

RESEARCH PROGRAM 2003-2006

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Current Research Focus:

- NEURAL SYSTEMS REGULATING DOPAMINE REWARD PATHWAYS AND ACTIONS OF DRUGS OF ABUSE
- DEVELOPMENT OF NEUROPHARMACOLOGICAL TREATMENTS FOR NEUROPSYCHIATRIC DISORDERS

Basic research continues to become more complex with grant funding agencies expecting investigators to take a much greater multi-disciplinary approach in their investigations. Over the last three years I have forged a number of important collaborations with prominent researchers in my field of study to expand the capability of my lab and fulfillment of my research goals. For example, I have collaborated with 19 different research institutions and 3 commercial organizations on several integrated research projects. These collaborative investigations have led to important discoveries on the neural and receptor mechanisms that regulate dopaminergic neurotransmission in normal and neuropsychiatric disease states. Some of the important outcomes of my recent studies are summarized below:

NEURAL SYSTEMS REGULATING DOPAMINE REWARD PATHWAYS AND ACTIONS OF DRUGS OF ABUSE:

1. How visual stimuli activate dopamine reward pathways at sub-second latencies

Sensory stimuli that are biologically salient by virtue of their novelty, intensity, or reward value elicit a stereotyped phasic increase in firing rate of mammalian midbrain dopamine neurons. This and other evidence has suggested that dopamine neurons provide the brain reinforcement information that may be used to adjust future behavioral response probabilities. Surprisingly, little is known about the sources of sensory input to dopamine neurons. We show for the first time that dopamine neurons receive a direct projection from the superior colliculus, a midbrain structure that receives direct input from the eyes. This enables the superior colliculus to serve as a critical relay in the pathway that transmits in sub-second latencies visual information to dopamine neurons (Dommett et al., 2005, *Science*, 307:1476-1479).

2. A new target for the pharmacological treatment of cocaine abuse: Dopamine autoreceptors coupled to G_z proteins

G_z is a member of the G_i protein family whose *in vivo* functions remain unknown. Locomotor activity induced by the psychostimulant cocaine has been shown to be significantly enhanced in mice lacking the alpha subunit of G_z. Since cocaine-elicited locomotor activity relies on the integrity of dopaminergic system, modification of dopamine receptor functions in G_z knockout mice may account for this altered psychostimulant-induced behavioral response. Our recent studies have shown for the first time that the G_z protein is functionally coupled to dopamine D2 autoreceptors *in vivo* and suggest that this second-messenger protein may serve as a potential drug target when subtle fine-tuning of dopamine receptor mediated functions are required to treat drug abuse (Leck et al., 2006, *Neuropharmacology*, 51:597-605).

3. The rewarding effects of drugs of abuse require activation of a specific type of acetylcholine receptor on dopamine neurons in the midbrain

GABAergic and glutamatergic inputs from various cortical and subcortical regions strongly regulate dopamine cell random and burst firing. An important additional source of regulation may also include acetylcholine neurons in the laterodorsal and pedunculo pontine tegmentum of the mesopontine (hindbrain region). Several of our recent studies have examined the role of these acetylcholine neurons in regulating dopamine neurotransmission via activation of nicotinic and muscarinic receptors in the midbrain. Collectively, our studies have discovered a novel subtype muscarinic receptor called M5 on midbrain

dopamine cells that plays a role in mediating prolonged dopaminergic activity and the response of dopamine neurons to various drugs of abuse, including cocaine, amphetamine and heroin (Miller and Blaha, 2005, *European Journal of Neuroscience*, 21:1837-1846; Miller et al., 2005, *Neuroscience*, 136:531-538; Forster et al., 2003, *Journal of Neuroscience*, 22:RC190, 1-6; Wang et al., 2004, *Neuropsychopharmacology*, 29:2126-2139; Alderson et al., 2004, *Neuroscience*, 125:349-358).

4. Development of a selective neurotoxin to study the regulation of midbrain dopamine reward pathways by hindbrain acetylcholine neurons

Previous studies examining the effects of hindbrain lesions of acetylcholine neurons that project to midbrain dopamine neurons have had to rely on the use of neurotoxins such as ibotenate which non-selectively destroy neuronal cells. Unfortunately, neurotoxins such as DSP4 or saporin, that have been shown to selectively destroy cholinergic neurons in the forebrain, fail to selectively destroy cholinergic neurons in the hindbrain. The lack of a selective toxin for hindbrain acetylcholine neurons has thus hampered investigations of the function of these neurons. Additional studies in my laboratory have revealed the importance of the neuropeptide urotensin as a modulator of these cholinergic neurons (Clark et al., 2005, *Brain Research*, 1059:139-148). These recent findings have led to the development of a neurotoxin (a diphtheria-urotensin II fusion toxin) that represents the first toxin that can be used to selectively lesion hindbrain acetylcholine neurons and as such, has opened the door to specific studies of the role of these neurons in modulating dopamine neurotransmission and drug-related behaviors.

