed many behavioral abnormalities relevant to schizophrenia. For example, mice with reduced expression of NMDA receptors displayed increased motor activity and stereotypy and deficits in social interactions[75]. Also, NMDA receptor antagonists such as PCP and MK-801 were used to induce positive and negative schizophrenia-like behaviors in animals, and to test the effect of potential antipsychotic drugs on these behaviors[76, 77]. In these models, atypical antipsychotics such as clozapine had a better effect at ameliorating abnormal behaviors than typical neuroleptics, supporting an abnormality in other neurotransmitter systems in schizophrenia.

9. Animal Models of the Neurodevelopmental Hypothesis of Schizophrenia:

With the emergence of the neurodevelopmental hypothesis of schizophrenia, many factors that have been proposed to interfere with normal brain development to increase one's risk of developing schizophrenia have been studied using animal models. The functions of various susceptibility genes for schizophrenia that may be involved in neurodevelopment have been explored using genetically altered mice. These models permit the study of variations in multiple alleles and their interactions that may increase the risk of developing schizophrenia. For example, mice heterozygous for mutations in domains of Neuregulin-1 (nrg-1+/-) exhibit PPI deficits, abnormal exploratory behavior,

deficits in latent inhibition, and decreased expression of NMDA receptors[78]. Some of the behavioral abnormalities in these mice are ameliorated with clozapine, suggesting that variations in NRG-1 may contribute to some of the abnormalities seen in schizophrenia. Another model involves mice with a deletion in the gene for neural cell adhesion molecule 180 (NCAM-180), which is involved in neural migration. These mice show abnormal neural migration within the subventricular zone, enlarged ventricles, morphological abnormalities of the hippocampus, and PPI deficits[79]. In another model, mice with a genetic mutation that results in the expression of only a small percent of NR1 subunits of the NMDA receptor show spontaneous hyperlocomotion that attenuates after treatment with haloperidol, and social behavioral deficits that can be reduced with clozapine[80].

The significance of prenatal and early postnatal environments in neurodevelopment has also been investigated through environmental animal models of schizophrenia. One such model involves deprivation of nutrients, such as dietary protein, in rats during these early stages of brain development. This nutritional deficiency shows abnormal development and function of the hippocampus in mature rats which may relate to deficits in learning and memory[81-83]. Some studies on the effect of prenatal viral infection on cortical and hippocampal neurodevelopment have shown decreased area of these brain regions and a low number of reelin-positive cells, implicating a defect in neuronal migration[84]. Animal models of chronic placental insufficiency, involving a unilateral ligation of the uterine artery, have demonstrated fetal growth restriction observed in the form of enlarged lateral ventricles and reduced

hippocampal volume without signs of gliosis, along with abnormal neuronal migration in the cortex and some behavioral abnormalities analogous to symptoms of schizophrenia[85-87]. Other animal models that involve disruption of early neurodevelopment include prenatal injection of methylazoxymethanol (MAM), a toxin that kills actively replicating cells, which leads to abnormal social behaviors and brain morphology in mature animals[88-90]. Certain strains of rat have shown post-pubertal abnormalities in sensorimotor gating and spatial learning following maternal deprivation early in postnatal life[91], indicating that maternal deprivation may be responsible for some of the cognitive deficits seen in certain groups of schizophrenia patients.

10. Neonatal Ventral Hippocampus Lesion Rat Model of Schizophrenia:

The neonatal ventral hippocampus (nVH) lesion rat model was introduced in order to test the neurodevelopmental hypothesis that early aberrations to the hippocampus and its connections may lead to delayed behavioral and molecular abnormalities similar to those seen in schizophrenia[92]. This model was based on the results of independent studies on the neuropathology of schizophrenia which have provided convergent data indicating an abnormality in the structure and function of the hippocampal formation in patients suffering from schizophrenia.

10.1. The Hippocampus in Schizophrenia:

Magnetic resonance imaging (MRI) studies of the brain of schizophrenia patients show decreased volume of the hippocampus and abnormal hippocampal shape[93, 94]. These abnormalities are consistent in prodromal and first-episode subjects, removing any confound of anti-psychotic medication[95, 96]. Unaffected twins, who have a genetic predisposition to schizophrenia, have also shown decreased hippocampal volume in comparison to controls, although not as much as that of their affected twin[97]. Other studies have shown reduced neuronal markers, such as N-acetyl aspartate (NAA), reduced metabolic activity, and altered neurochemical activity in the hippocampus of schizophrenia patients[98, 99]. Furthermore, electrical stimulation of the parahippocampal gyrus in normal individuals causes psychic phenomena[100]. Together, these results suggest that the hippocampus may be central to the pathophysiology of schizophrenia.

The hippocampal formation, which includes the dentate gyrus, Ammon's horn (CA1 – CA3) and the subiculum, receives most of its input from the entorhinal cortex[101]. The hippocampus, in turn, has projections to the prefrontal cortex, the amygdaloid complex, the nucleus accumbens, and the hypothalamus[102-104]. Due to the vast connectivity of the hippocampus, disruption of this area may cause abnormal functioning of connecting brain regions.

Morphological studies have shown misplaced and aberrantly clustered pre-alpha cells in the entorhinal cortex[105] and disarray in the orientation of pyramidal neurons of the hippocampus[106] of schizophrenia patients. Since neuronal migration occurs very

early in development, these results suggest that an aberration in the development of the hippocampal formation may be an important neuropathology in schizophrenia. In fact, high levels of stress have been shown to disrupt hippocampal neurogenesis, reduce dendritic density, and lead to atrophy of existing cells[107]. One possibility is that an interaction of genetic and environmental insults may stress the nervous system during critical stages of neurodevelopment, leading to abnormality of the hippocampus and its connecting brain regions, and the emergence of some of the symptoms of schizophrenia.

10.2. The nVH Lesion Model:

Animal studies have demonstrated that bilateral excitotoxic lesion of the ventral hippocampus (VH) in the adult rat, which is analogous to the anterior hippocampus in humans, increases spontaneous locomotion and amphetamine-induced hyperlocomotion[108]. Dopamine levels in the nucleus accumbens of (NAcc) of lesioned animals are increased, while the levels of dopamine and its metabolites DOPAC and HVA are reduced in the medial prefrontal cortex (mPFC). Dorsal hippocampus lesions fail to induce these changes[109]. More interestingly, bilateral excitotoxic lesion of the VH during early postnatal life, i.e. postnatal day (PD) 7, leads to normal prepubertal behaviors but many abnormal behaviors after puberty[92]. At PD56, nVH lesioned rats show increased locomotor activity to mild stress such as novelty and saline injections in comparison to sham-lesioned animals. Also, AMPH-induced hyperlocomotion is enhanced in nVH lesioned rats as compared to controls. Pretreatment with haloperidol, a dopamine receptor antagonist, reverses these behaviors. nVH lesioned animals also show impaired grooming and social interaction[110, 111], reduction in reward-seeking

behaviors[112], as well as deficits in spatial learning[113], sensorimotor gating[114] and working memory[115].

Other interesting studies have shown that nVH lesion at PD3 also caused abnormal behaviors that are almost all exclusively post-pubertal[116]. nVH lesions at PD14, however, showed behavioral abnormalities similar to rats lesioned in adulthood within 3 weeks of surgery (PD35). Interestingly, transient inactivation of the VH at PD7 using tetrodotoxin (TTX) resulted in enhanced stress- and amphetamine-induced hyperactivity compared to controls, which were not seen when VH of animals were infused with TTX in adulthood[117]. These results demonstrate that a 'critical period' exists within which a loss of function of the VH may cause developmental aberrations leading to various abnormal post-pubertal behaviors analogous to symptoms of schizophrenia. It is known that during the period of late gestation to early postnatal weeks, the hippocampus of rats undergoes extensive neurogenesis and strengthens its active synapses with other brain regions such as the prefrontal cortex, amygdala, nucleus accumbens, and thalamus[118, 119]. This period is considered equivalent to the second half of human fetal development[120, 121]. Disruption of the hippocampus during this period may interfere with normal synaptic strengthening and pruning leading to misconnections which may play a role in the abnormal development and function of connecting brain regions, and the aberrant behaviors analogous to symptoms of schizophrenia.

Studies on the different effects of nVH lesions in male and female rats have shown that although both sexes showed increased post-pubertal locomotor activity only male rats showed a significant deficit in spatial working memory[122]. Furthermore, while female rats showed a decrease in the number of social interactions, males showed a decrease in the duration of these interactions. These studies demonstrate a differential effect of VH abnormality on the behavior of different sexes.

In addition to sex differences, nVH lesions also have a differential effect on different strains of rat[123]. Different strains of rat vary in many respects, such as their behavioral and neuroendocrine responsiveness to stress, and preference for drugs of abuse. Fischer rats are on one end of the spectrum, being highly sensitive to stressful situations, and relatively resistant to inflammatory diseases. Lewis rats, on the other hand, show reduced responsiveness to stress, high susceptibility to autoimmune diseases, and very high sensitivity to drugs of abuse. Sprague-Dawley rats show moderate levels of all three phenomena. nVH lesion on each of these strains of rat demonstrates an interaction of genetic makeup with environmental manipulations, where nVH lesions caused exclusively post-pubertal disruptions in behavior in Sprague-Dawley rats, while the same lesions disrupted pre-pubertal and post-pubertal behaviors in Fischer rats. nVH lesions, however, had no significant effect on the behavior of Lewis rats at any age. These results support theories of gene-environment interactions in complex mental disorders by demonstrating that genetic differences that influence differential responsiveness to neurodevelopmental disruption may determine the emergence of abnormal behaviors later in life.

10.3. The Medial Prefrontal Cortex in nVH Lesioned Animals:

Early abnormality of the VH is thought to also affect development of connecting brain regions, which may be responsible for many of the abnormalities seen later on in life. Of these connecting brain areas, the medial prefrontal cortex (mPFC), which receives afferent projections from the hippocampus[124], is largely implicated in the neuropathology of schizophrenia. Post-mortem studies of brains of schizophrenia patients have shown a reduction in size of cortical neurons and a decreased number of cortical GABAergic neurons[125-130]. PET studies have revealed lower activity of the frontal brain regions during various cognitive tasks, which has been correlated with negative symptoms of schizophrenia[131, 132].

In the nVH lesion model of schizophrenia, rats display many cognitive deficits, such as impairments in working memory, attention and learning, suggesting abnormal mPFC function in these animals[113, 114]. Lesion of the mPFC in adult nVH lesioned rats reverses the post-pubertal hypersensitivity to novelty-stress and AMPH seen in these animals[133]. These results propose that development in the absence of a normal hippocampus leads to disruption of normal neuronal circuits, particularly in the mPFC, which may be responsible for the abnormal behaviors seen in nVH lesioned rats.

On a molecular level, nVH lesions are shown to induce rapid and transient DNA damage in the striatum and NAcc[134], possibly through connections via the mPFC. In vivo levels of N-acetylaspartate (NAA), a chemical marker of neuronal integrity, is reduced in the mPFC of nVH lesioned rats following puberty without a significant

change in cortical area size[135]. Glutamate binding is increased post-pubertally in the mPFC, while D1, D2, and D3 dopamine receptor binding sites are decreased in the striatum[136, 137], possibly as compensatory mechanisms for a hypoactive glutamatergic and hyperactive dopaminergic systems, respectively. Expression of neurotrophic factor BDNF and transcription factor NGFI-B, which play a role in neurodevelopment and responses to stress and injury[138], are also reduced in the PFC of nVH lesioned animals post-puberty[139, 140]. Decreases in the expression of glutamic acid decarboxylase-67 (GAD-67), a precursor of GABA, have also been found in the mPFC[141]. The enhanced mPFC metabolic response to VTA stimulations in nVH lesioned rats suggest a higher energy demand for a hyperactive mPFC in these animals[142]. Aberrations in intracellular signal transduction mechanisms have also been demonstrated through an attenuation of AMPH-induced increase in c-fos mRNA expression in mPFC, striatum and NAcc as compared to sham animals[143].

Morphological studies have demonstrated a reduction in dendritic length and branching, and spine density in the mPFC of adult nVH lesioned rats, as well as a decrease in spine density in the NAcc[144] suggesting altered neuronal plasticity in these brain regions due to a decrease of excitatory projections from the VH. Electrophysiological studies have shown increased spike firing in the mPFC[145] and NAcc[146] of nVH lesioned rats in response to VTA stimulation, demonstrating an abnormal response of these brain regions to dopamine release during stress. The increased NAcc firing in response to VTA stimulation was reversed by mPFC lesions[147], which is in correlation with behavioral data that has shown a reversal of

locomotor deficits following mPFC lesion[133], suggesting that abnormal activation of the mPFC may be, at least partially, responsible for the abnormal behaviors seen in nVH lesioned animals during periods of stress.

10.4. Dopaminergic Abnormalities in nVH Lesioned Animals:

Initially, the neurochemical basis for hyperresponsiveness of nVH lesioned animals to psychostimulants was thought to be increased dopamine levels in the NAcc. However, studies have demonstrated that nVH lesioned rats show similar baseline and AMPH-induced levels of dopamine and its metabolites, DOPAC and HVA, in the NAcc as the control group before and after puberty[148]. More surprisingly, another study has shown an attenuated increase in AMPH-induced release of DA in nVH lesioned rats[149]. In support of this data, another study has shown that DA release in the NAcc in response to stress is attenuated in nVH lesioned rats, contrary to expected results considering behavioral hyperresponsiveness to stress[150]. These results suggest that increased DA transmission and hyperlocomotion seen in lesioned rats may not be due to increased presynaptic DA release in the NAcc, but a dysfunction in postsynaptic DA receptors. Subsequent studies have shown that D2/D3 dopamine receptor agonist, quinpirole, but not D1 receptor agonist, SKF38393, increases locomotor activity in both sham and lesioned groups, with a higher increase in locomotion in lesioned animals[151]. Also, AMPH-induced hyperlocomotion in lesioned and sham animals was blocked by raclopride, a D2 dopamine receptor antagonist. Ligand autoradiography of the NAcc of nVH lesioned animals have shown that, following puberty, levels of D3 dopamine receptors are markedly reduced while levels of D2 dopamine receptors are

unchanged[137]. These results suggest that locomotor hyperactivity to stress and AMPH seen in nVH lesioned animals may be, at least in part, due to postsynaptic hypersensitivity of D2 dopamine receptors that have a stimulatory role on locomotor behavior, and/or decreased levels of D3 receptors that inhibit locomotion, at the level of NAcc.

11. Interaction of Other Neurotransmitter Systems with the Dopaminergic System:

In addition to the role of the dopaminergic system in behaviors abnormal in nVH lesioned animals, other neurotransmitter systems have also been shown to interact with the dopaminergic system in order to modulate these behaviors. For example, 5-HT(1B) serotonin receptors have been shown to play a permissive role in the locomotor response to acute AMPH, as well as in the development of sensitization to chronic AMPH[152]. Other studies have demonstrated that memory impairments caused by muscarinic acetylcholine receptor antagonists can be ameliorated by D2 dopamine receptor agonists in the hippocampus, indicating an interaction of the cholinergic and dopaminergic systems in mnemonic processing[153]. Another neurotransmitter system that has been shown to play a vital role in dopamine-related behaviors is the noradrenergic system, which, more recently, has been suggested to be abnormal in schizophrenia as well as in some animal models of schizophrenia. Due to increasing support for a role of the noradrenergic system in various behaviors disrupted in schizophrenia, and its direct relevance to the present study, a more detailed description of this neurotransmitter system is provided below.

