TYROSINE HYDROXYLASE INCREASES IN LOCUS COERULEUS AFTER CHRONIC NMDA BLOCKADE IN THE VENTRAL STRIATUM: COMPARISON TO OLFACTORY BULBECTOMY

by

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(Under the Direction of Philip V Holmes)

ABSTRACT

Olfactory bulbectomy (OBX) results in many neurochemical and behavioral changes that model depression. To test the possibility that glutamatergic dysfunction causes these changes, the present study aimed to create a pharmacological manipulation that would be comparable to the OBX model. To this end, we chronically injected the competitive NMDA antagonist, AP-5 (2.5, 5.0, or 10.0µg/day) directly into the olfactory tubercle for a period of 14-17 days followed by in situ hybridization measuring galanin (GAL) and tyrosine hydroxylase (TH) mRNA in the locus coeruleus (LC). Autoradiography revealed that TH mRNA levels were significantly increased in the LC of rats in the AP-5 10.0µg/day group compared to all other groups. No significant change was found in GAL mRNA levels. These results suggest that glutamatergic disruption in the ventral striatum leads to increased NE activity in the LC, thereby stimulating dopaminergic activity in the VTA, which may result in the hyperactive locomotor response observed following this manipulation or OBX.

INDEX WORDS: Olfactory bulbectomy, NMDA receptor, Dopamine, Ventral tegmental area, Norepinephrine, Locus Coeruleus

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DEDICATION

I would like to dedicate this thesis to my son, Aidan Dante, and to my husband, Sean. Sean, I want to thank you for being fully supportive and always believing in me and my ability to actually finish this thesis. And Aidan, although you've kept me up countless nights in the process of my finishing this thesis, you are the inspiration for all the things I plan to accomplish. I love you both.

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CHAPTER 1

INTRODUCTION

Structure of Thesis

The following thesis consists of two main components, which include a literature review and a manuscript to be submitted for publication. The literature review covers important and relevant background information pertaining to this study. It begins with a description of the olfactory bulbectomy model of depression, followed by a review of the anatomical connections of the olfactory bulb. The next section describes changes in glutamatergic and dopaminergic activity following bulbectomy. The rationale of the present study is then presented, followed by a review of the regulatory role of glutamate on dopamine activity in the ventral striatum. Finally the literature review concludes with a description of the mechanisms by which the ventral tegmental area and locus coeruleus affect dopaminergic activity in the ventral striatum. The manuscript portion of this thesis consists of an introduction, a materials and methods section, a results section, and a discussion section, and will be submitted to *Psychopharmacology*. The figures referred to in the results section are presented immediately after the discussion section.

Purpose of Study

The purpose of the experiments presented in the manuscript was to create a pharmacological manipulation that would be comparable to the olfactory bulbectomy model of depression, in order to clarify the role of glutamatergic deafferentation to the ventral striatum, mainly the olfactory tubercle. In a previous experiment in our lab, we showed that chronic administration of the competitive NMDA receptor antagonist, AP-5, into the ventral striatum

resulted in hyperactivity, similar to bulbectomy, but in situ hybridization analyses in the olfactory tubercle revealed no change in neurochemicals reflective of dopaminergic activity (i.e. D2 receptor or prepro-enkephalin). Therefore, this study sought to determine if neurochemical changes occurred upstream from the site of drug administration, in areas that have a regulatory function on dopamine release in the striatum, mainly the locus coeruleus and the ventral tegmental area.

CHAPTER 2

LITERATURE REVIEW

Olfactory Bulbectomy Model of Depression

The olfactory bulbectomy (OBX) is an animal model of depression validated by its capability to detect antidepressant treatment effects after chronic, but not acute administration (Kelly, Wrynn, & Leonard, 1997). The ablation of the olfactory bulbs results in physiological, neuroendocrine, and many neurochemical changes due to the disrupted connections between the bulbs and other brain regions, as well as concomitant behavioral changes. These changes associated with OBX cannot be attributed to anosmia, since behavior is altered in tasks that do not require olfactory senses (Jesberger & Richardson, 1988; Kelly et al., 1997).

Neurochemical disruptions induced by OBX include reduced norepinephrine (NE) in the telencephalon, reduced serotonin (5-HT) in numerous brain regions, decreased or increased dopamine (DA) levels in the telencephalon and striatum (see Kelly et al., 1997 or Song & Leonard, 2005 for review), and an imbalance between excitatory (glutamate) and inhibitory (glycine) amino acids (Cairncross et al., 1975; Halaxz & Shepherd, 1983; Edwards et al., 1977; Jersberger & Richardson, 1988; Kelly et al., 1997). OBX seems to increase levels of glycine, while decreasing the levels of glutamate in various brain regions (Kelly et al., 1997; Redmond et al., 1997). Neuropeptide changes are also observed following bulbectomy. These include increased levels of prepro-enkephalin (ENK) mRNA and neuropeptide Y (NPY) mRNA in various regions to which the olfactory bulbs project (Holmes et al., 1998; Primeaux & Holmes,

2000; Rutkoski et al., 2002), and also increased prepro-galanin levels in the locus coeruleus (Holmes & Crawley, 1996).

The neurochemical changes induced by OBX could result from retrograde, anterograde and transneuronal degeneration, as well as changes in synaptic number and function, including synaptic sprouting and denervation super-sensitivity. OBX causes a reduction in the number of synapses along with the number of dendritic spines and shafts in the piriform cortex and similar changes have been observed in the hippocampus (Song & Leonard, 2005). These, and the previously mentioned, neurochemical changes can be reversed following antidepressant treatment (Kelly et al., 1997).

The behavioral changes seen following OBX, often referred to as the bulbectomy syndrome, are also quite numerous including reduced sexual behavior, deficits in passive-avoidance tasks, increased irritability and increased open-field activity (Jesberger & Richardson, 1988; Kelly et al., 1997). The most extensively investigated behavioral change following bulbectomy has been the hyperactivity observed in the open-field, and this behavioral marker of OBX is most commonly used when determining the effects of antidepressant treatment in the reversal of OBX syndromes (Primeaux & Holmes, 1999).

While the effects of bulbectomy and its usefulness as a model of depression is well accepted, questions still persist as to how the destruction of the olfactory bulbs produces these "depressive-like" symptoms (Wrynn, et al., 2000). An explanation of the anatomical connections to the bulbs may be useful in beginning to elucidate these phenomena.

Olfactory Bulb Anatomy

The olfactory bulbs are considered part of the limbic system and comprise 4% of the total mass of the rat brain (Jesberger & Richardson, 1988; Kelly et al., 1997; Song & Leonard, 2005).

The main olfactory bulb consists of six cell layers divided into external and inner layers, which contain mitral, tufted, and periglomerular cells (Song & Leonard, 2005). The mitral and tufted cells are the projection neurons of the bulbs, sending glutamatergic projections through the olfactory tract and terminating in diverse brain regions (Halasz & Shepherd, 1983; Kelly et al., 1997).

The mitral and tufted cells project to the pyriform cortex, amygdala, hippocampus, entorhinal cortex, bed nucleus of the stria terminalis, hypothalamic areas, and the olfactory tubercle. The bulb has extensive efferent connections with mesocortical and subcortical brain regions that contribute to the impact of bulbectomy upon major brain functions (Song & Leonard, 1995). The olfactory bulbs receive afferent projections from the locus coeruleus, raphe nuclei, piriform cortex, diagonal band of Broca, hypothalamus, hippocampus, and medial amygdaloid nucleus (Jesberger & Richardson, 1988; Kelly et al., 1997; Scalia & Winans, 1975).

Cholinergic and GABAergic input is received from the diagonal band, while serotonergic afferents come primarily from the raphe nucleus. The locus coeruleus projects noradrenergic neurons into the bulbs (Halasz & Shepherd, 1983; Jesberger & Richardson, 1988; Kelly et al., 1997; Nesterova et al., 1997; Shipley et al., 1985; Wirth et al., 2000). The medial forebrain bundle (MFB) is the major source of noradrenergic and serotonergic projection into the olfactory bulbs (Kelly et al., 1997). The bulbs are extensively connected to various limbic regions, and therefore the depressive-like effects observed after bulbectomy may be attributed to the disruption of these connections.

Glutamate and Dopamine Changes Following OBX

As previously stated, the olfactory bulb sends out glutamatergic efferents to many subcortical limbic structures, which are transected by bulbectomy. This denervation causes

changes in glutamatergic function (Ho et al., 2004; Song & Leonard, 2005). A reduction in glutamate content occurs in the olfactory cortex after OBX (Ho et al., 2001, 2004). Changes in the glutamate NMDA receptor densities (Ho et al., 2004; Robichaud et al., 2001; Song & Leonard, 2005) have also been reported following bulbectomy. NMDA receptor densities have been found to decrease in the prefrontal and piriform cortexes, lateral amygdaloid nucleus and thalamic nucleus (Robichaud et al., 2001; Song & Leonard, 2005). Behavioral changes in the OBX rat are also influenced by NMDA receptors, evidenced by the ability of MK-801, a non-competitive NMDA antagonist, to suppress OBX-induced hyperactivity and muricidal behavior (Ho et al., 2001, 2004; Redmond et al., 1997).

