



Vanderbilt University Medical Center

PND Association
PO Box 180622
Delafield, WI 53018
262-646-5133
July 1, 2009

Re: Letter of Intent, 2009 PND Research Grant Program

Title: Dopaminergic Effects on the Formation of Brain Architecture (G.D. Stanwood)

1. Objective:

The overall goal of my research program is to understand pleiotropic functions of biogenic amine neurotransmitters during brain development. To date, we have focused on the dopamine system, because we have found the cellular distribution and G-protein coupling of D₁ receptors to be permanently altered following a transient developmental insult. We believe this to be a unique cellular response that occurs developmentally. In an animal model used in the laboratory, we administer cocaine to pregnant dams during midgestation, resulting in alterations in dendritic morphology, disruptions in interneuron differentiation, and behavior for the lifetime of offspring. Similar anatomical changes are present in D₁ receptor null mice, suggesting that genetic or environmental regulation of dopaminergic tone during development alters the functional connectivity of the forebrain. Ongoing work in primary cultures being carried out in my laboratory suggests that dopamine receptor stimulation alters the rate of process outgrowth by these dopaminergic target neurons. The proposal to be presented to the PND Association would examine the effects of hyperdopaminergia, produced by genetic deletion of the dopamine transporter, on the formation and function of cortical and striatal circuits *in vivo*.

2. Which specific PND disease the research relates to and/or relevance to PND's:

The proposal is of broad basic relevance to understanding the developmental effects of a neurotransmitter previously linked to a variety of inborn errors of metabolism and pediatric neurotransmitter diseases.

3. Hypothesis or hypotheses to be tested:

We have previously observed that alterations in biogenic amines during brain development produce specific and permanent alterations in dendritic morphology, disruptions in interneuron differentiation, and abnormal cognitive and motor behavior. In the current proposal, we will test the hypothesis that hyperdopaminergia specifically is the primary mediator of these deleterious effects on forebrain development. First, we will assess the morphology of apical dendrites of pyramidal neurons in the frontal cortex of dopamine transporter (DAT) $+/+$, $+/-$ and $-/-$ mice using immunohistochemistry and dye-labeling techniques. Loss of the DAT protein eliminates the primary mechanism by which dopamine is removed from the extracellular space and thus results in dramatic elevation of extracellular dopamine levels and prolonged signaling. We therefore hypothesize that DAT $-/-$ will exhibit increased dendritic length and decreased bundling. DAT haploinsufficiency ($+/-$ mice) should also be sufficient to induce phenotypic differences in dendritic

trajectories. Next, we will examine the mice for proper development of cortical and striatal GABAergic interneurons. Work by our lab and others have shown previously that proper dopaminergic specification required for normal differentiation of a specific subtype of interneuron, that being those expressing parvalbumin as a phenotypic marker. Our initial anatomical studies will focus on two ages – P90 (adult) and P14 (peak of synaptogenesis). If differences across genotypes are observed, then additional ages will be added to more comprehensively assess changes over developmental time. Finally, we will perform a selected behavioral screen focused on detailed studies of cognitive and locomotor functions in the mutant line using sensitive video-based measurement techniques. My laboratory has expertise in all of the proposed techniques and the mice are already present in my animal colony (the line was graciously provided to me by Dr. Marc Caron, Duke University).

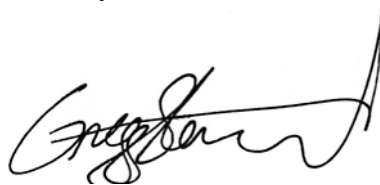
These studies will allow us to mechanistically tie our previous observations specifically to the dopamine system, and position my laboratory to utilize multiple animal models to identify the mechanisms by which neurodevelopmental dopaminergic dysfunction alters neuronal differentiation, connectivity and behavioral function.

4. Approximate amount of funding requested: \$25,000

5. Approximate timeline for research project: 1 year

I hope to be allowed to provide you with a detailed research proposal for your consideration.

Sincerely,

A handwritten signature in black ink, appearing to read "Gregg Stanwood". The signature is fluid and cursive, with a long, sweeping tail on the final letter.

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