12. The Noradrenergic System:

Cell bodies of the central noradrenergic system originate in the locus coeruleus (LC) in the brainstem, which sends projections to various regions throughout the forebrain, including the hippocampus, amygdala, NAcc and cerebral cortex[154]. These neurons release noradrenaline (NA), also known as norepinephrine, which is a catecholamine involved in many brain functions such as sleep-wakefulness, attention, novelty-induced behaviors, stress response, learning and memory[155]. NA interacts with three families of G-protein linked adrenergic receptors (AR), each with three subtypes – α-1 AR (α-1A, α-1B, and α-1D), α-2 AR (α-2A, α-2B and α-2C) and β AR (β1, β2, and β3)[156, 157]. NA has highest affinity for the α-2 ARs, followed by α-1 ARs, and lowest affinity for β ARs. α-1 ARs are linked to Gq proteins that activate the phospholipase C pathway which leads to activation of protein kinase C (PKC). α-2 ARs are linked to Gi/o proteins that inhibit adenylyl cyclase leading to reduced activation of protein kinase A (PKA). β AR are linked to Gs proteins that activate adenylyl cyclase leading to activation of PKA. Activated PKC and PKA then phosphorylate other proteins involved in a variety of neuronal events, such as ion flux, gene transcription, etc.

12.1. Development of the Noradrenergic System:

In the rat brain, noradrenergic neurons differentiate between gestational day (GD) 10 and 13, and NA can be detected in the brain soon after[158]. Noradrenergic cell bodies begin to proliferate and their axons project to various regions of the brain by the last week of gestation. Projections of noradrenergic axons from the locus coeruleus to the cerebral cortex occurs between GD16-18. By the first postnatal week, the number of

noradrenergic cell bodies has reached adult levels; however, the axon terminals continue to proliferate and form axonal varicosities. The spontaneous firing rate of the locus coeruleus is low during the first week after birth, and then significantly increases during the following weeks until it reaches adult firing rates by PD20.

Interestingly, many known animal models of schizophrenia interfere with brain development during crucial time points of development of the noradrenergic system. For example, in the MAM-model of schizophrenia, pregnant dams are injected with MAM at GD16-17[159], the period when noradrenergic cell bodies send their axonal projections to the cerebral cortex. Hypoxia is administered during birth or during the first week after birth[160], which may disrupt proliferation of noradrenergic cell bodies. Similarly, nVH lesions should be performed within the first week after birth in order to lead to exclusively post-pubertal behavioral abnormalities[161]. These associations of time-frames for brain insult in animal models of schizophrenia, and periods of development of the noradrenergic system suggest that some abnormalities in schizophrenia may be a result of aberrations in normal development of the adrenergic system.

At birth, α-1 ARs are found at very low levels in the rat brain, which increase to above adult levels by PD20, and then decrease steadily to adult levels[162]. Some subtypes of α-2 AR (α-2A and C) are found at high levels in the brain at birth and decrease slightly by adulthood, while others (α-2B) are found at lower levels at birth and then increase to adult levels[163-165]. β-ARs are found at very low levels at birth, but then

rapidly increase until PD20 to above adult levels, followed by a decrease to adult levels[166].

12.2. Interaction of the Noradrenergic System with the Dopaminergic System:

Investigation into the dynamics of the noradrenergic system and its interaction with the dopaminergic system in stress response has revealed that stress-induced release of NA in the mPFC leads to stress-induced release of DA in the NAcc[167]. Other studies have shown that cortical NA release leads to cortical DA release through activation of α-1 AR, while cortical DA release leads to cortical NA release via activation of D1 dopamine receptors[168]. Prazosin, an α-1 AR antagonist, reduces hyperlocomotion induced by d-amphetamine[169] and dose-dependently decreases burst firing of dopamine neurons in the VTA[170].

A very interesting study on the dynamics of NA and DA release in response to stress has shown that restraint stress leads to an initial, short-lived increase in NA release in the mPFC which corresponds with, and is necessary for, increased DA release in the NAcc[171]. This is followed by a delayed and sustained increase in DA release in the mPFC which corresponds with, and is necessary for, a decrease in DA release in the NAcc to below resting levels. One speculation may be that the initial stress-induced NA release in the mPFC may be required for the necessary accumbens and behavioral response to stress, while the delayed DA release may be required for restoring balance in the system after its reaction to the stressful stimulus. A balance between the noradrenergic and dopaminergic systems at the level of the mPFC may be important for

a normal accumbens stress-response, and this balance may be disrupted in psychiatric illnesses such as schizophrenia.

II. RATIONALE:

1. The Noradrenergic System in Schizophrenia:

In the past decade, atypical neuroleptics have gained a lot of attention due to their higher efficiency in ameliorating positive as well as negative and cognitive symptoms of schizophrenia[172]. These antipsychotic drugs act on many other neurotransmitter receptors in addition to dopamine receptors, such as serotonin, acetylcholine and noradrenaline receptors[173-175], leading to the hypothesis that other neurotransmitter systems, and perhaps their interaction with the dopaminergic system, may also be disrupted in schizophrenia. Among these neurotransmitter systems, the noradrenergic system has received some modest attention due to its role in arousal, attention, and stress response. Recent hypotheses state that an aberration in the noradrenergic system in schizophrenia patients may be associated with the observed deficits in attention and information processing, and abnormal response to stress[176]. In support of this hypothesis, molecular studies have demonstrated increased concentrations of noradrenaline (NA) in the cerebrospinal fluid (CSF) of patients with schizophrenia when compared to the CSF of normal individuals and individuals with other psychiatric illnesses[177]. Other studies have shown a positive correlation between negative symptoms of schizophrenia and NA levels in the CSF of patients during relapse[178]. Such findings compel us to research the role of the noradrenergic system in the pathology of schizophrenia.

More recently, the significance of the noradrenergic system in mediating behaviors disrupted in schizophrenia has been studied in animals. Pharmacological

studies have revealed that α-2 AR agonists, such as clonidine and guanfacine, reverse working memory impairments in aged primates and rats[179, 180], while α-1 AR agonists, such as phenylephrine and cirazoline, impair spatial working memory[181, 182]. In addition, α-1 AR antagonist, urapidil, reverses working memory deficits due to stress[183]. Other studies have shown that β AR antagonist, betaxolol, improves working memory performance in rats and monkeys[184]. These results suggest that moderate levels of NA released during normal wakefulness enhance cognitive function by binding to and activating α-2 ARs, while higher levels of NA, released during stress, activate α-1 ARs and β AR, impairing cognitive function. In addition to working memory, α-1 ARs have also been shown to play an important role in other cognitive functions such as sensorimotor gating. α-1 AR agonists cirazoline, phenylephrine, and methoxamine have been shown to reduce PPI in rats via activation of central α-1 AR[185]. The noradrenergic system also plays an important role in locomotor behavior. Modafinil, a drug used in the treatment of depression, increases locomotor activity in rats, mice and monkeys through activation of α-1 ARs[186, 187]. Also, blockade of α-2 AR in the PFC of monkeys has been shown to increase locomotor behavior[188].

2. Abnormal Noradrenergic System in Animal Models of Schizophrenia:

In the MAM model of schizophrenia, rats that were exposed to MAM prenatally showed an exaggerated increase in NA in the mPFC following post-pubertal injections of MK-801, a noncompetitive NMDA receptor antagonist[159]. MK-801 also induces an exaggerated hyperlocomotion in MAM-exposed animals, suggesting that the

enhancement of NA release in the mPFC due to stress and drugs may be responsible for the abnormal locomotor behaviors seen in these animals. In the PCP animal model of schizophrenia, rats pretreated with prazosin show a reversal of PPI deficits induced by PCP, suggesting a role of α-1 AR in mediating negative symptoms of schizophrenia[189].

Previous autoradiography studies in our lab on the noradrenergic system in nVH lesioned animals revealed increased binding of [^{3}H]prazosin to α-1 AR in the PFC of post-pubertal nVH lesioned animals, in comparison to sham animals[190]. No difference in α-1 AR binding was seen in the NAcc of nVH lesioned animals. An increase in binding of norepinephrine transporter (NET) was seen in the mPFC and NAcc of nVH lesioned animals. However, no significant change in the expression of α-2 AR was seen in either brain regions of the lesioned animals. Also, behavioral data showed that pretreatment with α-1 AR antagonist prazosin reduces AMPH-induced hyperlocomotion in sham rats but fails to do so in nVH lesioned rats. These results suggest a possible increase in the sensitivity of α-1 AR in nVH lesioned animals following puberty, such that the doses of prazosin used in this study was insufficient to antagonize α-1 AR in these animals. An alternative hypothesis is that nVH lesions may lead to dysfunctional α-1 AR, such that these receptors do not modulate AMPH-related behaviors as they do in control animals.

III. OBJECTIVES:

My project was to study the initial hypothesis that nVH lesions may lead to a postpubertal increase in sensitivity of α-1 AR. I hypothesized that post-pubertal nVH lesioned animals may show an increased responsiveness to stimulation of α-1 AR. Since these receptors play an important role in locomotor activity and sensorimotor gating, I decided to study these behaviors in nVH lesioned animals in response to α-1 AR agonist cirazoline.

Locomotor activity is widely considered an indicator of mesolimbic dopamine function. As mentioned earlier, nVH lesioned animals have consistently shown abnormal locomotor response to novely, stress and AMPH, with some conflicting data on the role of the mesolimbic dopamine system in these abnormal behaviors. Since the noradrenergic system interacts with the dopaminergic system in modulating locomotor activity, this behavior was studied in nVH lesioned animals in response to α-1 AR agonist cirazoline to investigate whether a potential hyperactive α-1 AR system in nVH lesioned animals may play a role in locomotor activity of these animals.

Sensorimotor-gating mechanisms are a fundamental component of information processing in the brain, necessary for stimulus recognition and sequential organization of behavior[191]. Prepulse inhibition (PPI), a measure of sensorimotor gating, is a phenomenon whereby a weak non-startling stimulus attenuates the startle response to a subsequent, more intense stimulus, representing normal preattentional information processing that is necessary for proper cognitive functioning. Abnormalities in PPI may

indicate dysfunction of the limbic system, including the mPFC and the NAcc, and perhaps the neurotransmitter systems that regulate normal functioning of these brain regions. Since PPI is disrupted in nVH lesioned animals and α-1 ARs have been shown to modulate PPI, this behavior was studied following treatment with agonist cirazoline to investigate whether a potential hyperactive α-1 AR system in nVH lesioned animals may play a role in PPI in these animals.

Also, as an extension to the previous autoradiography study conduced in our lab, I studied whether the prefrontal cortical changes in α-1 AR in post-pubertal nVH lesioned animals may be due to alterations in the transcription of specific α-1 AR subtypes, i.e. α-1A, α-1B and α-1D AR mRNA. The region of focus in my studies was the mPFC due to its role in modulating behaviors that are abnormal in nVH lesioned animals, and the abnormalities seen in ligand binding of this receptor in this brain region.

In other words, I hypothesized that:

- nVH lesioned animals may show increased locomotor activity in response to α-1 AR agonist cirazoline, in comparison to sham animals.
- nVH lesioned animals may show increased deficits in prepulse inhibition in response to cirazoline, in comparison to sham animals.
- nVH lesioned animals may show subtype-specific alterations in mRNA levels of α-1 ARs in the mPFC.

IV. METHODS:

1. Animals:

Male (300-350g) and female (250-300g) Sprague-Dawley rats were obtained from Charles River Laboratories, Inc. (Wilmington, MA) and housed in groups of two in a temperature- and humidity-controlled environment at the animal facility of Douglas Hospital Research Centre on a 12h light/dark cycle with free access to food and water. The animals were allowed to acclimatize to this new environment for a week, after which one male rat was placed in a cage of two female rats and allowed to mate. After fourteen days of being paired together, female rats were visually checked for protrusion of the abdomen, indicating pregnancy. Pregnant females were then housed individually and monitored for delivery. Four days after birth, litters of 8 pups, consisting of a maximum of 7 males, were culled to maintain similar litter size across dams. All animal procedures were in strict accordance with the guidelines of the Canadian Council for Animal Care, and the McGill University Animal Care Committee.

2. Drugs:

Ibotenic acid was obtained from Sigma-Aldrich Corp. (St. Louis, MO), and dissolved in 0.1M phosphate-buffered saline (PBS) to a final concentration of 10 mg/ml. Prazosin (Sigma-Aldrich), an α-1 AR antagonist that crosses the blood-brain barrier (BBB), was dissolved in DMSO and diluted to 1.0 mg/ml in distilled water. Cirazoline, an α-1 AR agonist that crosses the BBB, was obtained from Tocris (Ellisville, MO) and dissolved in sterile isotonic (0.9%) saline to make final concentrations of 0.1, 0.3, 0.45, and 0.6 mg/ml. The injection volume for systemic

administration of prazosin and cirazoline, which readily cross the blood-brain barrier, was 1ml/kg, i.p.

3. Surgery:

nVH lesions were conducted as described by Flores, et al (1996)[137] with minor modifications. On postnatal day (PD) 7, male pups from each litter were weighed and those within 15-17g were randomly assigned a status of 'sham' or 'lesion'. Pups were anesthetized by hypothermia by placing them on wet ice for 18-20 min, and were positioned on a platform fixed to a stereotaxic Kopf instrument (Stoelting Co, IL). An incision was made on the head of the pup, and holes drilled in the skull at the area above the VH. A sterile 30-gauge stainless steel canula was inserted into the brain and positioned at: anteroposterior -3.0mm, mediolateral +/-3.5mm to bregma, and dorsolateral -5.0mm from dura. The canula released 0.3µl of either ibotenic acid (10 mg/ml, for lesion group) or vehicle (0.1M PBS, for sham group) into each VH over 2 min, and remained in place for an additional 2 min to allow the drug to diffuse into the brain. After the canula was removed, the incision was treated with antibiotic powder (Cicatrin) and the incision was sutured and placed on a heated pad for recovery, after which they were returned to their respective dams. On PD21, the males were weaned and housed in groups of two, consisting of one sham and one lesioned rat.

4. Locomotor Behaviour:

At post-pubertal age PD56, sham and lesioned animals were individually and randomly placed in one of twelve activity chambers (30 x 40 x 40 cm) housed in a

dimly lit room. Each chamber was equipped with two photoelectric switches; light beam interruptions from each chamber were monitored and stored in a computer (ACTANAL software, Concordia University). 'Locomotor activity' was measured by consecutive breaking of the two photoelectric switches in a box; breaking only one switch was considered 'other activity', and total activity was the sum of these two measures. Measurements of total activity were reported every 10 min. The animals were allowed to habituate to their environment for 60 min, after which they each received intraperitoneal (i.p) injections of saline (0.9%). Locomotor activity of each animal was tested for 60 min. Next, each animal received i.p injections of cirazoline (0.1 mg/kg), and after 5 mins, they were placed again in their respective boxes and cirazoline-induced locomotion was recorded for 60 min. This procedure was repeated after one week, with a cirazoline dose of 0.3 mg/kg, and after an additional week with 0.6 mg/kg, to test locomotor activity in response to a range of doses of cirazoline. Locomotor experiments were conducted between 9am and 3pm, during the light phase of the animals.

5. Prepulse Inhibition (PPI):

PPI of the acoustic startle response (ASR) was studied in post-pubertal animals using a commercially available system (SR-LAB; San Diego Instruments, San Diego, CA) comprised of two sound-attenuating chambers, each equipped with a cylindrical Plexiglas animal enclosure (length, 16 cm; inner diameter, 8.2 cm). A 70dB background noise was provided by a small electric fan used for ventilation. A speaker positioned 24cm directly above the enclosure provided the broadband tone pulses. A piezoelectric

accelerometer affixed to the animal enclosure frame was used to detect and transduce motion resulting from the animals' startle response. Tone pulse parameters were controlled by a microcomputer using a commercial software package (SR-LAB) and interface assembly that also digitized (0-4095), rectified, and recorded stabilimeter readings.