The direction of change that glutamate function takes following OBX is not clear. A hypoactive glutamatergic system has been implicated in OBX rats, based on findings that locomotor stimulation induced by phencyclidine is reduced in rats with olfactory bulbectomy (Song & Leonard, 2005). Robichaud et al. (2001) found that OBX rats had significantly decreased NMDA receptor binding in several cortical and subcortical regions to which the bulbs project, and when challenged with a dose of MK-801 (0.2mg/kg, i.p.) one month after surgery, the OBX rats did not exhibit increased open-field locomotor activity, whereas sham operated rats challenged in the same way showed significant increases in locomotion in the open-field. This observation is consistent with a decrease in NMDA receptor binding, while the downregulation of NMDA receptors is consistent with an increase in glutamatergic activity in the OBX rat. It can be concluded from these studies that glutamate and NMDA receptors are related to some behavioral changes in the OBX rat (Ho et al., 2004).

Microdialysis studies examining changes in neurotransmitter release following bulbectomy found increases in glutamate release in the striatum in response to novelty exposure

(Ho et al., 2000). This increase in glutamate release was, however, only seen in the first 10 minutes after novelty exposure, but corresponds to the OBX-induced hyperactivity. Thus, it can be deduced that striatal glutamatergic functions contribute to motor control in stressful situations. Another microdialysis study measured monoamine content in the OT and dorsal striatum following OBX and found that dopamine levels in the OBX rats were significantly increased in both the OT and dorsal striatum compared to sham rats (Masini et al., 2004).

Dopamine dysregulation is observed, in addition to glutamatergic dysfunction, in rats following bulbectomy . OBX results in decreased dopamine turnover in the striatum and increased tyrosine hydroxylase activity in the midbrain (Holmes, 1999). Song and Leonard (1997) found a decrease in dopamine within the brain stem following OBX, along with decreases in NA in the hypothalamus and amygdala. Holmes (1999) found that dopamine receptor D2 mRNA levels were increased in the OT, but not in the nucleus accumbens, of OBX rats. Furthermore, prepro-enkephalin mRNA and peptide levels were found to increase after OBX, suggesting dopamine depletion or dysfunction, since ENK is inversely related to DA in the striatum (Holmes, 1999; Primeaux & Holmes, 2000). These results imply that the behavioral effects produced after OBX may be a result of decreased dopamine activity due to deafferentation produced by OBX (Holmes, 1999).

Rationale of Present Study

This study attempted to create a pharmacological manipulation that would be comparable to the OBX model, in order to clarify the role of glutamatergic deafferentation to the ventral striatum, mainly the olfactory tubercle. In order to accomplish this, we chronically injected a competitive NMDA receptor antagonist, AP-5, directly into the ventral striatum, at coordinates corresponding to the olfactory tubercle. This manipulation is meant to pharmacologically

induce, in one brain area, the glutamatergic dysfunction that would otherwise be produced by OBX. The olfactory tubercle was chosen instead of the nucleus accumbens (NAC), because the olfactory bulbs project extensively to the tubercle and not the NAC. Because this study sought to remain comparable to the OBX model, AP-5 was injected into the OT using osmotic minipumps for a time period of 14-17 days, which is around the duration of time it takes for the bulbectomy syndrome and neurochemical changes to occur following OBX. The brains of the rats were extracted 14-17 days post-surgery, and in situ hybridization was performed in the locus coeruleus (LC). This brain area was chosen for analysis because of its known influence on dopamine regulation through its projection to the ventral tegmental area (VTA).

In a previous study in our lab, chronic administration of the competitive NMDA antagonist, AP-5, resulted in hyperactivity, similar to OBX rats, but there were no detectable differences in D2 receptor or ENK mRNA levels in the olfactory tubercle of rats given AP-5 compared to shams (unpublished data). These results, thus, suggest that a similar behavioral effect as OBX is produced using this pharmacological manipulation, but the same neurochemical changes as seen after OBX were not found in the OT. As a follow up, this experiment sought to determine if neurochemical changes occurred upstream from the site of drug administration, in areas that have a regulatory function on dopamine release in the striatum, mainly the VTA and LC. Tyrosine hydroxylase (TH), the rate-limiting enzyme in the biosynthesis of catecholemines (Jones & Hess, 2003), including norepinephrine and dopamine (Arias-Montano et al., 1992; Counts et al., 2002), and galanin mRNA levels were measured in the LC following chronic treatment with AP-5. TH is a sensitive index of neuronal activity in dopaminergic and noradrenergic neurons (Ericson & Ahlenius, 1999). The following will be a discussion of the

anatomy and cellular function of glutamate and dopamine in the striatum, and how the neurochemicals in the VTA and LC are involved in striatal dopaminergic regulation.

Glutamate and Dopamine in the Striatum

Glutamate is the main excitatory neurotransmitter in the striatum receiving extensive glutamatergic input from the neocortex (Schmidt & Kretschmer, 1997) and the thalamus (Kotter, 1994). Glutamate (GLU) in the striatum is released from corticostriatal terminals which form synapses with the dendritic spines and dendritic shafts of the medium spiny neurons that make up the majority of the neostriatal cell population (Calabresi et al., 1997; Kotter, 1994). The striatum also receives dense dopaminergic afferents from the midbrain (Kotter, 1994; Schmidt & Kretschmer, 1997). The major synaptic target of the dopaminergic input into the striatum is the medium spiny projection neurons (Calabresi et al., 1997). The dopaminergic terminals are located at the necks of the dendritic spines of these neurons, where they form symmetrical contacts, while corticostriatal glutamate terminals form asymmetrical contacts with the heads of dendritic spines (Calabresi et al., 1997; Garside et al., 1996). Therefore, DA and GLU form synaptic contacts in the striatum, without any direct synapses (Kulagina et al., 2001; Segovia & Mora, 2001), instead they make numerous appositional contacts (Borland & Michael, 2004).). The close proximity of cortical glutamate and midbrain dopamine (DA) terminals suggests an anatomical basis for a possible physiological (Calabresi et al., 1997) and behaviorally relevant (Vezina & Kim, 1999) interaction at the level of the nerve terminals.

Anatomical Connections of the Dorsal and Ventral Striatum

The striatum is divided into dorsal and ventral compartments, which differ in the origin of afferents and destination of efferents (Kiyatkin, 2002). The dorsal striatum consists of the caudate-putamen and receives dopaminergic afferents primarily from the substantia nigra pars

compacta (SNC) (Kotter, 1994) and GLU from the cerebral cortex and thalamus (Kiyatkin, 2002), and the neurons of the dorsal striatum project to the substantia nigra reticulata (SNR) and dorsal pallidum.

The ventral striatum's main structures include the nucleus accumbens and olfactory tubercle, and it receives its dopaminergic input mainly from the VTA DA perikarya via the medial forebrain bundle (MFB), while the GLU input comes from prefrontal cortex, hippocampus, amygdala, (Healy & Meador-Woodruff, 1996; Ikemoto, 2002; Kiyatkin, 2002; Schmidt & Kretschmer, 1997; Vezina & Kim, 1999; You et al., 2001), and olfactory bulb (Shepherd, 1972). The neurons in the ventral striatum project primarily to the VTA and ventral pallidum (Kiyatkin, 2002). The mesocorticolimbic system refers to the integration of these connections. The ventral striatum, particularly the nucleus accumbens, is thought to play an important role in the generation of locomotor activity, most likely influenced by the convergence or interaction of mesolimbic dopamine and corticostriatal glutamate input from limbic areas onto the same dendritic spines of the medium spiny neurons in this structure (Segovia & Mora, 2001; Vezina & Kim, 1999).

Glutamatergic Regulation of Dopamine in the Ventral Striatum

Glutamate and dopamine appear to function together in the ventral striatum to produce locomotor behavior and regulate mood and affect (Breysse et al., 2002), though the complexity of their possible interaction is controversial (Borland & Michael, 2004; Vezina & Kim, 1999). There is substantial evidence showing that dopaminergic pathways in the striatum are under the regulation of glutamatergic efferents from the prefrontal cortex (Borland & Michael, 2004; Kulagina et al., 2001; Healy & Meador-Woodruff, 1996; Hu & White, 1997; Whitton et al., 1994; Wu et al., 2000). Moreover, these efferents project to the vicinity of mesolimbic

dopamine neuron soma and terminals, which suggests the regulation of dopamine by glutamate can occur by various routes (Wu et al., 2000). It has been proposed that the interaction between glutamate and dopamine may occur at the presynaptic level in the cortex or striatum, postsynaptically, or both pre- and postsynaptically (List et al., 1995).

Several studies have demonstrated the role of glutamate in dopamine release using in vitro (Wu et al., 2000) and in vivo techniques (Borland & Michael, 2004; Kulagina et al., 2001). Glutamate at corticostriatal terminals and glutamate agonists injected directly into the striatum have been found to elicit dopamine release through an impulse-independent mechanism, also referred to as tonic release, which involves the reversal of the dopamine transporter (DAT) or the non-burst firing activity of midbrain dopamine neurons (Borland & Michael, 2004; Kulagina et al., 2001). Furthermore, microdialysis studies have shown that glutamate antagonists cause extracellular dopamine levels to increase (Breysse et al., 2002; Kulagina et al., 2001) or decrease (Borland & Michael, 2004; Whitton et al., 1994). Systemic administration of a non-competitive NMDA receptor antagonist has been shown to cause an increase in DA release in the NAC, through the facilitation of burst firing of VTA neurons (Kretschmer, 1999). In situ hybridization showed that dopamine receptor D1 and D2 mRNA increases in the striatum and D2 receptor mRNA increases in the VTA after MK-801 treatment (Healy & Meador-Woodruff, 1996).