5. Adolescent exposure to alcohol induces hypersensitivity of dopamine reward pathways to alcohol and addictive behaviors in adulthood

The abuse of alcohol typically begins during adolescence and predicts further abuse in adulthood. Unfortunately, as many natural changes in central neurological and endocrine systems occur during this critical period of development these systems are at risk of being modified or disrupted by overt alcohol abuse. One neuronal system that is at particular risk to the effects of repeated alcohol exposure during adolescence is the mesoaccumbens dopaminergic system. This system is a critical substrate for the control of motivated and goal-directed behaviors. Studies have shown that following withdrawal from repeated exposure to various drugs of abuse (e.g., amphetamines, heroin, and nicotine) this system exhibits a long-lasting hyper-responsiveness (sensitization) to the stimulatory effects of these drugs. Therefore, drug-induced sensitization is considered a critical step in the development of drug addictive behavior. Our recent studies (Tinsley et al., 2006, *Society for Neuroscience*, 477.6) have provided neurochemical and behavioral evidence that alcohol exposure in adolescent rats produces long-lasting functional changes in mesoaccumbens dopamine neurotransmission and alcohol-related behaviors that persist into adulthood.

DEVELOPMENT OF NEUROPHARMACOLOGICAL TREATMENTS FOR NEUROPSYCHIATRIC DISORDERS:

6. Neurotransmitter mechanisms involved in deep brain stimulation for neurological diseases

The precise mechanism whereby continuous high-frequency electrical stimulation of the subthalamic nucleus ameliorates motor symptoms of Parkinson's disease is unknown. We examined the effects of high-frequency stimulation of regions dorsal to and within the subthalamic nucleus on dopamine release in the striatum of urethane anesthetized rats using constant potential amperometry. Complementary extracellular electrophysiological studies determined the activity of subthalamic nucleus neurons in response to similar electrical stimulation of the subthalamic nucleus. Our studies demonstrate for the first time that high-frequency electrical stimulation of dopaminergic axonal fibers of passage dorsomedial to the STN optimally enhance dopamine transmission in the basal ganglia. These data suggest an important mechanism whereby deep brain stimulation ameliorates symptoms of Parkinson's disease in patients with

a partially functional nigrostriatal dopaminergic system (Lee et al., 2006, *European Journal of Neuroscience*, 23:1005-1014).

7. Reduction of L-DOPA-induced dyskinesia with entacapone: A new treatment strategy for Parkinson's patients

Long-term palliative treatment of Parkinson's disease with the dopamine precursor L-DOPA is compromised by the occurrence of motor complications, most notably motor fluctuations and involuntary movements (L-DOPA-induced dyskinesias). Our recent work has shown that the combination of L-DOPA with the COMT inhibitor entacapone prolongs striatal dopamine levels and increases the behavioural response to L-DOPA (Gerlach et al., 2004, *Naunyn-Schmiedeberg's Archive of Pharmacology*, 370:388-394). From a clinical point of view, this finding suggests that administration of a COMT inhibitor should allow a decrease in the frequency of L-DOPA administration to Parkinson's patients that in turn reduce the risk of dyskinesia induction.

8. Clathrin-assembly processes in dopamine nerve terminals: A novel target for preventative treatment in the early stages of Parkinson's disease

Elevated brain levels of iron in Parkinson's disease patients are thought to increase oxidative stress-induced death of nigrostriatal dopamine neurons. Using www.genenetwork.org, we have identified a mouse gene (clathrin-assembly *Picalm*) with expression levels in mouse forebrain and striatum that inversely correlate with brain iron content. Mice with a truncated *Picalm* allele show abnormal iron disposition in multiple body tissues. We determined whether mutations in this gene are also associated with a dysfunction in striatal dopamine transmission *in vivo*. Our studies have shown for the first time that a mutation in the clathrin-assembly *Picalm* gene leads to an upregulation in dopamine terminal autoreceptor sensitivity, dopamine transporter capacity, and dopamine uptake efficiency in the striatum that, in turn, leads to a marked reduction in dopamine transmission. Our recent findings (Duboue et al., 2006, *Society for Neuroscience*, 173.14) have important implications with respect to the impact of elevated iron levels and brain dopamine function and suggest that clathrin-assembly mechanisms may serve a novel targets for preventative therapeutic drug treatment of Parkinson's disease.

9. A unique mouse model to study the role of cerebellar and cortical dysfunction in autism

Stereotyped behavior (excessive production of repetitious behaviors) is a prominent symptom of developmental disorders that also involve pathology of the cerebellum (e.g., autism). We have hypothesized that Purkinje cells in the cerebellum may be able to regulate dopamine release in the prefrontal cortex which is known to be responsible for the expression of stereotyped behaviors. Using mutant mice expressing different degrees of Purkinje cell loss in the cerebellum, we were able to determine a close association with the number of these cerebellar cells and the ability of the cerebellum to mediate prefrontal cortex dopamine release and, in turn, stereotypic behavior. Our recent studies (Blaha et al., 2006, *Society for Neuroscience*, 805.10) present a unique mouse model with cerebellar deficits that have behavioral and anatomical features characteristic of autistic patients and that these cognitive deficits may result from specific disruptions in cerebellar-mediated prefrontal cortex dopamine transmission.