All PPI studies were conducted between 9am and 5pm. Post-pubertal animals (PD 56-60) of both sham and lesioned groups were equally assigned one of two groups – the 'treatment' group received i.p injections of either cirazoline (0.3, 0.45 or 0.6 mg/kg), prazosin (1.0 mg/kg), or prazosin followed by cirazoline, while the 'vehicle' group was injected systemically with 0.9% saline. Saline-treated and drug-treated animals were then randomly placed in the Plexiglas enclosure and allowed to acclimatize to the environment with background noise for 5 min before being tested during 27 discrete trials. On the first two trials, the magnitude of the startle response to a 50ms duration, 120 dB tone was measured. These first two startling tones were presented to habituate the animals to the testing procedure and thus were omitted from the data analysis. On the subsequent 25 trials, the startle tone was either presented alone or 100 ms after presentation of a 30 ms duration prepulse with intensity ranging from 3 to 15 dB above background noise (73-85 dB) varied randomly between trials in 6 dB steps. ASR was measured at each of the three prepulse intensities on five trials; animals were randomly presented with the startle tone alone during the other 10 trials. The same stimulus condition was never presented on more than two consecutive trials. The interval between each trial was programmed to a variable time schedule with an average

duration of 15s (range, 5-30 s). A measure of ASR amplitude was derived from the mean of 100 digitized data points collected from stimulus onset at a rate of 1 kHz.

6. In situ Hybridization:

6.1. Tissue Preparation:

Animals were decapitated in a clean, dry area and their brains were quickly removed, snap-frozen in isopentane cooled on dry ice (-40°C) and stored in aluminum foil at -80°C. Using a cryostat, coronal sections (14μm thickness) at the level of the mPFC were collected on pre-cleaned, pre-coated X-tra® microscope slides (Surgipath, Winnipeg, MB) (three sections per slide) and stored at -80°C in tightly sealed boxes to prevent RNase contamination.

6.2. Oligonucleotide Probes:

Oligonucleotide probes for α-1A, α-1B, and α-1D AR mRNA were obtained from GeneDetect.com Ltd. Antisense (AS, probe) and sense (S, control) sequences for α-1A AR mRNA were 45 base pairs (bp) in length, and derived from rattus norvegicus adrenergic receptor, alpha 1a (Adra1a), mRNA at bases 36-80. Probe and control sequences for α-1B AR mRNA were also 45 base pairs (bp) in length, and derived from rattus norvegicus adrenergic receptor, alpha 1b (Adra1b), mRNA at bases 397-441. Probe and control sequences for α-1D AR mRNA were 47 base pairs (bp) in length, and derived from rattus norvegicus adrenergic receptor, alpha 1d (Adra1d), mRNA at bases 241-287. Each of the probes showed a sequence specificity of 100%. The sequences of each probe are as follows:

α-1A AS:

5’GGTGCAGTTGGAGCCTTCCGAAGCATTTTCAGAGAGAAGCACCAT

α-1A control:

5’CCACGTCAACCTCGGAAGGCTTCGTAAAAGTCTCTCTTCGTGGTA

α-1B AS:

5’CCCAGTGGGCAGGTGCTGATGTGTTGTGGCCGGTGTCCAGATCGG

α-1B control:

5’GGGTCACCCGTCCACGACTACACAACACCGGCCACAGGTCTAGCC

α-1D AS:

5’GAAGGTCCCAATGGGAACGTTGGTGTGAGGGAACCGACTCCGGCGGT

α-1D control:

5’CTTCCAGGGTTACCCTTGCAACCACACTCCCTTGGCTGAGGCCGCCA

6.3. Preparation of Digoxigenin-labeled Oligonucleotide Probes:

Lyophilized oligonucleotide probes were reconstituted with sterile Tris-EDTA buffer to a final concentration of 10 pmol/µl. A DIG oligonucleotide 3’-end labeling kit (Roche Applied Science) was used to label the oligonucleotide probes by adding, in sequence, 4µl 5x reaction buffer (1M potassium cacodylate, 0.125M Tris-HCl, 1.25 mg/ml bovine serum albumine, pH=6.6), 4µl 25µM $CoCl_2$ solution, 1µl 1mM digoxigenin-ddUTP solution, 1µl terminal transferase (in 60mM K-phosphate (pH=7.2), and 150mM KCl, 1mM 2-Mercaptoethanol, 0.5% Triton X-100, 50% glycerol) to 10ul of 100 pmol oligonucleotide probe. The solutions were then mixed briefly and

incubated at 37°C for 15min, and placed on ice. The labeled probe was then either used immediately, or stored in a tightly sealed box at -80°C until use.

6.4. Pre-hybridization Steps:

Only sterilized apparatuses and solutions were used in this experiment. Frozen mPFC sections were immediately fixed in 4% paraformaldehyde (in 0.1M phosphate buffer) at 4°C for 10-15 min, then washed three times for 5min in 0.1M phosphate buffer (NaH_2PO_4, Na_2HPO_4). Next, the sections were dehydrated for 5 min in 100% ethanol and then air dried.

Hybridization buffer (5M NaCl, 1M Tris (pH=7.5), 0.5M EDTA (pH=8.0), 100x Denhardt's solution, 10 mg/ml ssDNA, 1 mg/ml tRNA, 7% Dextran sulphate) was mixed with deionized formamide in a 1:1 ratio, and incubated at 37°C for a few minutes to reduce viscosity. The buffer was then separated into vials labeled for each sense and antisense oligonucleotide probe. 3.5µl of the respective DIG-labeled oligonucleotide probe was added to each 1ml of hybridization buffer, and mixed thoroughly.

The slides were then placed in a humidified hybridization box, and 200µl of the respective hybridization buffer was pipetted onto each section. Parafilm was lightly placed over each section and the humidified chamber was tightly sealed and incubated overnight at 37°C.

6.5. Post-hybridization Steps:

The next day, the sections were washed thoroughly in 2x SSC (at 55°C) twice for 15min, and in 0.1x SSC (at 55°C) twice for 15min. Then, the slides were placed in blocking solution containing TBS (100mM Tris-HCl (pH=7.5), 100mM NaCl, 2mM $MgCl_2$) containing 1%BSA for 30min, after which they were placed face-up in the humidified hybridization box. 200µl of AP conjugated anti-DIG antibody (Roche Applied Science, Indianapolis, IN) dissolved in the above TBS/BSA solution (1:500) was added to each section and fresh parafilm was lightly placed over each section. The slides were incubated for 2h at room temperature. Next, the slides were washed thrice for 15 min with TBS (pH=7.5, without BSA), and once for 15 min with TBS (100mM Tris-HCl (pH=9.5), 150mM NaCl, 5mM Mg Cl_2) at room temperature. Then, 200µl of a staining solution containing nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate, toluidine salt (NBT/BCIP, Roche Applied Science, Indianapolis, IN) dissolved in TBS (pH=9.5) (1:50) was added to each section and incubated in the humidified box at room temperature until coloration appeared. The reaction was stopped by placing slides in sterile water for 10 min, after which the slides were air dried. The sections were then mounted with DPX permount (Sigma-Aldrich, St. Louis, MO), and cover slips were placed on top of each section. Labeling was analyzed using a light microscope and Nikon Eclipse 50i software (Nikon, Japan).

7. Lesion Analysis:

To check lesions, 35µm thick coronal sections at the level of the hippocampus were obtained from sham and nVH lesioned animals and treated with serial dilutions of

ethanol – 2 min in 75% ethanol, 2 min in 95% ethanol, and 2 min in 100% ethanol. Next, the slides were immersed for 10 min in 0.1M phosphate buffer, followed by 3 min in 0.05% cresyl violet staining solution. The slides were then washed in serial dilutions of ethanol – 1 min in 75% ethanol, 2 min in 95% ethanol, 2 min in 100% ethanol, and 2 min in fresh 100% ethanol, after which it was immersed in xylene for a minimum of 3 min. Finally, sections were mounted with permount and cover slips were placed over each slide. Sections were analyzed using a light microscope and MCID software. Hippocampal sections that showed bilateral neuronal loss and atrophy of the VH, while sparing the dorsal hippocampus were considered as good nVH lesions. The hippocampus of sham animals was also checked for the absence of lesion.

8. Data Analysis:

For locomotion experiments, total locomotor activity of each animal was calculated by adding the total number of single beam crossings and the total number of double beam crossings. Locomotor activity was then compared between groups using two-factor repeated-measures analysis of variance (ANOVA) with treatment as a within-subjects factor, followed by ***post hoc*** analyses using Tukey's ***t***-tests. The α level of significance was set at 0.05.

For PPI experiments, startle response was recorded for each Pulse-Alone and Pre-pulse + Pulse trial. Startle magnitude was calculated as the average of the startle responses to the Pulse-Alone trials. Prepulse effectiveness in suppressing ASR was expressed as a percentage based on the mean amplitude of responses to the startle tone

alone (***n*** = 10) relative to those recorded under the three prepulse conditions (***n*** = 5 per condition) where:

$$\%PPI = 100 - \left(\frac{\text{Startle response for Pre-pulse+Pulse trial}}{\text{Startle response for Pulse-Alone trial}}\right) \times 100\%.$$

Startle response data was analyzed using two-factor ANOVA with status and drug as between-subjects factors. PPI data was analyzed with three-factor repeated measures ANOVA, with status and drug as between-subjects factors and prepulse intensity as within-subjects factor. Post hoc analyses were done using Tukey's ***t***-tests. The α level of significance was set at 0.05.

For in situ hybridization experiments, mRNA expression was analyzed using SPOT Software 4.1 – Advanced version (Diagnostic Instruments Inc., Sterling Heights, MI). The number of cells were counted that were positively labeled for each α-1 AR subtype in the mPFC region of the left and right hemispheres of prefrontal cortical sections (corresponding to Plate 8-9 of Paxinos and Watson Rat Brain Atlas, 1986) labeled with antisense probes. Cells were considered positively labeled if their optical density was significantly greater than that of control sections (labeled with sense probes). Data from three sections per animal, and four sham and four nVH lesioned animals per subtype were used in the analysis. Results of in situ hybridization experiments were analyzed using Student's t-tests, and the means of the total number of labeled cells were compared between sham and nVH lesioned animals for each α-1 AR subtype.

V. RESULTS:

1. Lesion Verification:

Bilateral lesions of the VH were verified using Cresyl violet staining of hippocampal sections from the brains of sham and nVH lesioned animals. As depicted in Figure 1, bilateral neuronal loss, atrophy, retraction of the hippocampal formation, and sometimes cavities, were observed in the ventral hippocampus (CA1, CA2, and some of the CA3) and the ventral subiculum of nVH lesioned animals, but not of sham-operated animals. In addition, this lesion is contained within the VH and does not extend to adjacent areas such as the dorsal regions of the hippocampus, the thalamus, or the amygdala.

2. Locomotor Activity:

2.1. Locomotor Response to α-1 AR Agonist Cirazoline (0.1 mg/kg):

Sham and nVH lesioned animals (***n*** = 9–10) were placed individually in a locomotor box and were allowed to habituate to the environment for 60 min. Locomotor activity was monitored in both groups of animals for 60 min following systemic injections of saline, and for an additional 60 min following systemic injections of 0.1 mg/kg cirazoline, i.p (Fig. 2). Two-way repeated-measures ANOVA of the data on locomotor activity obtained during the 60 min following saline and cirazoline injections revealed no significant main effect of lesion ($F(1,17)=1.839$, $P=0.192$), drug ($F(1,17)=0.095$, $P=0.761$), or lesion x drug interaction ($F(1,17)=1.132$, $P=0.3021$).

2.2. Locomotor Response to α-1 AR Agonist Cirazoline (0.3 mg/kg):

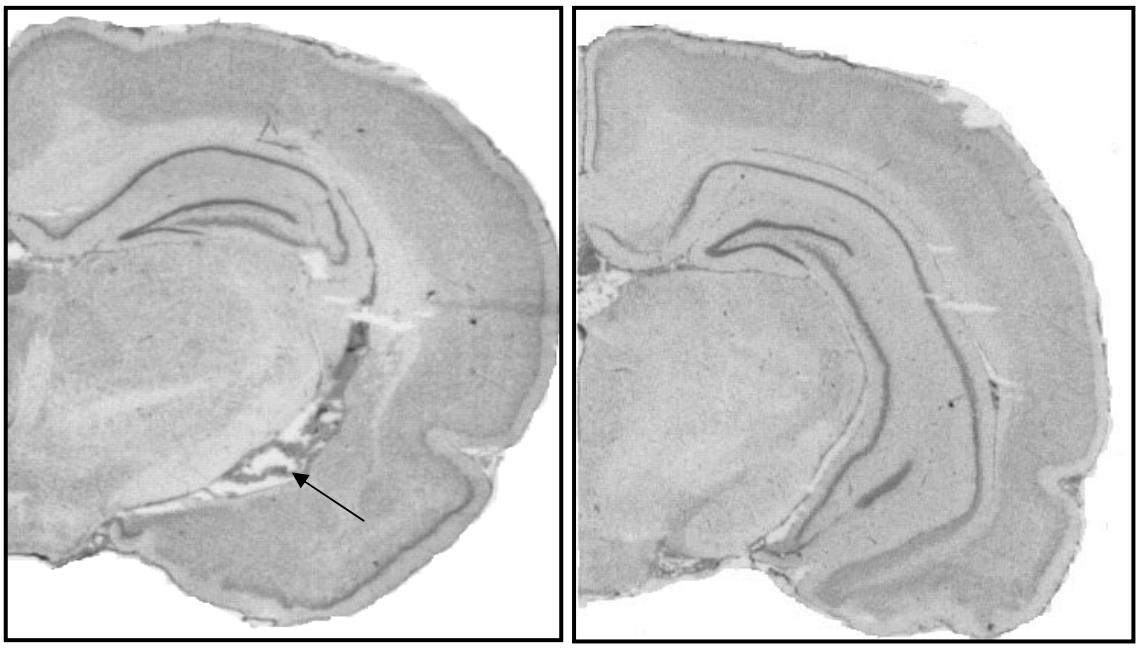
One week later, the animals were again tested as described above, with 0.3 mg/kg cirazoline, i.p (Fig. 3). Two-way repeated-measures ANOVA of data on locomotor activity during the 60 min following saline and cirazoline injections revealed no significant main effect of lesion ($F(1,19)=0.257$, $P=0.617$), drug ($F(1,19)=2.481$, $P=0.1317$), or lesion x drug interaction ($F(1,19)=0.008$, $P=0.929$).

2.3. Locomotor Response to α-1 AR Agonist Cirazoline (0.6 mg/kg):

After another week, the animals were again tested as above, with 0.6 mg/kg cirazoline, i.p (Fig. 4). Two-way repeated-measures ANOVA of locomotor activity during the 60 min following saline and cirazoline injections revealed significant main effects of lesion ($F(1,17)=5.471$, $P=0.031$), and drug ($F(1,17)=15.960$, $P<0.001$), but no significant lesion x drug interaction ($F(1,17)=0.906$, $P=0.354$). ***Post hoc*** analysis revealed a significant increase in locomotor activity in nVH lesioned animals in comparison to sham animals ($P=0.007$), and a significant increase in locomotor activity following cirazoline treatment ($P=0.001$).

Thus, stimulation of α-1 AR through systemic injection of receptor agonist cirazoline increased locomotor activity in both sham and nVH lesioned animals only at high doses of 0.6 mg/kg, without any differential effect on locomotor activity of nVH lesioned animals.