Another study found that ionotropic glutamate receptor agonists (kainate, AMPA, and NMDA in the absence of Mg2+) caused an inhibition of dopamine release using voltammetric measures (Wu et al., 2000), although excitatory actions of glutamate on dopamine release have also been reported (Hu & White, 1997; Kulagina et al., 2001; Wu et al., 2000). Arias-Montano and colleagues (1992) found that glutamate dose-dependently stimulated striatal tyrosine hydroxylase activity in vitro. These studies reflect a role for glutamate in dopaminergic

regulation, albeit with sometimes conflicting results. It is possible that endogenous glutamate exerts an effect with a dual nature of inhibitory and excitatory action on dopamine release, highlighting the complexity of the mechanisms of glutamatergic regulation of dopamine (Kulagina et al., 2001).

Glutamate Receptors and Dopamine Activity in the Striatum

Glutamate receptors are found in high densities within the striatum and they have been implicated in regulating the release of dopamine (Schmidt & Kretschmer, 1997). Using voltammetry, Kulagina et al. (2001) suggest that glutamate receptors within the striatum control dopamine release based on their finding of increased release of dopamine in striatal slices in the presence of kynurenate, a broad-spectrum glutamate receptor antagonist. Using a conditioning paradigm, Saulskaya and Marsden (1995) showed that conditioned dopamine release could be abolished by the NMDA antagonist, MK-801, administered into the nucleus accumbens implying that NMDA glutamate receptors are involved in the conditioned increase in dopamine to a contextual cue. Further evidence implying a role for glutamate receptors in the regulation of dopamine release was presented by Feenstra et al., (2002) who also found that blocking glutamate receptors in the mPFC significantly increased extracellular dopamine release, while the same treatment in the NAC did not alter DA release.

Glutamate binds to several types of receptors including ionotropic and metabotropic glutamate receptors. Dopaminergic terminals in the striatum have both ionotropic NMDA and non-NMDA glutamate receptors (AMPA, kainate), by which glutamate can stimulate both the release and synthesis of dopamine (Arias-Montano et al., 1992). However, other studies suggest there are few glutamatergic receptors present on dopaminergic terminals and that this action occurs through alternative means in the striatum (Kulagina et al., 2001).

Glutamate Receptors and Dopamine in the Ventral Striatum: Effects on Locomotor Activation

Ionotropic glutamate receptors (iGluRs) in the nucleus accumbens are associated with the production of locomotor behaviors (Vezina & Kim, 1999). Enhanced motor activity is also associated with increased release of DA in the striatum (Ougazal & Amalric, 1995; Svensson et al., 1994; Wheeler et al., 1995). Increasing evidence suggests that agonists at the ionotropic glutamate receptors produce their effects on locomotion by interacting with DA neurotransmission (Gimenez-Llort et al., 2002; Vezina & Kim, 1999). Hyperactivity has been produced by using agonists of the NMDA and non-NMDA iGluRs injected into the nucleus accumbens (Breysse et al., 2002), moreover, these effects are attenuated by drugs that interfere with dopamine neurotransmission, such as haloperidol (Vezina & Kim, 1997).

Antagonists at the NMDA and non-NMDA receptors in the nucleus accumbens have also been shown to inhibit both the activation of locomotion and DA release caused by amphetamine and cocaine administration in this site, implying a glutamatergic and dopaminergic interaction in the nucleus accumbens that contributes to the production of locomotor activity (Vezina & Kim, 1999). In another study, a competitive and non-competitive NMDA receptor antagonist (APV, and MK-801, respectively) produced locomotor stimulation similar to that produced by dopamine injections into the ventral striatum, suggesting a functional antagonism between mesolimbic dopamine and corticostriatal glutamate input (Alamaric et al., 1994).

Although the olfactory tubercle has received less attention than the nucleus accumbens, it is a component of the ventral striatum that also receives rich dopaminergic afferents from the VTA (Holmes, 1999; Mooney et al., 1987) and is involved in locomotor activity (Ikemoto, 2002). Ikemoto (2002) found that local injections of amphetamine, cocaine, dopamine, and D1- and D2-type agonists into the medial portion of the tubercle produced a marked increase in locomotion

and rearing. Moreover, the olfactory tubercle was found to have among the highest NMDA receptor densities of all brain areas tested, based on [^{125}I]-iodo-MK-801 autoradiography (Robichaud et al., 2001), and glutamate has been shown to stimulate the release of radiolabeled dopamine in the site (Jones et al., 1993).

NMDA Receptor Modulation of Locomotor and Dopamine Activity: Competitive vs. Non-Competitive Antagonists

The NMDA receptor has a multitude of functions in the central nervous system, including regulating motor activity (Gimenez-Llort et al., 2002; Kretschmer, 1999), sensory-motor integration and cognitive functions (Ouagazzal & Amalric, 1995) and is known to play an important role in the interaction between glutamate and dopamine (Aris-Montano et al., 1992; Healy & Meador-Woodruff, 1996; Kretschmer, 1999; Murata et al., 2002, Saulskaya & Marsden, 1995) in the production of motor activity, primarily in the ventral striatum (Amalric et al, 1994; Feenstra et al., 2002). The non-competitive NMDA receptor antagonists, phencyclidine (PCP) or MK-801, which act directly in the PCP binding site located in the ion channel of the NMDA receptor complex, have been shown to induce locomotor hyperactivity and stereotypies which closely resemble the behavioral effects produced after administration of dopamine enhancing drugs (Amalric et al., 1994). Furthermore, the behavioral activation effects of the non-competitive NMDA receptor antagonists was decreased after blocking dopamine receptors or dopamine depletion, which suggests the effects of these antagonists are dopamine-dependent (Ouagazzal et al., 1994; Ouagazzal & Amalric, 1995). Non-competitive NMDA receptor antagonists also have been found to increase the firing rate of midbrain dopamine neurons (Ouagazzal & Amalric, 1995), and induce changes in striatal D2 dopamine receptor expression (Murata et al., 2002).

Intra-accumbens administration of competitive NMDA receptor antagonists have also been found to produce hyperactivity and stereotyped behavior (Ougazzal & Amalric, 1995; Schmidt & Kretschmer, 1997; Svensson et al., 1994). On the other hand, the ability of competitive NMDA receptor antagonists to produce these effects through a dopaminergic mechanism is still being questioned (Ougazzal & Amalric, 1995). For example, using microdialysis, Kretscher (1999) found that systemic administration of MK-801 (non-competitive) caused an increase in DA in the VTA and NAC, while the competitive NMDA antagonist, CGP40116 resulted in no change in DA levels in either of these brain areas. Alternatively, when Kretscher injected MK-801 (non-competitive) and AP-5 (competitive) locally into the VTA, MK-801 produced an increase in DA in the VTA, but no change in the NAC, while AP-5 injected locally in the same site produced the opposite effect, decreasing DA levels in both the VTA and NAC. The effects of these drugs on locomotor activity also differed, as seen by increased behavioral activity in rats systemically injected with MK-801, while rats that received CGP40116 or AP-5 showed no behavioral activation. These data clearly demonstrate the differences between the effects of competitive and non-competitive NMDA receptor antagonists.

Similarly, when using the competitive NMDA antagonist, AP-5 (10µg), Ougazzal & Amalric (1995) found that blocking dopamine receptors with haloperidol (5µg) prior to AP-5 infusion into the nucleus accumbens failed to affect the locomotor stimulation produced by AP-5, whereas, dopamine blockade in rats infused with MK-801 (10µg) in the same manner significantly reduced the locomotor activation induced by MK-801, again providing evidence against a dopamine-mediated effect on locomotor stimulation induced by the competitive NMDA receptor antagonist. Also in line with the non-dopamine dependent behavioral activation

of competitive NMDA antagonists, Feenstra et al., (2002) found that AP-5 infused bilaterally into the NAC markedly increased locomotor activity without effecting DA efflux in this site.

Even more convincing are studies using monoamine-depleted mice which are systemically administered competitive NMDA receptor antagonists and increased locomotor activity is nonetheless observed (Ougazzal & Amalric, 1995; Svensson et al., 1994). Moreover, Svensson and colleagues (1994) found that AP-5 (.125µg) injected bilaterally into the nucleus accumbens stimulated locomotor activity and was significantly more potent in monoamine-depleted mice compared to monoaminergically intact mice. Also, in monoamine-intact mice, this dose of AP-5 had no effect on locomotor stimulation. Thus, this data suggests that competitive NMDA receptor antagonists do not exert their locomotor stimulatory effects through a dopaminergic mechanism.

Influence of the Ventral Tegmental Area in Ventral Striatal Dopamine Release

The mesocorticolimbic system arises from the ventral tegmental area and projects to various limbic structures, including the nucleus accumbens (Adell & Artigas, 2004; Iravani et al., 1996). The ventral striatum receives dense dopaminergic projections from the VTA, which contributes to the regulation of dopamine neurons of the mesocorticolimbic system and the locomotor activity associated with dopaminergic function in this pathway (Adell & Artigas, 2004; Pickel et al., 2002). Dopamine release in the VTA has been shown to change under different behavioral and pharmacological conditions (Adell & Artigas, 2004). The dopaminergic neurons in the VTA are under tonic inhibition by somatodendritic D2 receptors (Pickel et al., 2002). Extracellular dopamine can activate local inhibitory D2 autoreceptors, thus inhibiting dopamine cell firing and release in the VTA and in the nerve terminals of cortical and limbic fibers (Adell & Artigas, 2004). D2 receptors are present in dopamine neurons throughout the

VTA, which has been demonstrated by measures of D2 immunoreactivity, mRNA, and binding sites (Pickel et al., 2002).