REPRESENTATIVE EXAMPLE OF HIPPOCAMPAL FORMATION IN NVH LESIONED ANIMALS



A **B**

Figure 1 – Lesion of the ventral hippocampus in the adult rat brain. (A) A representation of the hippocampal region of an nVH lesioned rat. The arrow points to the region of the ventral hippocampus that has been lesioned in nVH lesioned rats. (B) A representation of the hippocampal region of a sham-operated rat.

LOCOMOTOR ACTIVITY IN NVH LESIONED ANIMALS FOLLOWING 0.1 MG/KG CIRAZOLINE

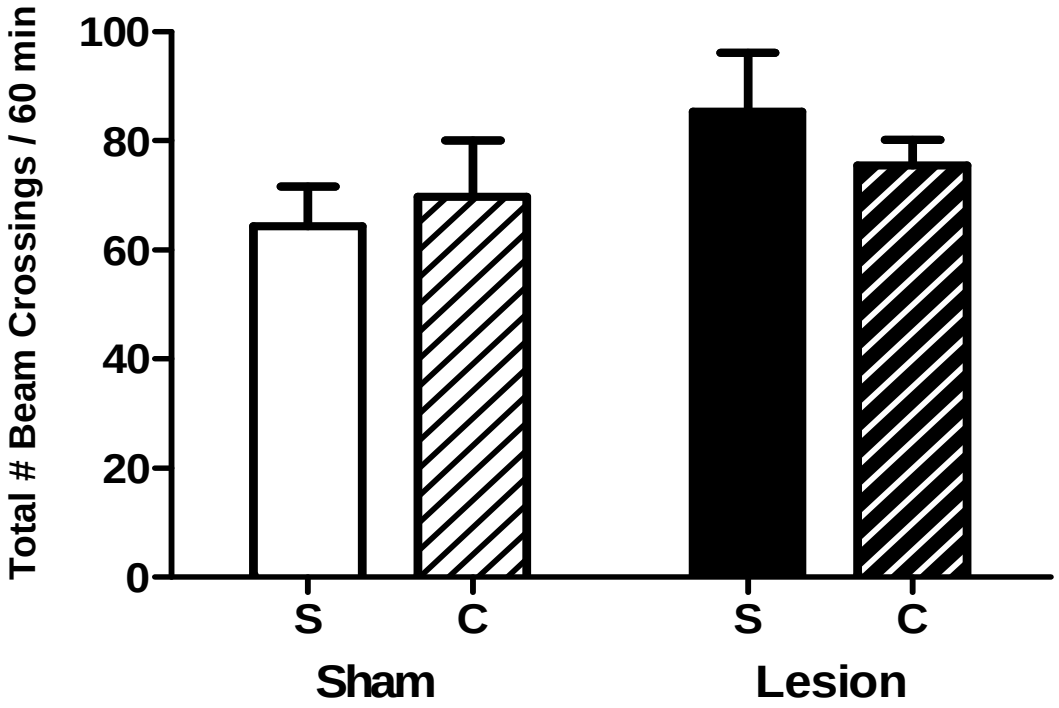


Figure 2 – Locomotor activity of adult sham and nVH lesioned animals (*n* = 9) in response to saline and 0.1 mg/kg cirazoline, i.p. Two-way repeated-measures ANOVA revealed no significant main effect of lesion, drug, or lesion x drug interaction effect. S= saline, C= cirazoline.

LOCOMOTOR ACTIVITY IN NVH LESIONED ANIMALS FOLLOWING 0.3 MG/KG CIRAZOLINE

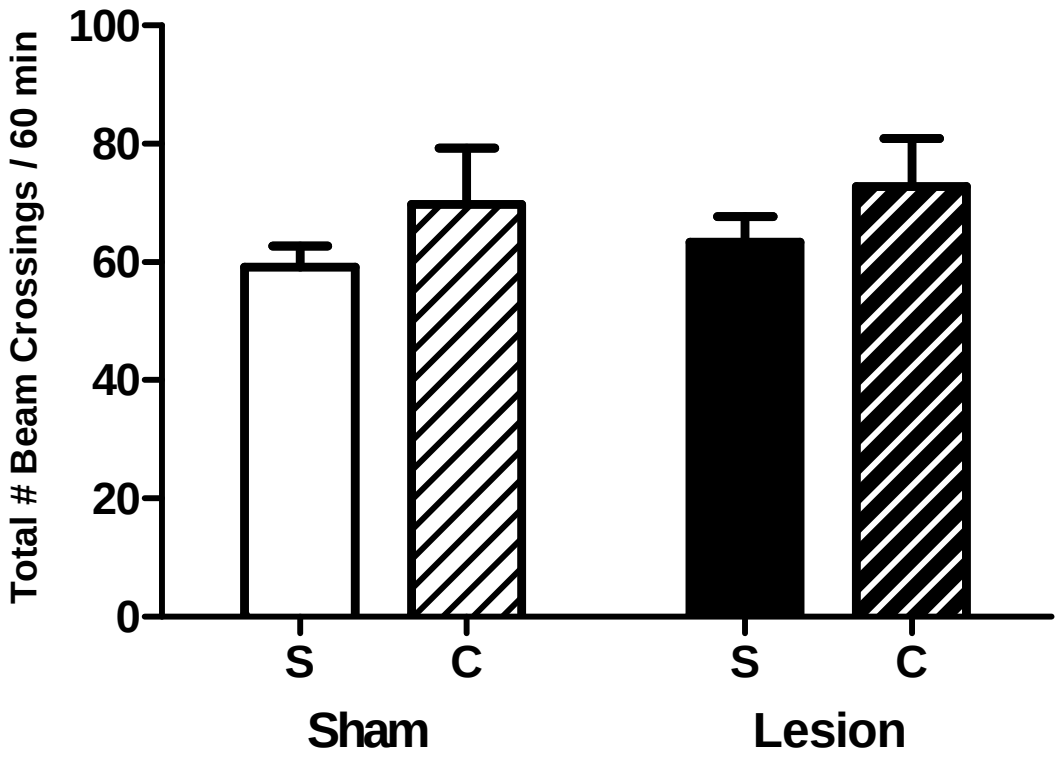


Figure 3 – Locomotor activity in sham and nVH lesioned animals (*n* = 10) in response to saline and 0.3 mg/kg cirazoline, i.p. Two-way repeated-measures ANOVA revealed no significant main effect of lesion, drug, or lesion x drug interaction effect. S= saline, C= cirazoline.

LOCOMOTOR ACTIVITY IN NVH LESIONED ANIMALS FOLLOWING 0.6 MG/KG CIRAZOLINE

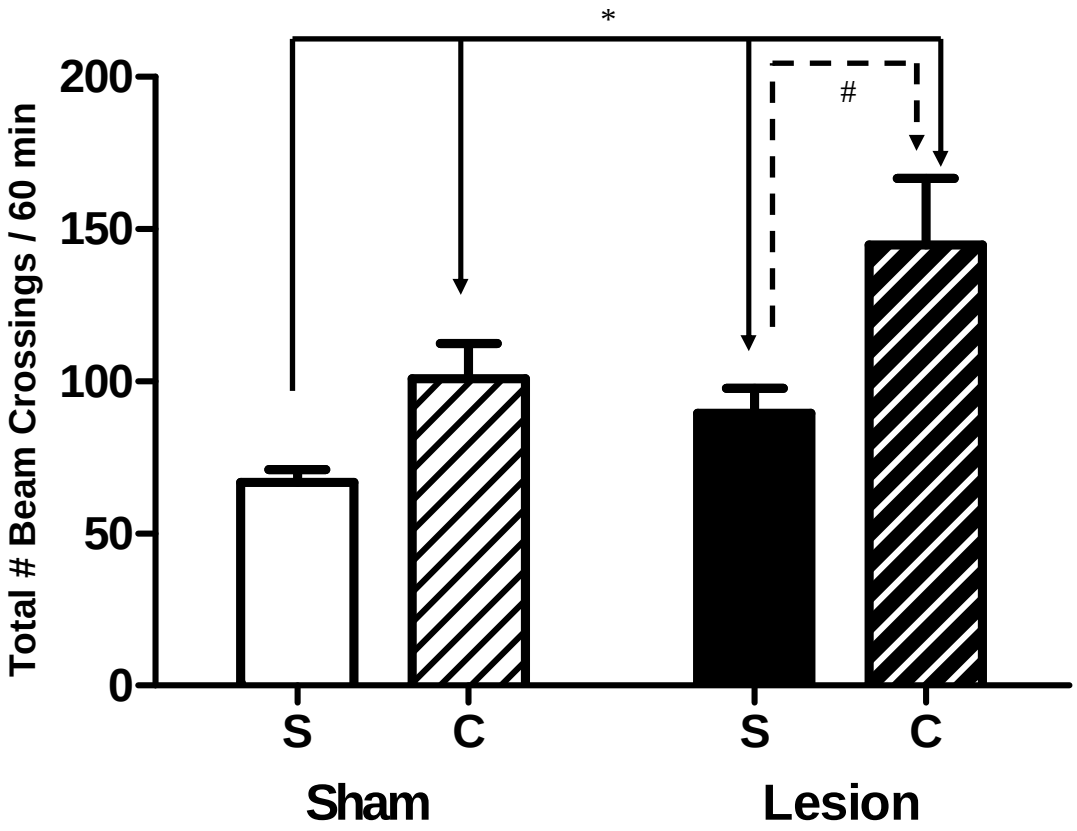


Figure 4 – Locomotor activity of sham and nVH lesioned animals (*n* = 9–10) in response to saline and 0.6 mg/kg cirazoline, i.p. Two-way repeated-measures ANOVA revealed a significant main effect of lesion, and drug, but no significant lesion x drug interaction effect. *Post hoc* analyses revealed that locomotor activity in nVH lesioned animals was significantly higher than that in sham animals following saline treatment. Also, cirazoline treatment increased locomotor activity significantly in both sham and nVH lesioned animals. S= saline, C= cirazoline. * P<0.05 in comparison to Sham(S), # P<0.05 in comparison to Lesion(S).

3. Sensorimotor gating:

3.1. Effect of α-1 AR Agonist Cirazoline (0.3 mg/kg) on PPI:

Sham and nVH lesioned animals (***n*** = 10–12) were injected with either saline or 0.3 mg/kg cirazoline, i.p, and placed individually into the startle chamber, where startle response was measured (Fig. 5). Two-way ANOVA of startle response showed no significant main effect of lesion ($F(1,37)=0.873$, $P=0.356$), drug ($F(1,37)=0.111$, $P=0.740$), or lesion x drug interaction effect ($F(1,37)=2.829$, $P=0.101$).

Three-way ANOVA of %PPI revealed significant main effects of lesion ($F(1,39)=6.48$, $P=0.014$), drug ($F(1,39)=15.93$, $P<0.001$), and prepulse intensity (PP) ($F(2,78)=59.11$, $P<0.001$). There was also a significant interaction between lesion and PP ($F(2,78)=28.409$, $P<0.001$), but no significant lesion x drug ($F(1, 39)=0.018$, $P=0.894$), drug x PP ($F(2,78)=2.335$, $P=0.103$), or lesion x drug x PP ($F(2,78)=3.050$, $P=0.053$) interaction effect. ***Post hoc*** analysis revealed that nVH lesioned animals have a significantly lower PPI than sham animals at PP3 ($P=0.04$), PP9 ($P=0.01$) and PP15 ($P<0.01$). Also, cirazoline treatment significantly reduces PPI ($P<0.01$). These results show that nVH lesioned animals have a lower basal PPI than sham animals, and 0.3 mg/kg cirazoline has a small effect in decreasing PPI in both groups at some PP intensities, without a significant differential effect in either group.

3.2. Effect of α-1 AR Agonist Cirazoline (0.45 mg/kg) on PPI:

One week later, sham and nVH lesioned animals were injected with either saline or 0.45 mg/kg cirazoline, i.p and placed into the startle chamber (Fig. 6). Two-way

ANOVA of startle response revealed no significant main effect of lesion ($F(1,31)=3.432$, $P=0.073$), drug ($F(1,31)<0.001$, $P=0.994$), or lesion x drug interaction effect ($F(1,31)=2.201$, $P=0.148$).

Three-way ANOVA of %PPI showed significant main effects of lesion ($F(1,32)=71.66$, $P<0.001$), drug ($F(1,32)=39.64$, $P<0.001$), and PP ($F(2,64)=91.60$, $P<0.001$), and a significant interaction effect of lesion x drug ($F(1,32)=39.64$, $P<0.001$). However, there was no significant lesion x PP ($F(2,64)=3.109$, $P=0.051$), drug x PP ($F(2,64)=0.852$, $P=0.431$), or lesion x drug x PP ($F(2,64)=0.464$, $P=0.630$) interaction effect. ***Post hoc*** analysis revealed a significant decrease in PPI in nVH lesioned animals compared to sham-lesioned animals after saline treatment ($P=0.02$) and cirazoline treatment ($P<0.01$). Interestingly, cirazoline significantly reduced PPI in nVH lesioned animals ($P<0.01$) but did not have a significant effect on PPI in sham animals ($P=0.54$). These results show that 0.45 mg/kg cirazoline had a significantly greater PPI-disruptive effect on nVH lesioned animals than sham animals.

3.3. Effect of α-1 AR Agonist Cirazoline (0.6 mg/kg) on PPI:

The following week, sham and nVH lesioned animals received another injection of either saline or 0.6 mg/kg cirazoline, i.p and their startle response was measured (Fig.7). Two-way ANOVA of startle response revealed no significant main effect of lesion ($F(1,34)=3.316$, $P=0.077$), drug ($F(1,34)=0.093$, $P=0.761$), or lesion x drug interaction effect ($F(1,34)=0.001$, $P=0.972$).

Three-way ANOVA of %PPI showed a significant main effect of lesion ($F(1, 34)=9.766$, $P=0.003$), a significant main effect of drug ($F(1,34)=11.79$, $P=0.001$), and a significant main effect of PP ($F(2,68)=239.13$, $P<0.001$). There was also a significant interaction effect between lesion and PP ($F(2,68)=6.731$, $P=0.002$), drug and PP ($F(2,68)=13.777$, $P<0.001$), and lesion, drug and PP ($F(2,68)=12.731$, $P<0.001$), but no significant lesion x drug interaction effect ($F(1,34)=1.86$, $P=0.181$). ***Post hoc*** analysis revealed a significantly lower PPI after saline treatment in nVH lesioned animals in comparison to sham-lesioned animals at PP3 ($P=0.04$) and PP15 ($P=0.04$), a significant decrease in PPI in cirazoline-treated sham animals compared to saline-treated sham animals at PP3 ($P=0.04$), PP9 ($P<0.01$) and PP15 ($P=0.03$), and a significant decrease in PPI in cirazoline-treated nVH lesioned animals compared to saline-treated nVH lesioned animals at PP3 ($P<0.01$), PP9 ($P=0.01$) and PP15 ($P<0.01$). These results show that 0.6 mg/kg cirazoline is a high enough dose to significantly reduce PPI in both sham and nVH lesioned animals.

Therefore, high doses of cirazoline disrupts PPI in both sham and nVH lesioned animals. However, an intermediate dose of cirazoline (0.45 mg/kg, i.p) that does not significantly alter PPI in sham-operated animals substantially decreases PPI in nVH lesioned animals. This differential effect may be due to increased responsiveness of α-1 AR in nVH lesioned animals.

3.4. Blockade of the Effect of α-1 AR Agonist Cirazoline (0.45 mg/kg) on PPI by Pretreatment with α-1 AR Antagonist Prazosin (1.0 mg/kg):

In order to study whether the above differential effect of an intermediate dose of cirazoline on PPI in nVH lesioned animals was due to stimulation of α-1 AR, sham and nVH lesioned animals (***n*** = 8–12) were injected either with saline, or 1.0 mg/kg prazosin followed by 0.45 mg/kg cirazoline, and startle response was measured (Fig. 8). Two-way ANOVA of startle response showed no significant main effect of lesion ($F(1,34)=2.055$, $P=0.160$), drug ($F(1,34)=0.904$, $P=0.348$), or lesion x drug interaction effect ($F(1,34)=3.578$, $P=0.067$).