D2 receptors can also function as heteroreceptors in non-dopaminergic neurons, and electrophysiological studies have found that dendritic release of dopamine may regulate the DA firing rate in VTA neurons through D2 receptors located in glutamatergic terminals (Adell & Artigas, 2004; Marinez-Fong et al., 1982). Glutamatergic input to the VTA originates in the medial prefrontal cortex (mPFC), and these afferents synapse on dopaminergic cells that project back to the mPFC and on GABAergic cells that project to the nucleus accumbens, therefore suggesting that glutamatergic control of DA activity can be achieved through the glutamate receptors in the VTA, (Adell & Artigas, 2004). Systemic injections of competitive NMDA receptor antagonists have been found to produce no change in the release of dopamine in the VTA and nucleus accumbens, in contrast to non-competitive NMDA receptor antagonists, which markedly induce burst firing of DA cells, as well as DA release in the VTA, nucleus accumbens, and mPFC (Adell & Artigas, 2004). In contrast to systemic administration, local injections of the competitive NMDA antagonist, AP-5, in the VTA, produced a reduction in the release of DA in this site and in the nucleus accumbens and mPFC (Adell & Artigas, 2004).

Dopamine Modulation by Norepinephrine and Galanin in the Locus Coeruleus

Dopaminergic neuron activity in the VTA, the midbrain structure which projects to the ventral striatum, can be modulated by noradrenergic afferents which originate in the locus coeruleus (LC) (Adell & Artigas, 2004; Herve et al.,1982). The stimulation of noradrenergic neurons in the LC has been found to increase the firing of VTA dopamine neurons (Harro et al., 2003; Lategan et al., 1990; Weiss et al., 2005). Herve et al. (1982) used 6-OHDA lesions to destroy the ascending noradrenergic input to the VTA, while preserving the DA levels in the

VTA by using a DA uptake inhibitor. Their results indicated that there was a reduced rate of DA utilization in the prefrontal cortex due to the destruction of the NA fibers projecting to the VTA, however they did not find a change in DA levels in the nucleus accumbens following this manipulation. Another lesion study using DSP-4, a noradrenergic neurotoxin, found that brain NE levels were reduced by 75%, along with a decrease in DA concentration in the caudate nucleus (52%) and nucleus accumbens (28%) (Lategan et al., 1992), thus supporting a role for noradrenergic facilitation of mesolimbic dopamine neurotransmission. Furthermore, DSP-4 lesions in the locus coeruleus were also found to increase the number of D2 receptor binding sites in the striatum (Harro et al., 2003).

Considering the modulatory effects of NE on dopaminergic activity, it is not surprising that the noradrenergic system also influences locomotor activity. Berridge and Waterhouse (2003) propose that the synergistic actions of α1- and β- adrenoreceptors play a role in the behavioral activation observed following amphetamine administration. Blocking the α1-adrenoreceptor in the LC with terazosin was shown to block exploratory behavior in a novel or home cage (Stone et al., 2004). In addition, Ihalainen and Tanila (2004) suggest modulation of locomotor activity is mediated by the alpha2A-adrenoreceptors, which regulate DA release in the NAC indirectly through their effect on DA neurons in the VTA. DSP-4 lesions have also been used to better understand the role of NE in the expression of locomotor stimulation in a mutant mouse strain which exhibits spontaneous locomotor hyperactivity (Jones & Hess, 2003). The DSP-4 lesions in this study caused a reduction in the hyperactivity of this mutant mouse strain and also slightly reduced locomotor activity in wild type mice, thus implying a role for NE in behavioral activation.

In contrast to the effects of norepinephrine on DA activity, VTA dopamine activity, as well as TH expression, is inhibited by galanin, a 29-amino acid neuropeptide, released from the LC (Counts et al., 2002). GAL is colocalized with NE in almost all LC cells and acts to hyperpolarize monoaminergic neurons, therefore inhibiting their activity (Weiss et al., 2005; Mu et al., 2001; Xu et al., 2005; Yoshitake et al., 2003). Tsuda et al. (1998) found that galanin inhibited stimulation-evoked [^{3}H]dopamine release in striatal sections. Ericson and Ahlenius (1999) looked at the effects of intracerebroventricular (i.c.v.) administration of GAL on DOPA, the biosynthetic byproduct of dopamine, levels in dopamine-dominated brain areas, such as the olfactory bulb, the olfactory tubercle, nucleus accumbens and neostriatum, and found that DOPA dose-dependently increased in these areas following this manipulation. Futhermore, when GAL was injected directly into the VTA, a significant increase in DOPA accumulation was found in the nucleus accumbens, however local administration of GAL into the nucleus accumbens itself did not produce changes in DOPA levels in this area, suggesting GAL exerts its effects at the somato-dendritic level in the mesolimbic dopaminergic system. Moreover, the authors suggest that the increase in DA synthesis is secondary to a decreased release of DA, which would result in the removal of D2 autoreceptor-mediated inhibition of DA synthesis (Erikson & Ahlenius, 1999).

The effects of GAL on dopamine activity carry implications of its effects on the modulation of locomotor activity. Weiss et al. (2005) infused GAL into the VTA and found that both ambulatory and rearing behavior were reduced in a novel environment, as well as reduced swim activity in a forced swim test paradigm. Furthermore, this effect was reversed in the forced swim test using the GAL antagonist, galantide, into the VTA. These behavioral measures are in line with the well known role of dopaminergic stimulation that causes increased motor activity,

which can be inhibited by GAL. Following i.c.v. injections of GAL, rats in an open-field displayed suppressed locomotor activity, along with increased DOPA accumulation in the nucleus accumbens, suggestive of decreased DA release from the VTA (Ericson & Ahlenius, 1999). This is also evidence of the locomotor suppression observed following inhibition of dopamine activity.

Conclusion

In light of the role of glutamate NMDA receptors and dopamine in the ventral striatum, the present study hypothesizes that chronic blockade of NMDA receptors in the ventral striatum using a competitive NMDA receptor antagonist, AP-5, will result in changes in the mRNA levels of neurochemicals, mainly galanin and tyrosine hydroxylase (TH), in the LC, which projects to the VTA, thereby influencing the dopamine release in the ventral striatum. These changes are believed to be concomitant to the enhanced locomotor activity observed following chronic blockade of NMDA receptors in the ventral striatum (unpublished data), and furthermore, we hypothesize that these neurochemical and behavioral effects are comparable to those observed following the glutamatergic deafferentation which results following olfactory bulbectomy.

The results of these experiments are reported in the following manuscript, along with a description of the methodology of our experimental manipulation and neurochemical analyses. The background provided in this literature review is integrated into the introduction and discussion sections of the manuscript.

CHAPTER 3

TYROSINE HYDROXYLASE INCREASES IN THE LOCUS COERULEUS FOLLOWING CHRONIC NMDA BLOCKADE IN THE VENTRAL STRIATUM: COMPARISON TO OLFACTORY BULBECTOMY[1]

1 Lelutiu, N.B. and P.V. Holmes. To be submitted to *Psycholpharmacology.*

Abstract

Rationale: Olfactory bulbectomy (OBX) results in many neurochemical and behavioral changes, one hallmark behavioral syndrome being hyperactivity in a novel open-field. Though the changes incurred following bulbectomy are well-documented, it is unknown precisely how these changes are produced. *Objectives*: In order to determine if the loss of glutamatergic input to the ventral striatum contributes to the locomotor activation and neurochemical changes following OBX, the present study aimed to create a pharmacological manipulation that would be comparable to the OBX model. *Method*: Male Sprague-Dawley rats were infused with one of three doses (2.5, 5.0, or 10.0 μg/day) of the competitive NMDA receptor antagonist, AP-5, directly into the right side of the ventral striatum for 14-17 days. Brains were then later sliced through the locus coeruleus and prepared for in situ hybridization in order to measure changes in radiolabelled tyrosine hydroxylase (TH) and galanin (GAL) mRNA levels in this area. *Results*: TH mRNA levels increased significantly in the LC of rats infused chronically with AP-5 at a dose of 10 μg/day compared to all other drug doses and vehicle. GAL mRNA levels did not significantly change in the LC of any group. *Conclusions*: These results suggest that glutamatergic disruption in the ventral striatum leads to increased noradrenergic activity in the LC, which would result in stimulation of dopaminergic activity in the VTA. This may be a possible explanation for the hyperactive locomotor response observed following NMDA receptor blockade in the ventral striatum, and thus comparably illustrating a mechanism for increased locomotor activity observed in the OBX rat.

KEYWORDS: Olfactory bulbectomy, NMDA receptors, Dopamine, Locus Coeruleus, Norepinephrine, Ventral Tegmental Area

Introduction

The olfactory bulbectomy (OBX) model of depression results in many neurochemical and behavioral changes, which can be reversed only by chronic administration of antidepressants (Kelly et al., 1997). The neurochemical disruptions which result following OBX are widespread, including reduced norepinephrine (NE) and serotonin (5-HT) in numerous brain regions and decreased or increased dopamine (DA) levels in the telencephalon and striatum (see Song & Leonard, 2005 for review). Neuropeptide changes following the ablation of the olfactory bulbs include increased prepro-enkephalin (ENK) and neuropeptide (NPY) mRNA in various olfactory bulb projection sites (Holmes et al., 1998; Primeaux & Holmes, 2000; Rutkoski et al., 2002), and also increased prepro-galanin levels in the locus coeruleus (Holmes & Crawley, 1996). The most extensively investigated behavioral change following bulbectomy has been the hyperactivity observed in the open-field. The mechanism by which bulbectomy causes these neurochemical and behavioral changes is still not well understood. One possibility is that the loss of glutamatergic input from the bulbs to various limbic structures causes these changes.

The olfactory bulb sends out glutamatergic efferents to many subcortical limbic structures, which are transected by bulbectomy. This denervation causes changes in glutamatergic function (Ho et al., 2004; Song & Leonard, 2005) and changes in glutamate NMDA receptor densities in various brain areas (Ho et al., 2004; Robichaud et al., 2001; Song & Leonard, 2005). Behavioral changes in the OBX rat are also influenced by NMDA receptors, evidenced by the ability of MK-801, a non-competitive NMDA antagonist, to suppress OBX-induced hyperactivity and muricidal behavior (Ho et al., 2001, 2004; Redmond et al., 1997).

Microdialysis studies looking at changes in neurotransmitter release following bulbectomy found increases in glutamate release in the striatum in response to novelty exposure

(Ho et al., 2000). Another microdialysis study measured monoamine content in the olfactory tubercle (OT) and dorsal striatum (DS) following OBX and found that dopamine levels in the OBX rats were significantly enhanced in both the OT and DS compared to sham rats, suggesting that increased dopamine activity corresponds to the hyperactivity in the open-field commonly observed in the OBX rat (Masini et al., 2004).

Dopamine dysregulation is observed, in addition to glutamatergic dysfunction, in rats following bulbectomy . OBX results in decreased dopamine turnover in the striatum and increased tyrosine hydroxylase (TH) activity in the midbrain (Holmes, 1999). Holmes (1999) found that dopamine receptor D2 mRNA levels were increased in the OT, but not in the nucleus accumbens, of OBX rats. Furthermore, prepro-enkephalin (ENK) mRNA and peptide levels were found to increase after OBX, suggesting dopamine depletion or dysfunction, since ENK is inversely related to DA in the striatum (Holmes, 1999; Primeaux & Holmes, 2000).

The present study attempted to create a pharmacological manipulation that would be comparable to the OBX model, in order to clarify the role of glutamatergic deafferentation to the ventral striatum, mainly the olfactory tubercle, and its effects on dopamine activity in this site. In order to accomplish this, we injected a competitive NMDA receptor antagonist, AP-5, directly into the ventral striatum, at coordinates corresponding to the olfactory tubercle. This manipulation is meant to pharmacologically induce, in one brain area, the glutamatergic dysfunction that would otherwise be produced by OBX. The olfactory tubercle was chosen instead of the nucleus accumbens, because the olfactory bulbs project extensively to the tubercle and not the NAC. Because this study sought to remain comparable to the OBX model, AP-5 was injected into the OT using osmotic minipumps for a time period of 14-17 days, which is around the duration of time it takes for the bulbectomy syndrome and neurochemical changes to occur

following OBX. The brains of the rats were extracted 14-17 days post-surgery, and in situ hybridization in the locus coeruleus (LC) was performed. This brain area was chosen for analysis because of its known influence on dopamine regulation.

In a previous study in our lab, chronic administration of the competitive NMDA antagonist, AP-5, resulted in hyperactivity, similar to OBX rats, but there was no detectable differences in dopamine D2 receptor or ENK mRNA levels in the olfactory tubercle of rats given AP-5 in this site compared to those receiving saline (unpublished data). These results, thus, suggest that a similar behavioral effect as OBX is produced using this pharmacological manipulation, but the same neurochemical changes which have been observed following OBX were not found in the OT. As a follow up, the current experiment sought to determine if neurochemical changes occurred upstream from the site of drug administration, in areas that have a regulatory function on dopamine release in the striatum, mainly the ventral tegmental area (VTA) and the locus coeruleus (LC). Tyrosine hydroxylase (TH), the rate-limiting enzyme in the biosynthesis of dopamine (Arias-Montano et al., 1992; Counts et al., 2002), and galanin (GAL) mRNA levels were measured in the LC following chronic treatment with AP-5. TH is a sensitive index of neuronal activity in dopaminergic and noradrenergic neurons (Ericson & Ahlenius, 1999). TH mRNA levels were also measured in the VTA, along with D2 receptor mRNA levels. The following briefly discusses the role of the LC in dopaminergic regulation.

Dopaminergic neuron activity in the VTA, the midbrain structure which projects to the ventral striatum, can be modulated by noradrenergic afferents which originate in the locus coeruleus (LC) (Adell & Artigas, 2004; Herve et al.,1982). The stimulation of noradrenergic neurons in the LC has been found to increase the firing of VTA dopamine neurons (Harro et al., 2003; Lategan et al., 1990; Weiss et al., 2005). Herve et al. (1982) used 6-OHDA lesions to

destroy the ascending noradrenergic input to the VTA, while preserving the DA levels in the VTA by using a DA uptake inhibitor. Their results indicated that there was a reduced rate of DA utilization in the prefrontal cortex due to the destruction of the NA fibers projecting to the VTA, however they did not find a change in DA levels in the nucleus accumbens following this manipulation. Another lesion study using DSP-4, a noradrenergic neurotoxin, found that brain NE levels were reduced by 75%, along with a decrease in DA concentration in the caudate nucleus (52%) and nucleus accumbens (28%) (Lategan et al., 1992), thus supporting a role for noradrenergic facilitation of mesolimbic dopamine neuerotransmission. Furthermore, DSP-4 lesions in the locus coeruleus were also found to increase the number of D2 receptor binding sites in the striatum (Harro et al., 2003).

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Materials and Methods

Subjects. Male Sprague-Dawley rats (Harlan Inc., Indianapolis, IN) weighing 250-335g at the time of surgery served as subjects. Rats were group housed two to four per cage and maintained on a 12-hour light/dark cycle (lights on at 0700hr). Food and water were available ad libitum. All procedures were carried out following NIH guidelines for the care and use of laboratory animals and were approved by the University of Georgia Animal Care and Use Committee.

Drugs. Animals received one of three doses (2.5µg/day, 5.0 µg/day, 10.0 µg/day) of the competitive NMDA receptor antagonist, DL-2-Amino-5-Phosphonovaleric Acid (AP-5, Sigma) or 0.9% saline. The drug or vehicle was injected through osmotic minipumps (Alzet Model 2002) for a duration period of 14-17 days. Animals were killed by rapid decapitation following the 14-17 day drug or vehicle administration. Brains were quickly extracted and blocked just anterior to the hypothalamus. The brains were then frozen in dry ice and stored at -80ºC until sectioning.

Surgical Procedures for Chronic NMDA Antagonist Administration. On the day of surgery, osmotic minipumps (Alzet Model 2002) with a pumping rate of 0.25µl/hr and a pump duration of 14-17 days, were pre-filled with either 0.9% saline or one of three doses (2.5µg/day, 5.0 µg/day, 10.0 µg/day) of the competitive NMDA receptor antagonist AP-5. Rats were anesthetized with pentobarbital (25mg/kg; Sigma) and ketamine hydrochloride (40mg/kg; Mallinckrodt) and placed in a stereotaxic device. A midline incision was made to expose the skull, a small burr hole was drilled and an injector cannula (Plastics One, Inc.) was lowered directly into the ventral striatum at the following coordinates from Bregma: +1.0mm AP, -2.5 mm Lat, and -8.5mm DV (Paxinos & Watson, 1986). Three additional burr holes were drilled for skull screws, and epoxy was applied to the skull to hold the injector cannula in place. The injector cannula was connected via Silastic tubing, underneath the skin, to the osmotic minipump, which was surgically implanted between the scapulae. A 2.5mg/kg dose of the analgesic Banamine was given immediately after surgery. The rats were allowed to recover 14-17 days before behavioral testing (data not reported) and brain extraction. Final number of rats per treatment group ranged from 4-9.

Verification of Cannulae Placement. The brains were stored at -80°C until sectioning on a Microm cryostat. Once the cannula track was visualized, a picture of the area was taken using a digital camera. For each brain, twelve-micron thick sections through the region of the cannula track were mounted on gelatin-coated slides and stained with thionin. Subjects' whose cannula tracks were not in the region corresponding with Plates 12-15 in the rat brain atlas of Paxinos and Watson (1986) were excluded from the study.

In Situ Hybridization. A Microm Cryostat was used to slice the brains into 12-μm sections at the level of the LC, in congruence with plate numbers 58, 59 of Paxino and Watson's (1986) rat brain atlas. Sections were thaw-mounted onto gelatin coated glass microscope slides (Fisher) and approximately 3 sections for each subject were stained with 0.1% thionin to verify anatomical location. Sections were taken for analyses of tyrosine hydroxylase (TH) and galanin (GAL) in the LC. Four sections (two slides) per animal were selected for each probe to be analyzed. Sections were fixed in a 4% formaldehyde in phosphate buffered saline (PBS, pH 7.4) for 5 minutes, rinsed twice in PBS, and placed in 0.25% acetic anhydride in 0.1 M triethanolamine HCl (TEA, pH 8.0) for 10 minutes. Sections were dehydrated using a series of ethanol washes (70, 80, 95, and 100%), delipidated in chloroform for 5 minutes, rinsed in ethanol (100%, 95%), and allowed to dry.