Three-way ANOVA of %PPI of sham and nVH lesioned animals showed significant main effects of lesion ($F(1,31)=16.57$, $P<0.001$) and PP ($F(2,62)=67.94$, $P<0.001$), and a significant interaction effect between lesion and PP ($F(2,62)=3.218$, $P=0.046$), but no significant main effect of drug ($F(1,31)=0.002$, $P=0.969$), or interaction effect between lesion x drug ($F(1,31)=0.208$, $P=0.651$), drug x PP ($F(2,62)=0.485$, $P=0.617$), or lesion x drug x PP ($F(2,62)=1.634$, $P=0.203$). ***Post hoc*** analysis revealed a significantly lower PPI in nVH lesioned animals compared to sham animals at PP3 ($P=0.03$), PP9 ($P<0.01$), and PP15 ($P=0.05$). However, there was no significant effect of prazosin+cirazoline treatment on PPI in either sham or nVH lesioned animals at any PP level. Thus, blockade of α-1 AR by pretreatment with prazosin blocked the PPI-disruptive effects of the intermediate dose of cirazoline in nVH lesioned animals.

3.5. Effect of α-1 AR Antagonist Prazosin (1.0 mg/kg) on PPI:

In order to study whether the disruption of PPI observed in nVH lesioned animals may be due to increased activity of α-1 AR, sham and nVH lesioned animals (***n*** = 10–12) received injections of either saline or 1.0 mg/kg prazosin, and their startle response was measured (Fig. 9). Two-way ANOVA on the startle response of sham and nVH lesioned animals to the 120 dB tone showed no significant main effect of lesion ($F(1,32)=0.035$, $P=0.851$), drug ($F(1,32)=3.482$, $P=0.071$), or lesion x drug interaction effect ($F(1,32)=3.632$, $P=0.065$).

Three-way ANOVA of %PPI of sham and nVH lesioned animals showed significant main effects of drug ($F(1,34)=14.77$, $P<0.001$), and PP ($F(2,68)=199.64$, $P<0.001$), and a significant lesion x drug interaction ($F(1,34)=17.02$, $P<0.001$), but no significant main effect of status ($F(1,34)=2.43$, $P=0.128$), or interaction effects between status x PP ($F(2,68)=0.699$, $P=0.500$), drug x PP ($F(2,68)=0.266$, $P=0.767$), or lesion x drug x PP ($F(2,68)=2.065$, $P=0.134$). ***Post hoc*** analysis revealed a significant reduction in PPI in saline-treated nVH lesioned animals in comparison to saline-treated sham animals ($P=0.04$). There was also a significant increase in PPI in nVH lesioned animals that received prazosin in comparison to saline-treated nVH lesioned animals ($P<0.01$). However, there was no significant difference in PPI in prazosin-treated sham animals in comparison to saline-treated sham animals ($P=0.78$), and no significant difference in PPI between prazosin-treated sham and nVH lesioned animals ($P=0.76$). These results suggest that some of the PPI-disruptive effects of nVH lesions may be due to increased activity of α-1 AR.

EFFECT OF 0.3 MG/KG CIRAZOLINE ON PPI IN NVH LESIONED ANIMALS

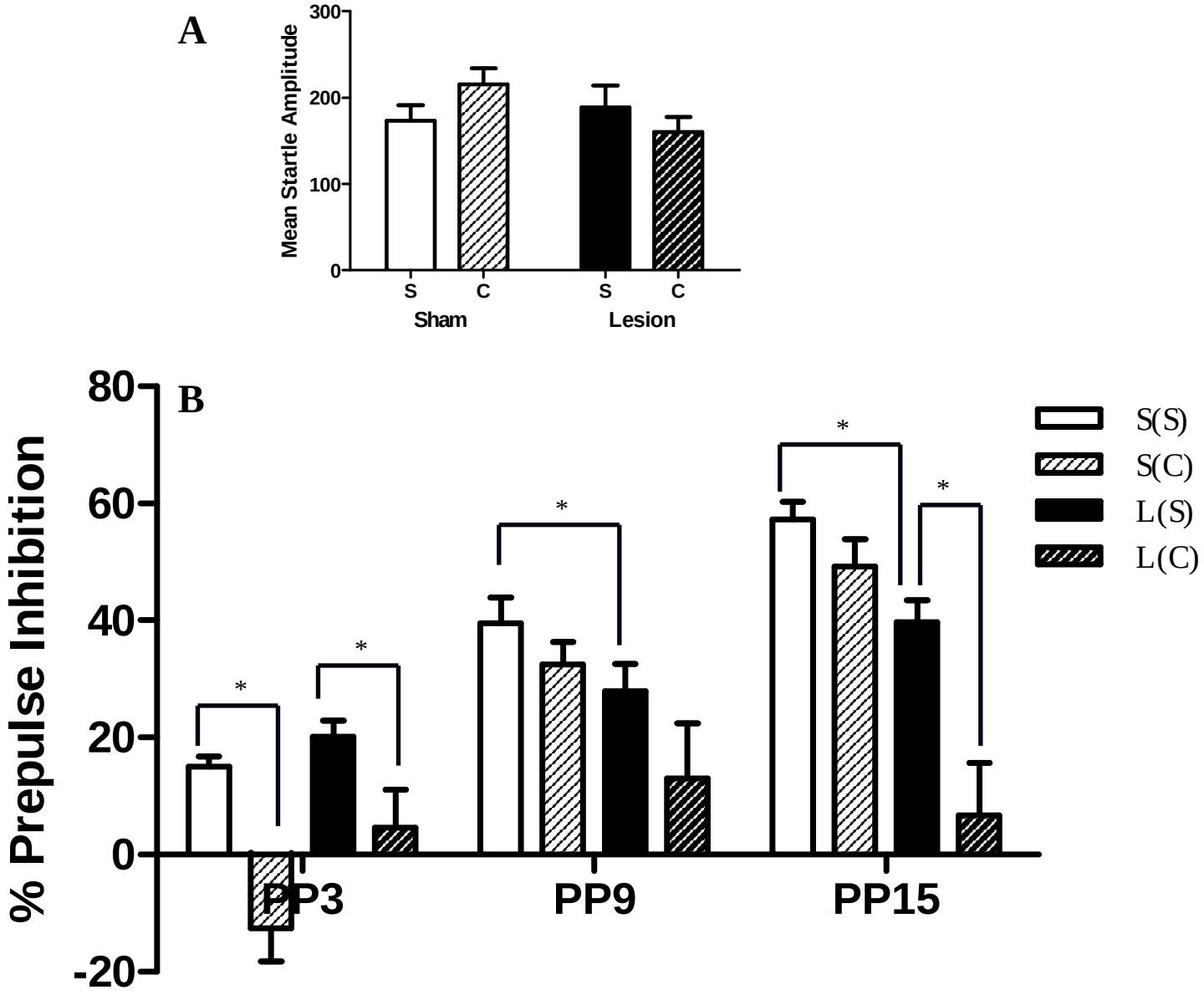


Figure 5 – Effect of systemic administration of 0.3 mg/kg cirazoline on startle response and PPI in sham and nVH lesioned animals (*n* = 10–12). (A) Startle response – Two-way ANOVA showed no significant main effect of lesion, drug, or lesion x drug interaction effect. (B) Pre-pulse inhibition – Three-way repeated-measures ANOVA showed significant main effects of lesion, drug and prepulse intensity, but no significant lesion x drug interaction effect. The asterisk represents significant difference between groups as revealed through *post hoc* analysis. S(S)= sham-operated (saline-treated), S(C)= sham-operated (cirazoline-treated), L(S)= nVH lesioned (saline-treated), L(C)= nVH lesioned (cirazoline-treated).

EFFECT OF 0.45 MG/KG CIRAZOLINE ON PPI IN NVH LESIONED ANIMALS

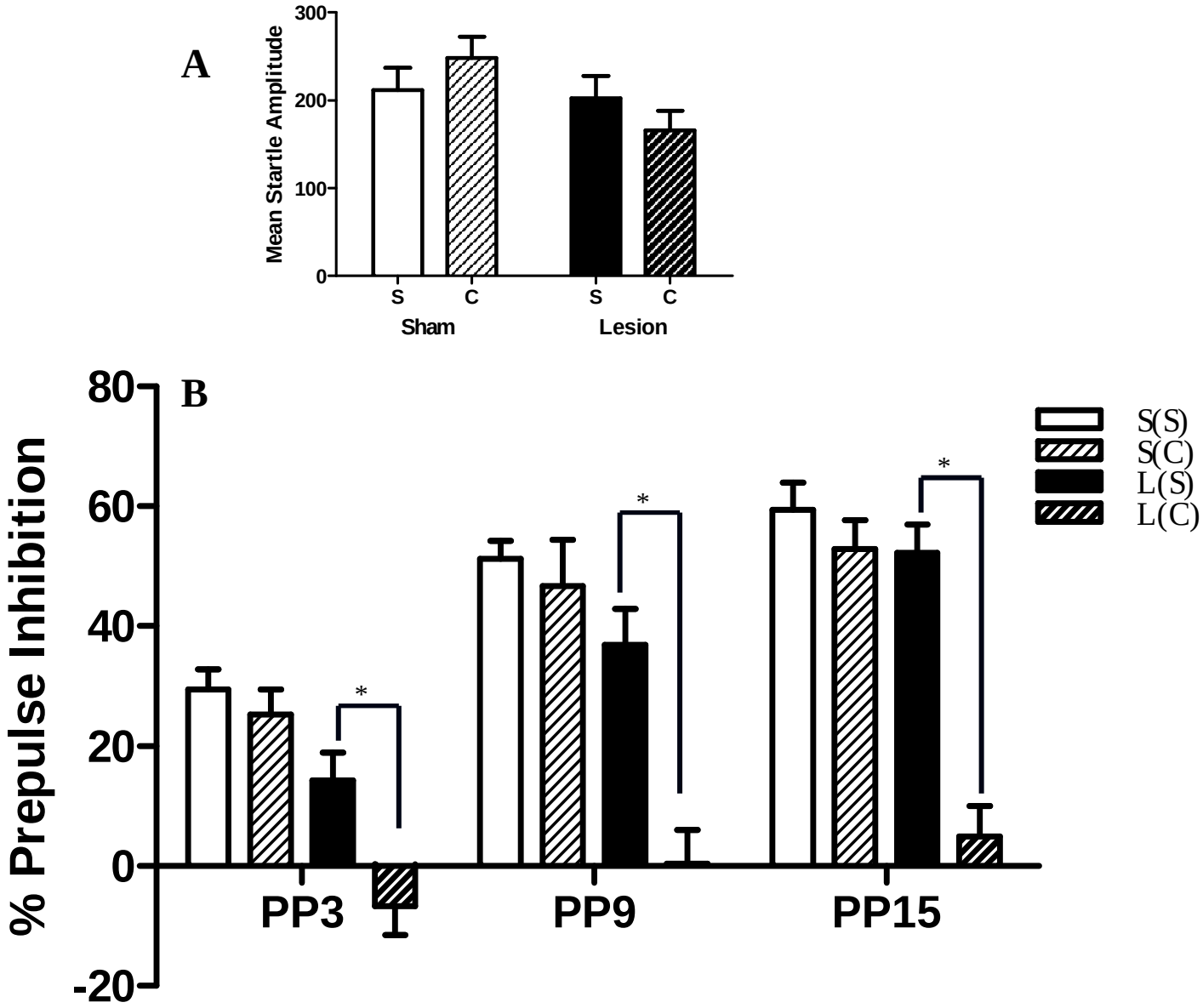


Figure 6 – Effect of systemic administration of 0.45 mg/kg cirazoline on startle response and PPI in sham and nVH lesioned animals (*n* = 9–10). (A) Startle response – Two-way ANOVA showed a trend of a main effect of lesion that did not reach significance, and no significant main effect of drug, or lesion x drug interaction effect. (B) Pre-pulse inhibition – Three-way repeated-measures ANOVA showed significant main effects of lesion, drug, prepulse intensity, and a significant lesion x drug interaction effect. The asterisk represents significant difference between groups as revealed through *post hoc* analysis. S(S)= sham-operated (saline-treated), S(C)= sham-operated (cirazoline-treated), L(S)= nVH lesioned (saline-treated), L(C)= nVH lesioned (cirazoline-treated).

EFFECT OF 0.6 MG/KG CIRAZOLINE ON PPI IN NVH LESIONED ANIMALS

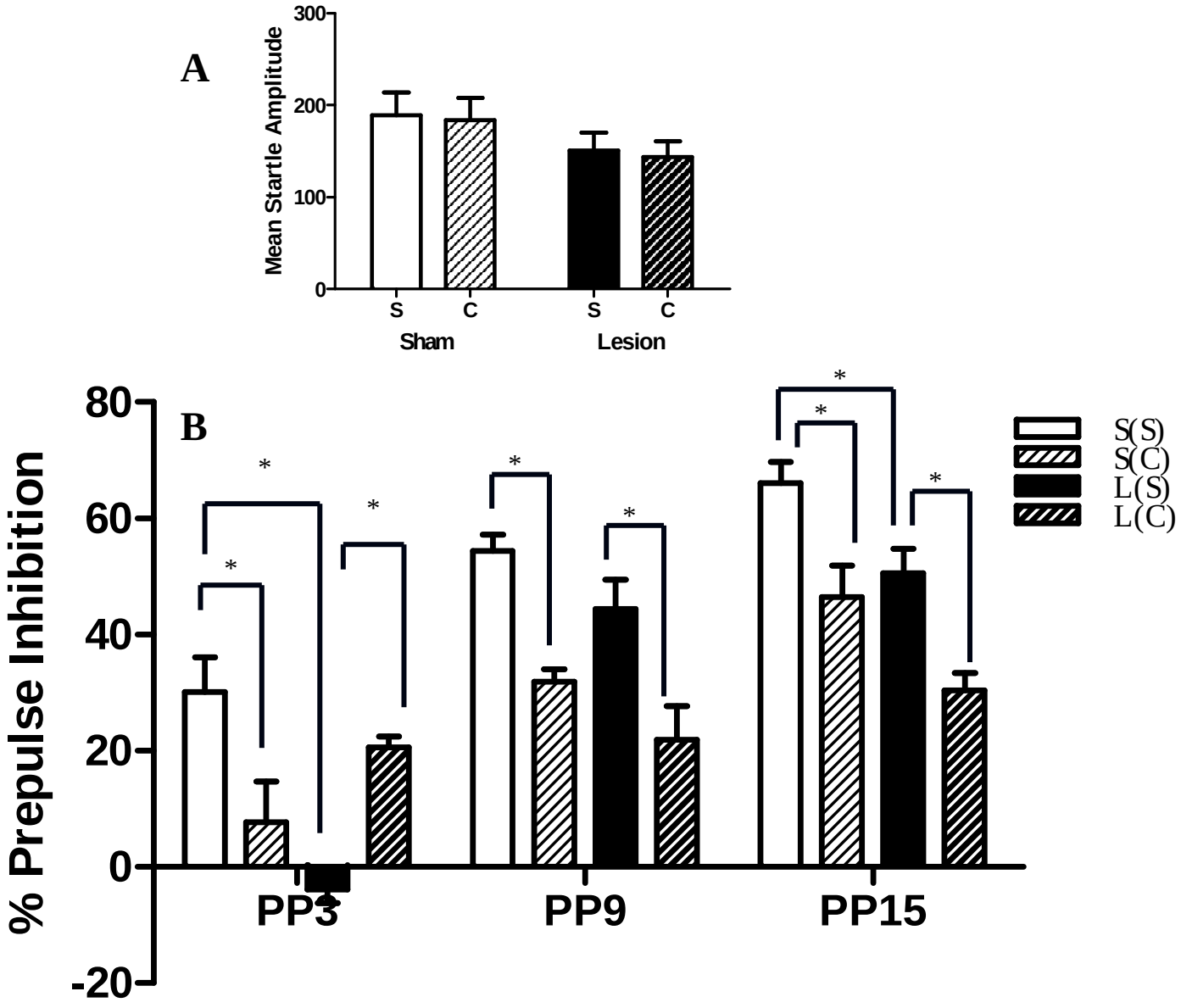


Figure 7 – Effect of systemic administration of 0.6 mg/kg cirazoline on startle response and PPI in sham and nVH lesioned animals (*n* = 8–11). (A) Startle response – Two-way ANOVA showed a trend of a main effect of lesion that did not reach significance, and no significant main effect of drug, or lesion x drug interaction effect. (B) Pre-pulse inhibition – Three-way repeated-measures ANOVA showed significant main effects of lesion, drug and prepulse intensity, but no significant lesion x drug interaction effect. The asterisk represents significant difference between groups as revealed through *post hoc* analysis. S(S)= sham-operated (saline-treated), S(C)= sham-operated (cirazoline-treated), L(S)= nVH lesioned (saline-treated), L(C)= nVH lesioned (cirazoline-treated).