Oligonucleotide probes were obtained from Oligos Etc. (Wilsonville, OR, USA). The TH probe was complementary to bases 1441-1488 of rat TH mRNA (5'-CGTGGGCCAGGGTGTGCAGCTCATCCTGGACCCCCTCCAAGGAGCGCT -3') (Grima et al., 1985). The Gal probe was complementary to bases 228-271of human Gal mRNA (5'-G AAG GTA GCC AGC GCT GTT CAG GGT CCA GCC TCT CTT CTC CTT T -3') (Kaplan et al., 1988). The probes were labeled at the 3'-end using [^{35}S]-dATP (1000 μCi/mmol;

PerkinElmer Life Sciences, Inc., Boston, MA, USA), terminal transferase, recombinant (400U/µl; Roche, Indianapolis, IN, USA), $CoCl^2$ (25mM, Roche), and 5x TdT reaction tailing buffer (Roche). Unincorporated nucleotides were removed from the DNA probes using Stratagene Nuctrap chromatographic columns (La Jolla, Ca, USA). Enough radiolabeled probe was used so that there would be 1.0×10^6 cpm per slide (0.5×10^6 cpm per section). Sections were hybridized with radiolabeled probes in hybridization solution containing 50% formamide, 600mM NaCl, 80mM Tris-HCl (pH 7.5), 4mM EDTA, 0.1% sodium pyrophosphate, 0.2% SDS, 0.2mg/ml heparin sulfate, and 10% dextran sulfate. Brain sections were incubated with the hybridization solution in a humid chamber at 37% for 20 hours. Sections then underwent a series of washes (three, 1X-SSC; one, 2X-SSC/50% formamide; three, 2X-SSC/50% formamide for 20 minutes at 40°C; two, 1X-SSC for 30 minutes at room temperature) to reduce nonspecific binding. Slides were rinsed in deionized water and 70% ethanol and allowed to dry. Sections were placed in cartridges and exposed to autoradiographic film (BioMax MR, Eastman Kodak, Rochester, NY, USA) and developed with Kodak GBX developer and fixer. Exposure time for GAL in the LC was 11 days. Exposure time for TH in the LC was 7 days.

Autoradiographic films were analyzed using a computerized image analysis system (IMAGE 1.38 software, Rasband, 1995, National Institute of Mental Health; Power Macintosh 8100 computer, Apple Computer, Cupertino, CA, USA; light box and camera, Imaging Research, St. Catharines, Ontario, Canada; video interface, Data Translation, Marlboro, MA, USA) to determine optical density (OD) within the LC. TH and GAL mRNA of each section was measured by determining the mean OD within a hand-drawn shape placed within the region of the LC bilaterally. Data was statistically analyzed by one-way analysis of variance (ANOVA) and Bonferroni post hoc analyses.

Results

The effects of chronic AP-5 infusion into the ventral striatum on TH mRNA levels in the LC are shown in Figure 3.1. Data was analyzed by one-way ANOVA, which revealed a significant main effect of drug treatment ($F=21.145$, $p < 0.01$). Bonferroni post-hoc analyses determined the significant effect was in the AP-5 (10µg/day) group only. This dose of the drug produced significantly higher TH mRNA levels in the LC compared to all other doses (AP-5 2.5 µg and 5.0µg/day) and vehicle. Figure 3.2 demonstrates, by computerized images of autoradiographic film, the difference in TH mRNA radiolabeling in the LC between the 10.0µg/day group and vehicle. No significant changes in TH mRNA occurred in rats given either 2.5µg or 5.0µg/day of the drug compared to vehicle.

AP-5 infusion produced no significant changes in GAL mRNA levels in the LC as revealed by one-way ANOVA ($F=0.189$, $p = 0.904$) (Fig. 3.3) at any dose tested, though a similar trend occurred as observed in TH mRNA levels, with AP-5 at the 10µg/day dose producing higher GAL mRNA levels in the TH. This did not, however reach significance.

Discussion

The current study attempted to determine if glutamatergic disruption caused by blocking NMDA receptors in the ventral striatum would result in changes in the LC thereby influencing dopaminergic activity in the VTA, which would be indicative of the changes which contribute to the increased locomotor activity associated with NMDA receptor blockade in the ventral striatum and comparable to the behavioral syndrome observed after olfactory bulbectomy. The blockade of NMDA receptors by AP-5 in the ventral striatum was intended to create in one brain area (ventral striatum), the changes that would otherwise occur in the OBX rat because of the glutamatergic deafferentation which occurs following the ablation of the olfactory bulbs. The

chronic infusion of AP-5 into the ventral striatum caused a significant increase in tyrosine hydoxylase (TH) mRNA levels in the LC, while exerting no significant effect on galanin gene expression in this site.

Previously in our lab, we have demonstrated that chronic blockade of NMDA receptors in the ventral striatum using AP-5 resulted in enhanced locomotor activity and stereotypic behavior in an open-field (unpublished data). Because we did not find an indicative change of dopaminergic activity in this brain area, we proposed that a change could have occurred upstream from the site of drug administration in areas which affect dopaminergic activity. The LC is one such site, which contains the majority of noradrenergic neurons which project to the VTA, thereby influencing the activity of dopaminergic activity through the stimulation of these neurons by NE (Harro et al., 2003; Lategan et al., 1990; Weiss et al., 2005) or inhibition of dopamine neurons by GAL (Counts et al., 2002). Our results suggest that the hyperactivity observed after chronically blocking NMDA receptors in the ventral striatum may be due to an increase in dopaminergic neurotransmission in the VTA caused by an increase in noradrenergic activity in the LC, which would therefore result in an increase in dopamine release in the ventral striatum. This change in the activity of dopamine in this pathway could be the cause of the behavioral stimulation observed following our pharmacological manipulation, thus implying changes in noradrenergic activity in the LC also contributes to the hyperactivity in an open-field commonly observed in the OBX model of depression.

It is well-established that dopamine in the ventral striatum contributes to enhanced locomotor activity (Ougazal & Amalric, 1995; Svensson et al., 1994; Wheeler et al., 1995). Ikemoto (2002) observed an increase in locomotion and rearing after local injections of dopamine into the olfactory tubercle. Similarly, microdialysis studies have shown that dopamine

release is increased in both the dorsal and ventral striata in OBX rats compared to sham (Masini et al., 2004). Hyperactivity in the open-field is a hallmark feature of the OBX model (Kelly et al., 1997). This hyperactivity could be a result of increased dopamine release in the ventral striatum, as these studies suggest. Our results are consistent with this type of dopaminergic dysfunction, implying that increased DA release in the ventral striatum is indirectly affected by increased noradrenergic activity in the LC. This increase in NE could act to stimulate dopamine neurons in the VTA, which project to the ventral striatum, thereby increasing DA activity in this site and thus causing hyperactivity.

The interaction between glutamate and dopamine in the ventral striatum is also well-documented, and both dopamine and glutamate NMDA receptors play a role in motor activity (Gimenez-Llort et al., 2002; Kretschmer, 1999; Vezina & Kim, 1999). This study attempted to clarify the role of glutamatergic deafferentation to the ventral striatum, which occurs in OBX, by blocking NMDA receptors in this site with a competitive NMDA receptor antagonist, AP-5. Both OBX (Ho et al., 2000; Redmond et al., 1997) and NMDA receptor blockade in the ventral striatum (Amalric et al., 1994; Ougazzal & Amalric, 1995; Schmidt & Kretschmer, 1997; Svensson et al., 1994) have been shown to produce behavioral stimulation. The results obtained here suggest that the loss of glutamatergic input to limbic structures, such as the ventral striatum, and the effect of this loss on dopamine activity may be the cause of the hyperactivity associated with OBX. Furthermore, these changes in dopamine activity may stem from increased noradrenergic activity, evidenced in this study by increased TH gene expression in the LC.

In contrast to studies finding competitive NMDA antagonists exert their effects on locomotor stimulation independent of dopaminergic influence (Feenstra et al., 2002; Kretscher, 1999; Ouagazzal & Amalric, 1995; Svensson et al., 1994), this study suggests that locomotor

activation through chronic NMDA receptor blockade with the competitive antagonist, AP-5 (10μg/day), may exert a change in dopaminergic activity indirectly by increasing noradrenergic activity in the LC. One possible explanation of these results may be that we chronically administered AP-5 into the ventral striatum, which differs from studies reporting no effect of competitive NMDA receptor antagonists on dopamine function (Feenstra et al., 2002; Kretscher, 1999; Ouagazzal & Amalric, 1995; Svensson et al., 1994). Further experimentation needs to be done in order to determine if this manipulation affected the release of dopamine or caused a change in dopamine receptors in the VTA. Though, it is quite likely that the increase in noradrenergic activity in the LC would stimulate dopaminergic neurotransmission in the VTA.

For example, Ihalaienen and Tanila (2004) suggest that locomotor activity is mediated by the alpha2A-adrenoreceptors, which regulate DA release in the NAC indirectly through their effect on DA neurons in the VTA. Similarly, bulbectomy has been shown to produce an increase in the number of alpha2-adrenoceptors in cortical regions (Song & Leonard, 2005). Furthermore, DSP-4 (a noradrenergic neurotoxin) lesions have been shown to reduce the hyperactivity associated with the mouse mutant coloboma, which exhibits spontaneous locomotor hyperactivity (Jones & Hess, 2003). These studies imply that increased locomotor activity may be a consequence of noradrenergic influence on dopamine activity.