BLOCKADE OF THE EFFECT OF CIRAZOLINE ON PPI WITH PRAZOSIN PRETREATMENT

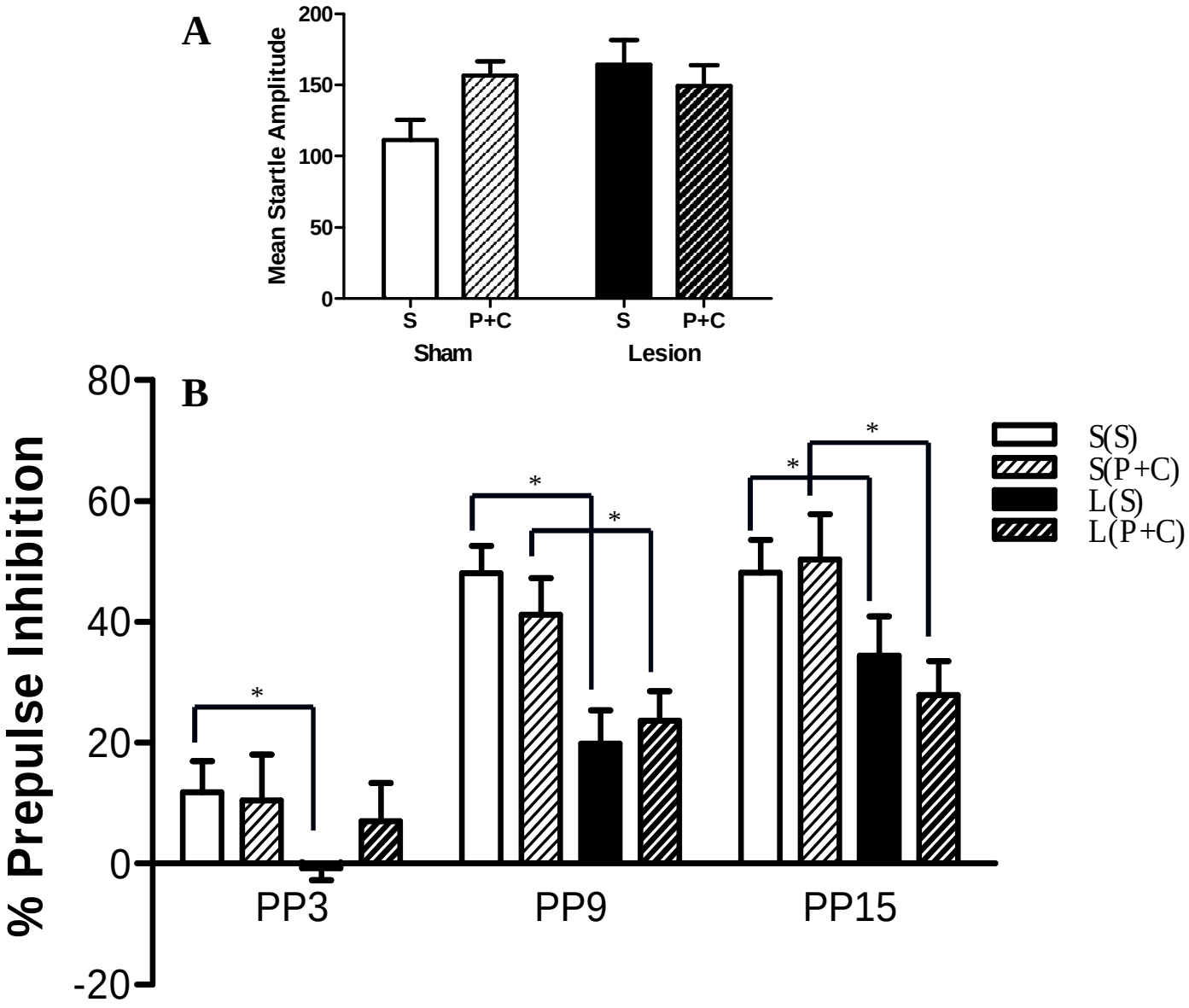


Figure 8 – Effect of systemic administration of 1.0 mg/kg prazosin followed by 0.45 mg/kg cirazoline on startle response and PPI in sham and nVH lesioned animals (*n* = 6–11). (A) Startle response – Two-way ANOVA showed no significant main effect of lesion or drug, and only a trend of a lesion x drug interaction effect that did not reach significance. (B) Pre-pulse inhibition – Three-way repeated-measures ANOVA showed significant main effects of lesion and prepulse intensity, but no significant main effect of drug or lesion x drug interaction effect. The asterisk represents significant difference between groups as revealed through *post hoc* analysis. S(S)= sham-operated (saline-treated), S(P+C)= sham-operated (prazosin+cirazoline-treated), L(S)= nVH lesioned (saline-treated), L(P+C)= nVH lesioned (prazosin+cirazoline-treated).

EFFECT OF 1.0 MG/KG PRAZOSIN ON PPI IN NVH LESIONED ANIMALS

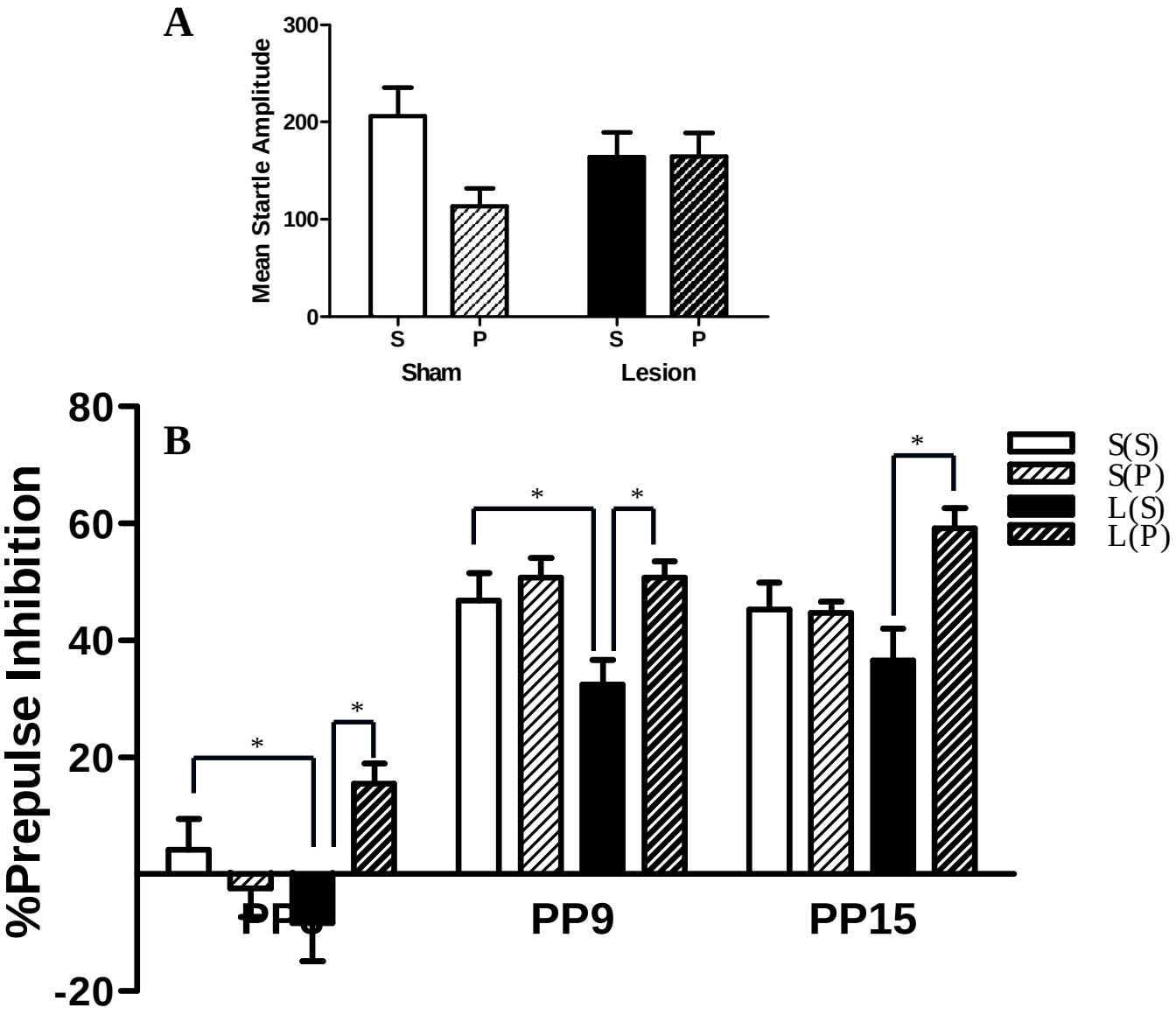
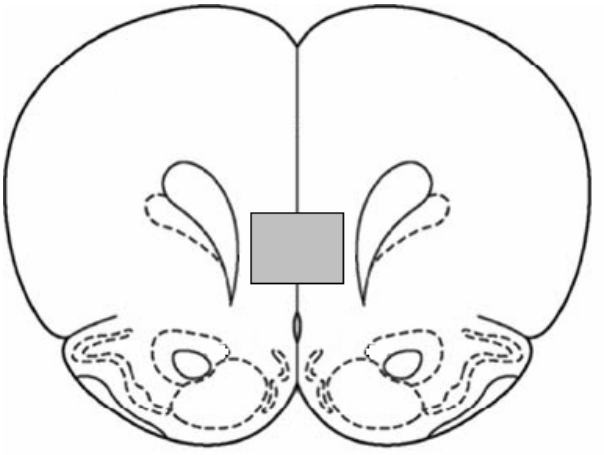


Figure 9 – Effect of systemic administration of 1.0 mg/kg prazosin on startle response in sham and nVH lesioned animals (*n* = 10–11). (A) Startle response – Two-way ANOVA showed no significant main effect of lesion, and only a trend of a main effect of drug and lesion x drug interaction effect that did not reach significance. (B) Pre-pulse inhibition – Three-way repeated-measures ANOVA showed no significant main effect of lesion, but significant main effects of drug and prepulse intensity, and a significant lesion x drug interaction effect. The asterisk represents significant difference between groups as revealed through *post hoc* analysis. S(S)= sham-operated (saline-treated), S(P)= sham-operated (prazosin-treated), L(S)= nVH lesioned (saline-treated), L(P)= nVH lesioned (prazosin-treated).

4. In situ Hybridization:

Prefrontal cortical sections of sham and nVH lesioned animals were hybridized with digoxigenin-labeled oligonucleotide probes specific for α-1A, α-1B or α-1D AR mRNA. Three sections from each animal (i.e. 16 sections total) were hybridized with each subtype-specific probe, and the mPFC region from left and right hemispheres were analyzed for positively-labeled cells. Statistical analysis using unpaired, two-tailed student's ***t***-test showed a significant difference in the number of cells labeled for α-1A ($P<0.01$) and α-1D ($P=0.04$), but not for α-1B ($P=0.57$), adrenergic receptor mRNA between sham and nVH lesioned animals, where the mPFC of nVH lesioned animals showed a significantly higher number of cells positively labeled for α-1A AR mRNA and a significantly lower number of cells labeled for α-1D AR mRNA compared to that of sham animals.

EXPRESSION OF α-1A, α-1B AND α-1D ADRENERGIC RECEPTOR MRNA IN MPFC OF NVH LESIONED ANIMALS



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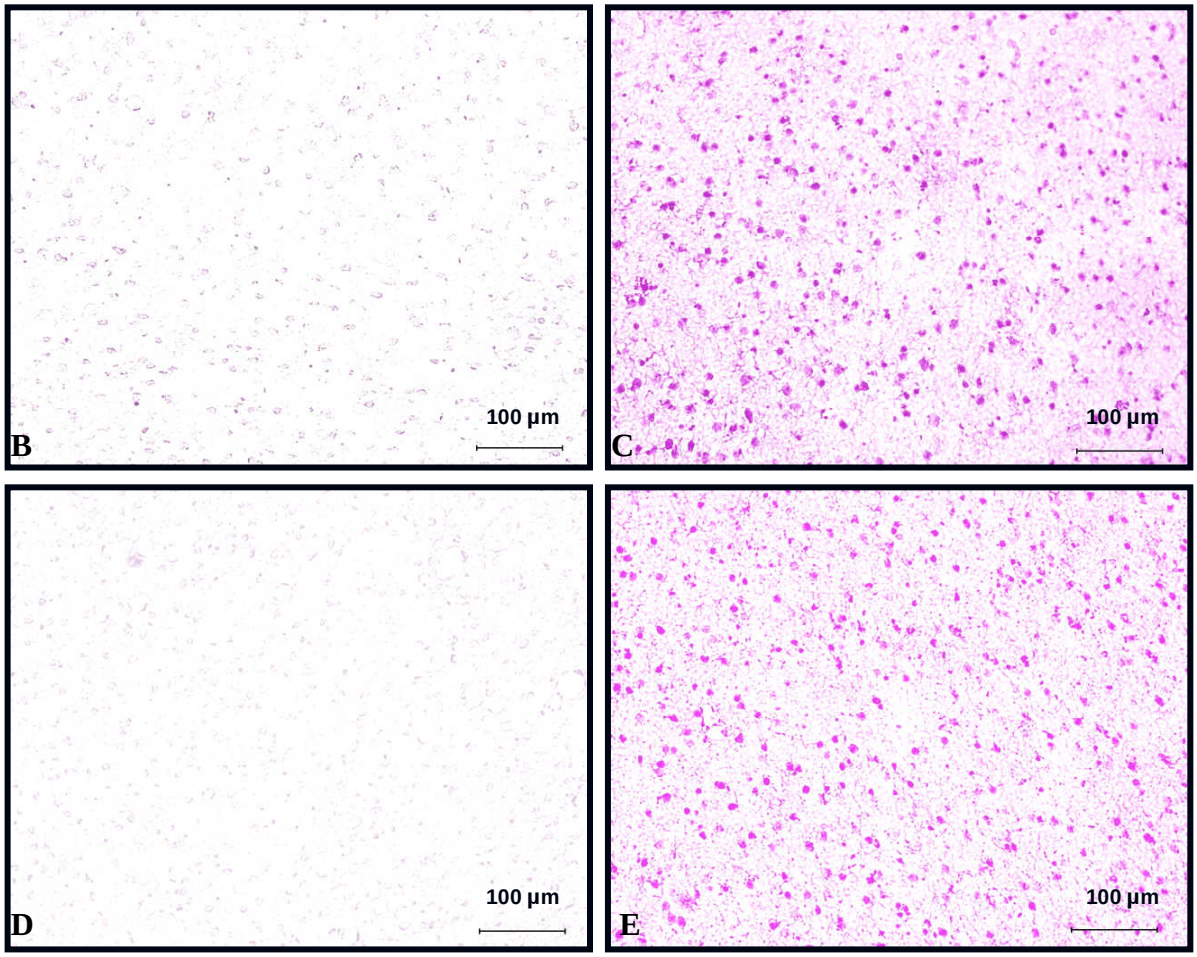

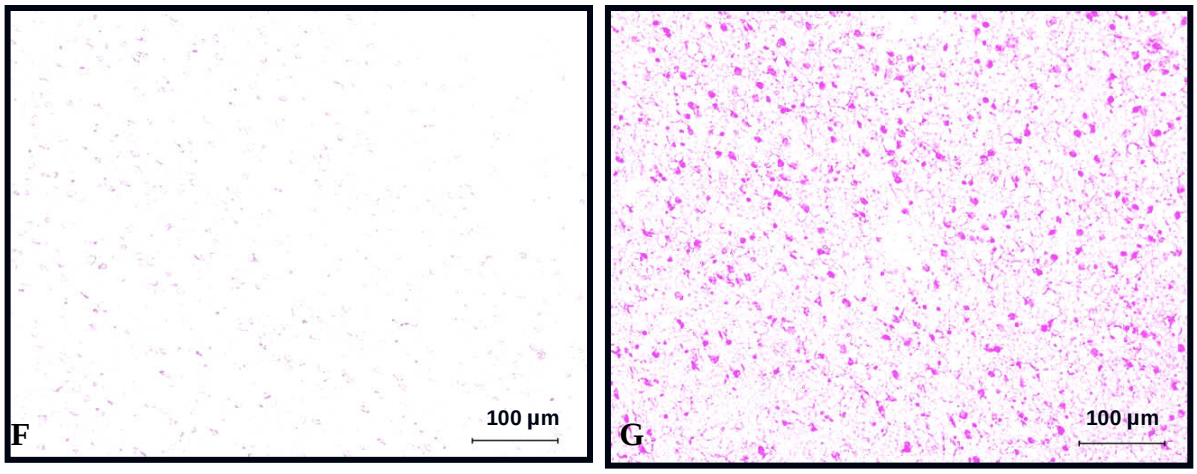

Figure 10 – In situ hybridization using DIG-labeled oligonucleotide probes for α-1A, α-1B and α-1D mRNA. (A) Schematic representation of a prefrontal cortical slice from a rat brain. The grey area represents the region of the mPFC within which DIG-labeled cells were analyzed. (B, D, F) Representative example of a 0.25 mm^2 mPFC region labeled with sense oligonucleotide probe for α-1A AR mRNA (B), α-1B AR mRNA (D), and α-1D AR mRNA (F) under 20x magnification. Sections hybridized with antisense probe were compared to sections hybridized with the respective sense probe when counting number of cells labeled. (C, E, G) Representative example of a 0.25 mm^2 mPFC region labeled with antisense oligonucleotide probe for α-1A AR mRNA (C), α-1B AR mRNA (E), and α-1D AR mRNA (G) under 20x magnification. The larger purple dots represent cells expressing mRNA for the respective α-1 AR subtype.

QUANTIFICATION OF EXPRESSION OF α-1A, α-1B AND α-1D ADRENERGIC RECEPTOR MRNA IN MPFC OF NVH LESIONED ANIMALS

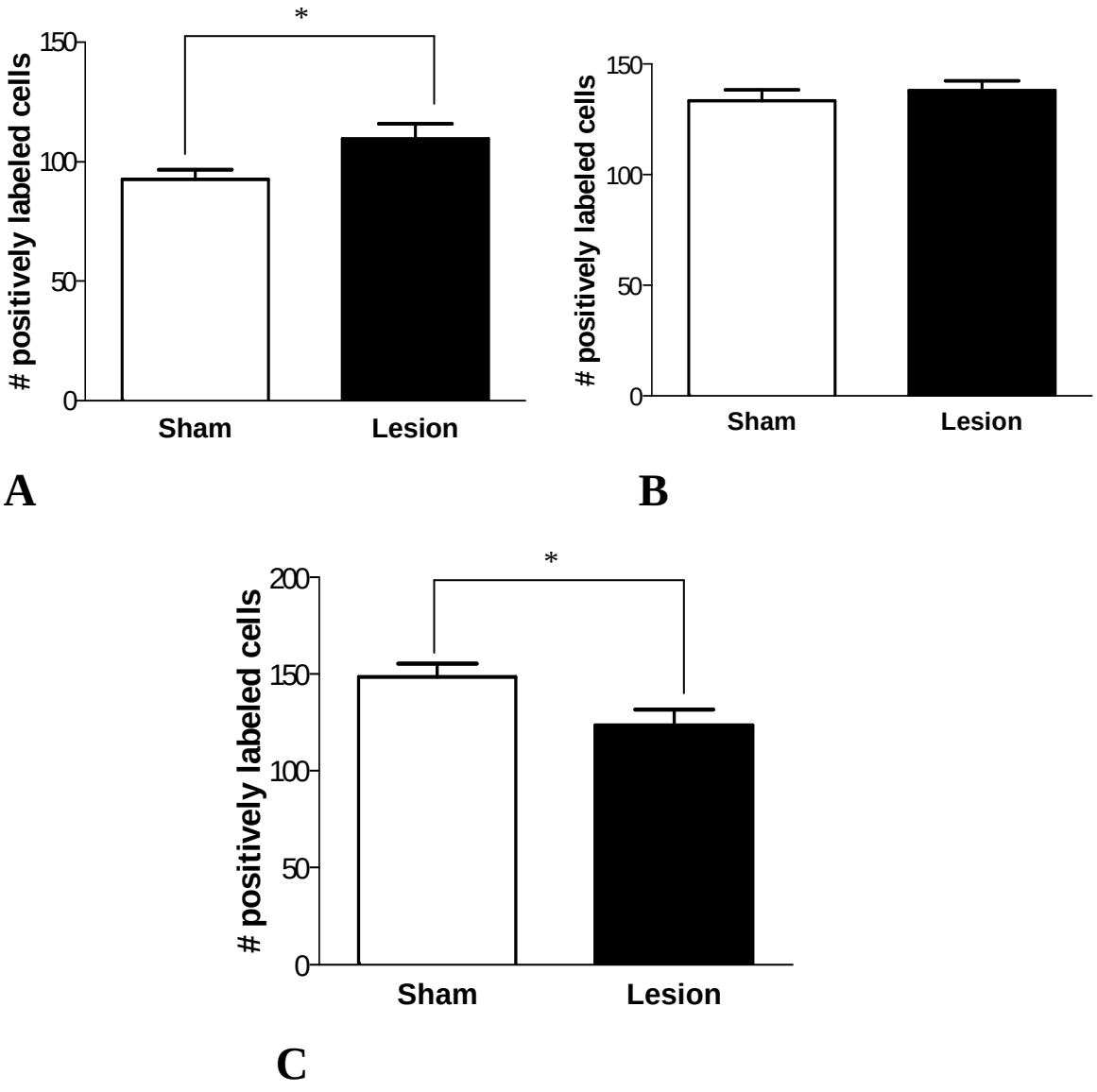

Figure 11 – Comparison of the number of cells positively labeled for mRNA for α-1 AR subtypes in the mPFC of sham and nVH lesioned rats (*n* = 4). (A) α-1A AR mRNA – nVH lesioned animals show a significantly higher number of mPFC cells labeled for α-1A AR mRNA compared to sham animals. (B) α-1B AR mRNA – There is no significant difference in the number of cells labeled for α-1B AR mRNA in the mPFC of sham and nVH lesioned animals. (C) α-1D AR mRNA – nVH lesioned animals show a significantly lower number of mPFC cells labeled for α-1D AR mRNA compared to sham animals. * P < 0.05

VI. DISCUSSION:

Experimental observations made with the neonatal ventral hippocampus lesion rat model strengthens the hypothesis that insult to the ventral hippocampus in rats, which corresponds to the anterior hippocampus in humans, during a critical period of early brain development leads to neurodevelopmental aberrations and delayed emergence of abnormal behaviors similar to some symptoms of schizophrenia. Due to the robust parallels in molecular and behavioral abnormalities seen in nVH lesioned animals and patients with schizophrenia, this model was used to further investigate potential abnormalities in schizophrenia.

The functional role of α-1 adrenergic receptors and its subtypes in the central nervous system is largely unknown, although these receptors vastly populate the brain[192-195]. This is mainly due to the inability of receptor-specific agonist and antagonist drugs to cross the blood-brain barrier. However, studies using intracranial injections of α-1 AR agonists and antagonists have revealed an important role of this receptor family in motor activity, cognitive function[180-182, 185, 196], and response to psychostimulants[169, 197, 198], many of which are abnormal in nVH lesioned animals.

Previous studies conducted in our lab showed an increase in ligand binding of α-1 adrenergic receptors in the prefrontal cortex of post-pubertal nVH lesioned animals[190]. As an extension to this study, I investigated the hypothesis that nVH lesions may lead to post-pubertal hyper-responsiveness of α-1 AR, and subtype-specific alterations in the transcription of α-1 AR in the medial PFC, a brain region that has been shown to play

an important role in modulating some of the behaviors that are abnormal in nVH lesioned animals. Previous studies have shown that stimulation of α-1 AR leads to increased locomotor activity[199, 200] as well as deficits in PPI[185]. Therefore, in order to test for potential hypersensitivity of α-1 AR in nVH lesioned animals, the effect of α-1 AR agonist, cirazoline, on locomotor behavior and sensorimotor gating in sham and nVH lesioned animals was tested. Also, potential alterations in transcription of α-1 AR in the mPFC of nVH lesioned animals was studied using in situ hybridization with digoxigenin-labeled oligonucleotide probes specific for mRNA of α-1 AR subtypes - α-1A, α-1B and α-1D.

1. Abnormal Function of α-1 AR in nVH Lesioned Animals May Not Affect Locomotor Activity:

Systemic injections of lower doses of cirazoline (0.1 and 0.3 mg/kg) did not alter locomotor behavior in either sham or nVH lesioned animals, but higher doses of cirazoline (0.6 mg/kg) increased locomotor activity in both groups, with no differential response of nVH lesioned animals to the drug. These results do not support our hypothesis, and suggest that although a high level of stimulation of α-1 AR influences locomotor activity, the α-1 ARs that enable locomotor activity may not be hyper-responsive in nVH lesioned animals.

Research on the brain regions where α-1 AR mediate behavioral activation has shown that terazosin, an α-1 AR antagonist, induces immobility when injected into the LC and NAcc, but does not affect behavior when injected into the PFC, VTA, and

amygdala[201]. These results were surprising as the PFC, VTA and amygdala contain many α-1 AR, and have been known to play a role in locomotion in response to specific stimuli such as psychostimulants and stress[202-207]. However, the inability of terazosin to induce immobility when infused into these brain regions suggests that α-1 AR in these particular brain regions may not be involved in enabling locomotor activation, per se, but may play a role in regulating locomotor response to other stimuli. Previous research from our lab has revealed that nVH lesioned animals show increased ligand binding of α-1 AR in the PFC, but not in the NAcc, and a dysfunction in α-1 AR regulation of AMPH-induced hyperlocomotion[190]. These results, along with the above behavioral data, suggest that in nVH lesioned animals, α-1 AR involved in locomotor stimulation, such as those in the NAcc, may not be altered, but those receptors involved in modulation of the locomotor response to AMPH, such as in the mPFC, may be abnormal. Future studies may test this hypothesis through intracerebral injection of α-1 AR agonists and antagonists into the mPFC and NAcc of nVH lesioned animals to study any aberrations in the role of these receptors in locomotor activation and the locomotor response to AMPH.

In naïve animals, α-1 AR play an important role in modulation of the mesoaccumbal dopamine response and locomotor response to psychostimulants. Studies have shown that AMPH injections into the mPFC releases DA which, through activation of D1 dopamine receptors, inhibits the locomotor-enhancing effect of AMPH in the NAcc[208]. However, AMPH also releases NA which stimulates α-1 AR in the mPFC that inhibit D1 receptor transmission[209] and, in turn, disinhibits mPFC pyramidal

neurons. Systemic administration of AMPH leads to activation of metabotropic glutamatergic receptors in the NAcc, perhaps via glutamatergic projections from the mPFC to the NAcc, which leads to an increase in the release of 'functional' DA in the NAcc that is associated with the observed increase in locomotor activity[210]. Studies have revealed a hyperexcitability of mPFC neurons in nVH lesioned animals in response to VTA stimulation[145], representing the abnormal response of these cortical neurons to psychostimulant effects. nVH lesioned animals also show increased levels of α-1 AR in the mPFC[190], which may be responsible for increased disinhibition/excitability of these neurons and perhaps some of the abnormal behaviors observed in these animals in response to psychostimulants.

An alternative interpretation of our results on locomotor activity may be that the doses of cirazoline used in this study were insufficient to capture any locomotor abnormalities in nVH lesioned animals. A previous study on the locomotor effects of cirazoline showed that a dose of 0.05 mg/kg significantly reduced locomotor activity in rats and this effect waned at higher doses of 0.1, 0.2 and 0.4 mg/kg[211]. In the present study, 0.1 mg/kg cirazoline showed a slight, albeit insignificant, decrease in locomotor activity in both sham and nVH lesioned animals. Perhaps, a more extensive dose-response study investigating locomotor activity in nVH lesioned animals in response to cirazoline at doses between 0-0.1 mg/kg may reveal a differential effect of cirazoline on locomotion in nVH lesioned animals.

Another possibility is that lower doses of cirazoline, such as 0.1 and 0.3 mg/kg, stimulate α-1 AR but do not affect locomotor behavior in rats, while higher doses of cirazoline, such as 0.6 mg/kg, may also activate other non-adrenergic receptors that may influence locomotor behavior. Cirazoline is an imidazoline ligand that has a high affinity for α-1 ARs, but also has lower affinity for other receptors with imidazoline binding sites[212]. Imidazoline binding sites are found in the striatum that, upon activation, enhance locomotor activity in rats[213]. It is possible that high doses of cirazoline activate non-α-1 AR imidazoline binding sites that modulate locomotion, but that are normal in nVH lesioned animals, thus, revealing no abnormality in locomotor responsiveness of nVH lesioned animals to cirazoline.