The fact that no significant changes were observed in GAL gene expression following chronic infusion with AP-5 is consistent with the role of GAL in the inhibition of dopamine (Erikson & Ahlenius, 1999; Tsuda et al., 1998) and locomotor activity (Erikson & Ahlenius, 1999; Weiss et al., 2005). GAL has been found to inhibit stimulation-evoked [^{3}H]dopamine release in striatal sections (Tsuda et al., 1998), and increase DA synthesis as a secondary effect of a reduction in dopamine release in the nucleus accumbens (Erikson & Ahlenius, 1999).

Morever, i.c.v. injections or local infusion of GAL into the VTA resulted in locomotor suppression when tested in an open-field (Erikson & Ahlenius, 1999) or forced swim test (Weiss et al., 2005), respectively.

This study supports the idea that increased dopamine activity leads to a hyperactive motor response, and that this change in DA can occur due to the loss of glutamatergic input to the ventral striatum, through antagonism or bulb ablation, and furthermore this increase in DA activity can be linked to the observed increase in noradrenergic activity, indicated by increased TH gene expression in the LC.

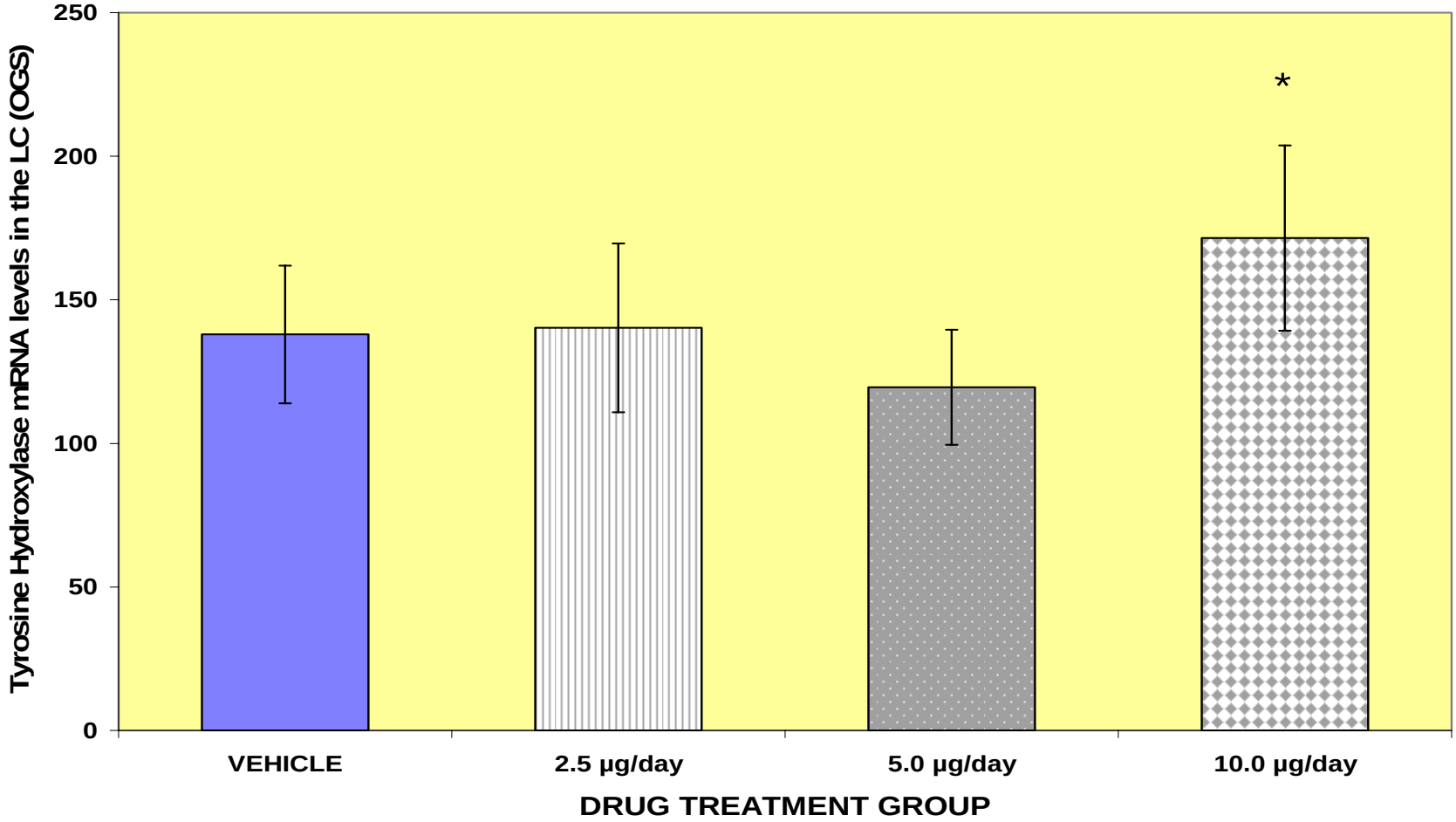
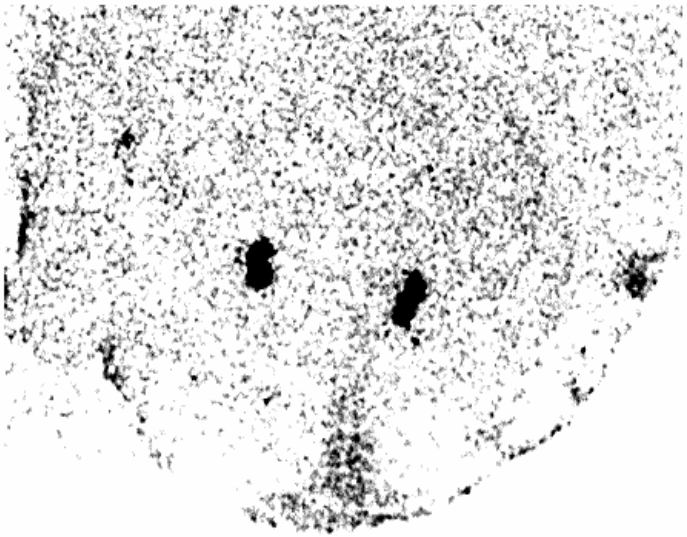
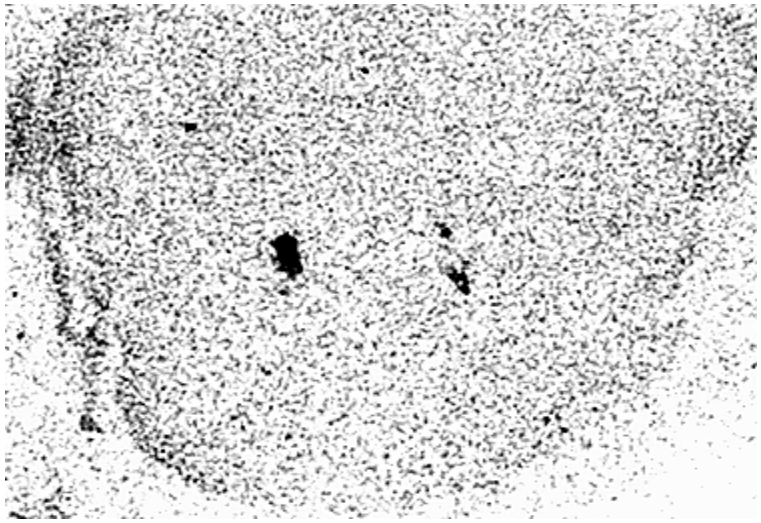


Figure 3.1. The effects of chronic AP-5 treatment (2.5µg/day, 5.0µg/day, and 10.0µg/day) on tyrosine hydroxylase (TH) mRNA levels in the locus coeruleus (LC). Rats received drug treatment or vehicle for 14-17 days after which they were killed. Brain sections of the locus coeruleus were hybridized with a radiolabelled oligonucleotide probe (rat TH). Data are presented as mean optical grey scale value (OGS) $\pm$ SEM. (*$p < 0.01$). The highest dose of AP-5 significantly increased TH radiolabeling in the LC compared to all other groups.



TH mRNA in LC
(AP-5 10µg/day)



TH mRNA in LC
(Vehicle)

Figure 3.2. Representative computerized images of autoradiographs from the brain of a rat which received 10ug/day AP-5 vs Vehicle hybridized for tyrosine hydroxylase (TH) mRNA in the LC. Dark areas represent TH mRNA in LC.