2. Abnormal α-1 AR in nVH Lesioned Animals may be Responsible for Sensorimotor Gating Deficits:

α-1 AR not only modulate locomotor activity, but also play an important role in sensorimotor gating. In order to further investigate whether nVH lesioned animals show hyperactive α-1 AR, we studied the effect of various doses of cirazoline on PPI in sham and nVH lesioned rats. Three doses of cirazoline were used in this study – 0.3, 0.45 and 0.6 mg/kg, i.p. – based on the doses used in previous PPI studies using cirazoline, where doses between 0.5 and 0.75 mg/kg formed the threshold for disruption of PPI in naïve animals[185]. Our results showed that a low dose of 0.3 mg/kg cirazoline reduced PPI in both sham and nVH lesioned animals at the lowest prepulse (PP) intensity. There was also a significant reduction in PPI in nVH lesioned animals at the highest PP value. However, statistical analysis did not reveal any significant differential effect of this

dose of cirazoline on PPI in nVH lesioned animals. Our results also showed that an intermediate dose of cirazoline (0.45 mg/kg) substantially reduced PPI in nVH lesioned animals, with little or no effect on sham animals. This enhanced effect of a moderate dose of cirazoline on PPI in nVH lesioned animals was reversed by pretreatment with prazosin, suggesting that this differential effect was due to hyperresponsiveness of α-1 ARs in nVH lesioned animals. Treatment with prazosin itself significantly increased PPI in nVH lesioned animals at some PP values, without a significant effect on PPI in sham animals. Similar to results of previous sensorimotor gating studies[185], 0.6 mg/kg cirazoline significantly decreased PPI in sham animals. Surprisingly, in nVH lesioned animals, this dose of cirazoline decreased PPI, but to a much smaller extent than seen with the 0.45 mg/kg dose. Although it may seem contradictory to what was expected, this could be due to several reasons. It is possible that cirazoline may have a U-shaped dose-response curve as reported in behavioral studies on cognitive functions such as spatial working memory (SWM), where low doses of cirazoline impaired SWM while increasing doses had no effect and even improved SWM[181]. Also, the dose-response characteristic of cirazoline may be due to its binding properties. Cirazoline is an imidazoline that is a potent α-1 adrenergic receptor agonist, but also has some affinity for α-2 adrenergic receptors containing an imidazoline binding site. Therefore, low doses of cirazoline may bind to α-1 adrenergic receptor, whereas higher doses may also bind to imidazoline/α-2 adrenergic receptors. In support of this possibility, the impairment of SWM in the above mentioned study by lower doses of cirazoline was blocked by pretreatment with prazosin, whereas the enhancement of SWM by higher doses of cirazoline was not blocked by prazosin but by idazoxan, an imidazoline/α-2

adrenergic receptor antagonist. Overall, these data suggest that disruption of the VH early in life may lead to hyperactivity of α-1 adrenergic receptors that may be responsible for some of the sensorimotor gating deficits observed in these animals.

Our study shows that 0.3 mg/kg cirazoline enhanced startle magnitude in sham, but not nVH lesioned, animals. However, 0.45 mg/kg and 0.6 mg/kg cirazoline did not have any significant effect on startle magnitude in either group of animals. Studies have shown that similar doses of cirazoline did not significantly alter startle magnitude in rats, although other studies using different α-1 adrenergic receptor agonists such as phenylephrine showed conflicting results[185, 214]. Also, prazosin reduced startle magnitude in both sham and nVH lesioned animals to similar extents, although it had a differential effect on PPI of nVH lesioned animals. Although the role of α-1 adrenergic receptors on startle magnitude is currently unclear, it should be noted that the effect of these receptors on PPI have been shown to be independent of its effect on startle magnitude[185].

The PPI pathway is modulated by the limbic cortex that involves the mPFC, hippocampus and amygdala, as well as the nucleus accumbens[215]. However, the specific role of each brain region in PPI is not yet well known. VH lesions in adult rats do not affect basal levels of PPI but enhance PPI deficits induced by apomorphine, a DA receptor agonist[216]. On the other hand, nVH lesioned rats show post-pubertal deficits in basal levels of PPI[217], as well as an increased sensitivity to the PPI-disruptive effects of apomorphine[218]. These studies imply an important role of the VH in regulation of the

dopamine pathway involved in PPI, and the development of other PPI regulatory brain sites. The mPFC is one such region that receives afferents from the hippocampus, and its development is thought to be affected by nVH lesions. It has been proposed that reduced dopaminergic tone and increased noradrenergic tone in the mPFC may disrupt PPI through disinhibition of descending glutamatergic projections and an increase in dopamine transmission in the NAcc. Reduction of dopaminergic tone through 6-OHDA lesions in the mPFC of naïve rats has been shown to reduce PPI, an effect that is reversed by haloperidol[219-221]. Also, studies on naïve human subjects have shown that blockade of NA reuptake using desipramine as well as blockade of DA receptors using haloperidol disrupt PPI, without any additive effects when both drugs were combined[222]. This suggests that the noradrenergic system and the dopaminergic system interact to modulate PPI most probably at the level of the mPFC where extracellular DA levels modulate NA levels and vice versa. Interestingly, the novel atypical antipsychotic drug iloperidone, that has a high affinity for α-1 ARs and acts as a dopamine/serotonin/norepinephrine antagonist, has been shown to prevent cirazoline-induced PPI deficits, suggesting that increased activity of α-1 ARs may play a role in sensorimotor gating deficits in patients with schizophrenia[223]. nVH lesioned animals show decreased DA release in the mPFC in response to stress[224] and increased excitability of PFC neurons in response to VTA stimulation[145] that has been shown to be responsible for the increased response of the NAcc to mesolimbic activation[147]. In addition, nVH lesioned animals display increased ligand binding of α-1 AR in the prefrontal cortex[190]. α-1 AR have been shown to induce excitatory currents in PFC neurons[225], suggesting that increased number or sensitivity of these receptors, along

with decreased DA transmission, may lead to disinhibition of PFC neurons, and perhaps some of the PPI deficits seen in these animals. It would be interesting to confirm whether the hyperresponsiveness of α-1 ARs observed in nVH lesioned animals can also be found at the level of the mPFC, and if these receptors play a role in the sensorimotor gating deficits observed in these animals.

3. Subtype-Specific Alterations of α-1 AR mRNA in mPFC of nVH Lesioned Animals:

In order to investigate whether the observed increase in number and/or affinity of α-1 ARs in the PFC of nVH lesioned animals is due to abnormal mRNA expression of specific subtypes of this receptor, we conducted in situ hybridization experiments on prefrontal cortical sections of sham and nVH lesioned animals using DIG-labeled oligonucleotide probes for α-1A, α-1B and α-1D mRNA. Our results indicate that the mPFC of nVH lesioned rats show a significantly higher number of cells positively labeled for α-1A AR mRNA, and a significantly lower number of cells positively labeled for α-1D AR mRNA, with no significant difference in the number of cells labeled for α-1B AR mRNA. These results suggest that nVH lesions lead to abnormal transcription of specific α-1 AR subtypes in regions of the adult mPFC.

Unfortunately, because of the technique used to label the oligonucleotide probes, only semi-quantitative analysis of these results could be performed. Therefore, it remains unknown whether nVH lesioned animals show changes in expression of α-1 AR subtypes ***within*** each cell. One method of detecting variation in receptor expression

within a cell would be through in situ hybridization experiments using [^{35}S]-labeled probes. However, this method proved to be difficult using the commercially available oligonucleotide probes. Perhaps radioactive-labeled ribonucleotide probes may be more efficient at detecting α-1 AR subtypes within the brain, while allowing a more quantitative analysis of changes in levels of expression. Immunohistochemistry studies on the expression of α-1A AR in the mouse brain have shown expression of this receptor subtype in neuronal cells, including NR1 NMDA receptor-containing neurons and GABAergic neurons of the cerebral cortex[226]. One interesting observation from our in situ studies is that the labeling for α-1A AR mRNA seemed to have a different shape and size compared to the labeling for α-1D AR mRNA, where α-1A AR mRNA labeling was larger and rounder than α-1D AR mRNA labeling which was much smaller and sometimes observed as small spots. It is possible that these AR subtypes are expressed in different types of neurons in the mPFC, or perhaps in very different quantities within the cell. Future studies using dual-labeled in situ hybridization can investigate the specific types of cells in the mPFC of nVH lesioned animals that show altered expression of α-1 AR subtypes, and perhaps PCR studies may provide better quantitative results of any differences in each subtype in the mPFC of sham and nVH lesioned animals.

The distribution of α-1 AR subtypes in the brain is not yet well known due to lack of commercially available subtype-specific antibodies. In situ hybridization studies on the distribution of mRNA of α-1 AR subtypes in the rat brain reveal that, unlike in the periphery where different tissues express different α-1 AR subtypes, central α-1 AR

subtypes may be co-localized in most tissues[192, 193, 227]. In the cerebral cortex, although mRNA for all α-1 AR subtypes are expressed, each subtype has a different pattern of expression in the different layers of the cortex. For example, mRNA for α-1A AR can be detected at high levels in the cerebral cortex, with highest labeling in layers II-III, lowest labeling in layer IV, moderate labeling in layers V and VI, and no labeling in layer I. α-1D AR mRNA can be detected at high levels in most areas of the cerebral cortex, mainly in layers V and VI, with lower labeling in layer II and III, very few cells labeled in layer IV, and little or no labeling in layer I. mRNA for α-1B AR are also found in the cerebral cortex, but not as abundant as those for α-1A and α-1D AR. The strongest labeling for α-1B AR mRNA is found in layers V and VI, with lighter labeling in layers II-IV, and no labeling in layer I.

The distinct pattern of expression of α-1 AR subtypes observed in the cerebral cortex suggests different functions of each receptor subtype. A few genetic studies have been conducted using mice mutant in specific α-1 AR subtypes, giving some idea of a role of each subtype in the brain. For example, knockout mice for the gene encoding α-1b AR show decreased sensitivity to locomotor-enhancing effects of AMPH, cocaine and morphine, as well as decreased rewarding effects of these drugs[198]. However, knockout mice for the gene encoding α-1d AR show decreased locomotor response to AMPH, but not cocaine, with no change in the rewarding effect of these drugs[197]. Moreover, stimulation of α-1b AR amplifies dopamine-mediated locomotion in rats and mice[228]. Although it is tempting to associate results from knockout studies with behaviors of nVH lesioned animals, it must be noted that knocking out a specific

receptor subtype genetically may lead to compensatory changes in other receptor subtypes, confounding the results observed in such experiments. However, these studies demonstrate that α-1 AR subtypes may have distinct functions in locomotor-enhancing and rewarding properties of specific psychostimulants.

In vitro studies on cortical neurons have shown that expression of α-1 ARs is regulated by neuronal excitability, where increased neuronal activity increases the expression of α-1 ARs[229]. Stimulation of α-1 ARs in the mPFC leads to release of serotonin[230] which may act on 5-HT2A receptors, and activation of these two types of receptors can induce excitatory postsynaptic currents in pyramidal neurons of the mPFC[231]. Converging evidence point to a hyperexcitable state of PFC neurons in nVH lesioned animals[142, 145], and it is possible that this increased cortical activity may be related to the increased expression of α-1A AR mRNA seen in these animals. This increase in cortical activity may either be a cause of the observed increased expression, where increased neuronal activity results in increased α-1A AR expression, or an effect of the increased α-1A AR expression, where low amounts of stress leads to increased activation of α-1A AR which, in turn, increases the activity of mPFC pyramidal neurons. In vitro studies on the cellular expression profiles of α-1 AR subtypes have shown that α-1A ARs in human embryonic kidney cells are expressed on the cell surface as well as intracellularly in a perinuclear orientation, while α-1D ARs are mainly expressed intracellularly[232]. Other studies on mouse neuroblastoma cells have shown that α-1D ARs are translocated to the cell surface when its long N-terminal tail is truncated, when it contains a signal peptide sequence on its N-terminus, when it

dimerizes with membrane-bound α-1B ARs, and when it is incubated with prazosin[233]. The externalization of α-1D AR following incubation with prazosin led to the suggestion that these receptors may be constitutively active, leading to their downregulation and internalization. Although expression profiles of these subtypes may be different in the postpubertal rat brain, one possibility is that nVH lesioned animals show increased activity of prefrontal cortical α-1 ARs, in particular α-1A ARs, which may lead to internalization of α-1D ARs and the observed downregulation of its mRNA expression. Furthermore, increased expression of α-1A AR seen in the mPFC of nVH lesioned animals may be, in part, responsible for the increased sensitivity of α-1 ARs that play a role in sensorimotor gating deficits in these animals. In fact, one study has shown an association of polymorphisms in the promoter region of the gene encoding α-1A ARs with schizophrenia in a Spanish isolate population[234], suggesting that alterations in the transcription of this receptor subtype may indeed play a role in some of the abnormalities seen in schizophrenia.

VII. CONCLUSION:

The results of my work suggest that post-pubertal nVH lesioned animals show increased sensitivity of α-1 ARs that modulate certain behaviors such as sensorimotor gating, but not locomotor activity. This increase in sensitivity may be related to the changes seen in the expression of specific subtypes of α-1 ARs, i.e. the increased expression of α-1A AR mRNA and decreased expression of α-1D AR mRNA, in the mPFC of these animals. Therefore, a disruption of the hippocampus early in neurodevelopment leads to hyper-responsiveness of α-1 ARs, and subtype-specific abnormalities in the transcription of these receptor genes in the mPFC, which are, at least partly, responsible for certain behavioral abnormalities similar to those seen in patients with schizophrenia.

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APPENDIX

Research Compliance Certificate for Animal Subjects

ACTION	✓	DATE
CC		
DB	✓	Oct 3rd 05
APPROVED		

www.mcgill.ca/rgo/animal/forms/

For Office Use Only:
Protocol #: 3283
Approval end date: Aug 31, 2006
Facility Committee: DH
Renewal#: 1st 2nd

McGill University Animal Care Committee
RENEWAL of Animal Use Protocol

For: Research ☒ Teaching ☐ project

Principal Investigator: Lalit K. Srivastava **Protocol #** 3283

Protocol Title: Role of cortical alpha-1 adrenergic receptors in delayed behavioral changes in rats with neonatal hippocampal lesions: Implications for developmental psychiatric disorders.

Phone: 514-761-6131 x2936

Unit, Dept. & Address: Douglas Hospital Research Centre **Fax:** 514-762-3034

Email: lalit.srivastava@mcgill.ca **Level:** D **Funding source:** CIHR

Start of Funding: Oct. 2004 **End of Funding:** Sep 2009

Emergency contact #1 + work AND home phone #s: Lalit Srivastava, 514-761-6131 x2936; 450-444-2412

Emergency contact #2 + work AND home phone #s: Sanjeev Bhardwaj, 514-761-6131 x3346; 450-678-6308

1. Personnel and Qualifications

List the names of the Principal Investigator and of all individuals who will be in contact with animals in this study and their employment classification (investigator, technician, research assistant, undergraduate/ graduate student, fellow). If an undergraduate student is involved, the role of the student and the supervision received must be described. Training is mandatory for all personnel listed here. Refer to *www.animalcare.mcgill.ca* for details. Each person listed in this section must sign. *(Space will expand as needed)*

Name	Classification	Animal Related Training Information	Occupational Health Program *	Signature *"Has read the original full protocol"*
Lalit Srivastava	PI	McGill course,Theory	yes	
Sanjeev Bhardwaj	Post-doc	McGill course, workshop	yes	
Aarthi Kamath	MSc	To attend Mcgill course	yes	
Irina Al-Khairi	MSc	To attend Mcgill course	yes	

* Indicate for each person, if participating in the local OHP Program, see http://www.mcgill.ca/rgo/animal/occupational/ for details.

Approved by:

2. Approval Signatures

Principal Investigator/ Course Director		Date: Aug. 30, 2005
Chair, Facility Animal Care Committee	Nicolas Cermakian	Date: Sept 9 05
UACC Veterinarian		Date: Sept 9, 2005
Chairperson, Ethics Subcommittee *(D level or Teaching Protocols Only)*		Date: 10/31/05
Approved Animal Use Period	Start: Sept 1, 2005	End: Aug 31, 2006

3. Summary (in language that will be understood by members of the general public)

AIMS AND BENEFITS: Describe, in a short paragraph, the overall aim of the study and its potential benefit to human/animal health or to the advancement of scientific knowledge *(was section 5a in main protocol)*.

Schizophrenia is a complex mental disorder affecting up to 1% of the Canadian population. The progress of

RJ. 1 OCT. 2005