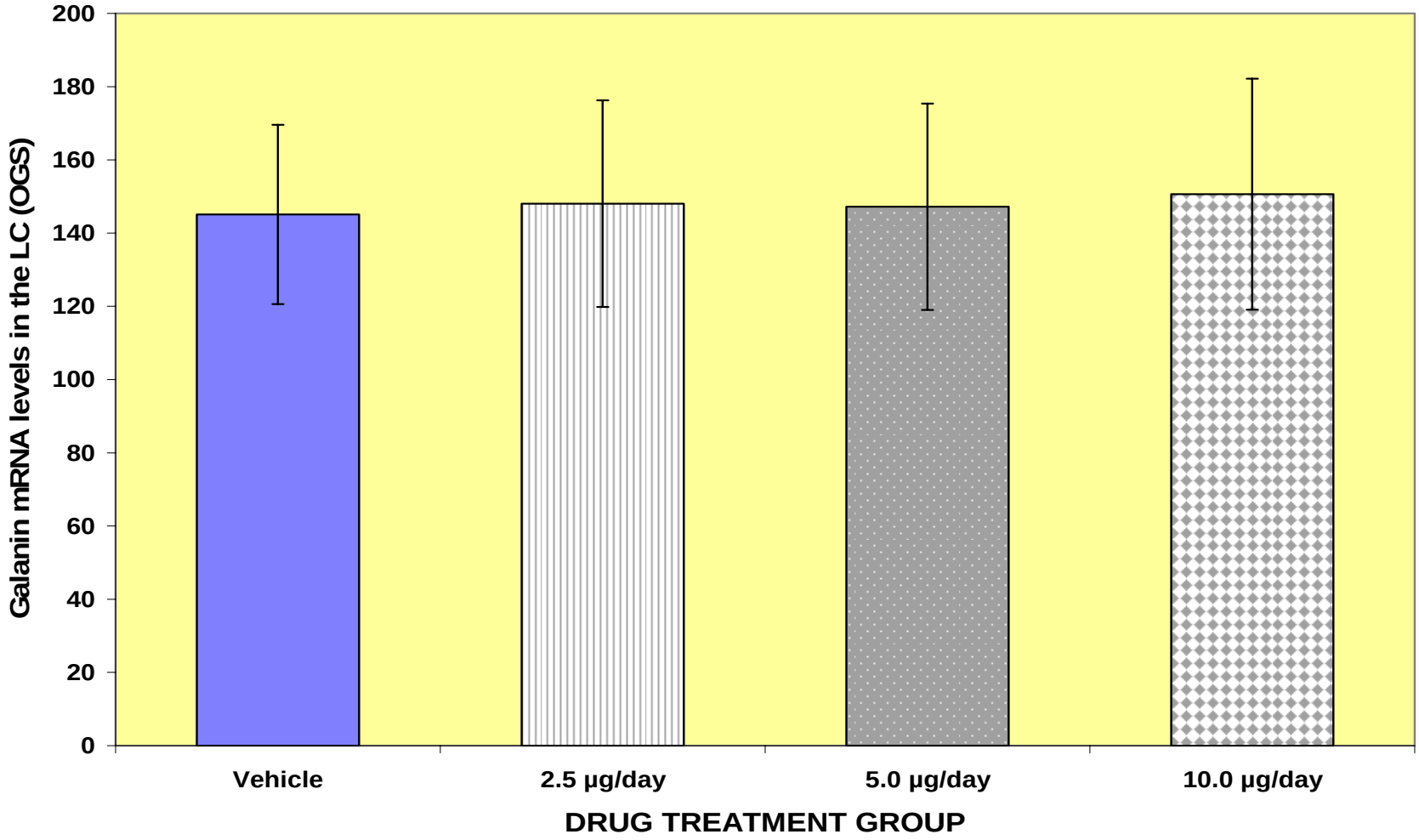


Figure 3.3. The effects of chronic AP-5 treatment (2.5µg/day, 5.0µg/day, and 10.0µg/day) on galanin (GAL) mRNA levels in the locus coeruleus (LC). Rats received drug treatment or vehicle for 14-17 days after which they were killed. Brain sections of the locus coeruleus were hybridized with a radiolabelled oligonucleotide probe (rat TH). Data are presented as mean optical grey scale value (OGS) $\pm$ SEM. There were no significant differences in GAL mRNA in the LC after drug treatment.

CHAPTER 4

CONCLUSION

As previously stated in the literature review, olfactory bulbectomy (OBX) leads to many neurochemical and behavioral changes. The most extensively investigated behavioral change following bulbectomy has been the hyperactivity observed in the open-field (Kelly et al., 1997). The mechanism by which bulbectomy causes these neurochemical and behavioral changes is still not well understood. One possibility, which is the basis of the present study, is that the loss of glutamatergic input from the bulbs to various limbic structures causes these changes, since the glutamatergic efferents are transected by bulbectomy.

The present study employed a pharmacological manipulation that would be comparable to the OBX model, in order to clarify the role of glutamatergic deafferentation to the ventral striatum, mainly the olfactory tubercle, and its possible effects on dopamine activity in this site. Using the competitive NMDA receptor antagonist, AP-5, chronically infused directly into the ventral striatum, we attempted to create the glutamatergic dysfunction that would otherwise be produced by OBX. The goal of this study was to determine if any changes occurred upstream from the site of drug administration, in areas that have a regulatory function on dopamine release in the striatum, mainly the ventral tegmental area (VTA) and locus coeruleus (LC). Tyrosine hydroxylase (TH) and galanin (GAL) mRNA levels were measured in the LC following chronic treatment with AP-5.

The results of this study, presented in the previous manuscript, determined that TH gene expression was significantly increased in the LC of rats receiving AP-5 at the highest dose

(10μg/day) compared to all other groups. GAL mRNA levels did not change significantly between groups, however. TH is a sensitive index of neuronal activity in dopaminergic and noradrenergic neurons (Ericson & Ahlenius, 1999). Our results indicate that one cause of the hyperactivity observed after chronic NMDA receptor blockade in the ventral striatum during a previous experiment in our lab, may be due to an increase in dopaminergic neurotransmission in the VTA caused by an increase in noradrenergic activity in the LC, which would therefore result in an increase in dopamine release in the ventral striatum. This change in the activity of dopamine in this pathway could be the cause of the behavioral stimulation observed following our pharmacological manipulation, thus implying changes in noradrenergic activity in the LC also contributes to the hyperactivity in an open-field commonly observed in the OBX model.

Dopamine in the ventral striatum is known to contribute to enhanced locomotor activity (Ougazal & Amalric, 1995; Svensson et al., 1994; Wheeler et al., 1995). Though the nucleus accumbens is most often discussed in the literature, increases in locomotion is also observed following local injections of dopamine into the olfactory tubercle (Ikemoto, 2002). Along these lines, an increase in dopamine release has been observed in the ventral striatum of OBX rats (Masini et al., 2004). The hyperactivity associated with OBX could be a result of increased dopamine release in the ventral striatum, as these studies suggest. The present results are consistent with this type of dopaminergic dysfunction, implying that increased DA release in the ventral striatum is indirectly affected by increased noradrenergic activity in the LC. This increase in NE could act to stimulate dopamine neurons in the VTA, which project to the nucleus accumbens, thereby causing hyperactivity.

The interaction between glutamate and dopamine in the ventral striatum is also well-documented, and both dopamine and glutamate NMDA receptors play a role in motor activity

(Gimenez-Llort et al., 2002; Kretschmer, 1999; Vezina & Kim, 1999). Moreover, both OBX (Ho et al., 2000; Redmond et al., 1997) and NMDA receptor blockade in the ventral striatum (Amalric et al., 1994; Ougazzal & Amalric, 1995; Schmidt & Kretschmer, 1997; Svensson et al., 1994) have been shown to produce behavioral stimulation. The results obtained here suggest that the loss of glutamatergic input to limbic structures, such as the ventral striatum, and the effect of this loss on dopamine activity may be the cause of the hyperactivity associated with OBX. Furthermore, these changes in dopamine activity may stem from increased noradrenergic activity, evidenced in this study by increased TH gene expression in the LC.

The previous literature review contains descriptions of many studies which found that competitive NMDA antagonists do not exert their effects through a dopamine-dependent mechanism (Feenstra et al., 2002; Kretscher, 1999; Ouagazzal & Amalric, 1995; Svensson et al., 1994). In contrast to these studies, the results obtained here suggest that locomotor activation through chronic NMDA receptor blockade with the competitive antagonist, AP-5 (10.0µg/day), may exert a change in dopaminergic activity indirectly by increasing noradrenergic activity in the LC. One possible explanation of these results may be that we chronically administered AP-5 into the ventral striatum, which differs from studies reporting no effect of competitive NMDA receptor antagonists on dopamine function (Feenstra et al., 2002; Kretscher, 1999; Ouagazzal & Amalric, 1995; Svensson et al., 1994). Further experimentation needs to be conducted in order to determine if this manipulation affected the release of dopamine or caused changes in dopamine receptors in the VTA. Though, it is likely that the increase in noradrenergic activity in the LC would stimulate dopaminergic neurotransmission in the VTA. For example, Ihalaienen and Tanila (2004) suggest that locomotor activity is mediated by the alpha2A-adrenoceptors, which regulate DA release in the NAC indirectly through their effect on DA neurons in the VTA.

Similarly, bulbectomy has been shown to produce an increase in the number of alpha2-adrenoceptors in cortical regions (Song & Leonard, 2005). Furthermore, DSP-4 (a noradrenergic neurotoxin) lesions have been shown to reduce the hyperactivity associated with the mouse mutant, coloboma, which exhibits spontaneous locomotor hyperactivity (Jones & Hess, 2003).

This study supports the idea that increased dopamine activity leads to a hyperactive motor response, and that this change in DA can occur due to the loss of glutamatergic input to the ventral striatum, through antagonism or bulb ablation, and furthermore this increase in DA activity can be linked to the observed increase in noradrenergic activity, indicated by increased TH gene expression in the LC.

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