Examining the Effects of Dopamine System Stimulation During Cortical Axon Guidance

**Stephanie Bronson* and Christine L. Konradi§**

Dopamine (DA) is a modulatory neurotransmitter that mediates motor function and emotion-based behaviors. Dopaminergic projections throughout the cerebral cortex innervate brain regions implicated in the pathophysiology of neuropsychiatric illnesses such as Parkinson’s disease (PD), schizophrenia, and mood disorders. DA is vital to normal brain function and is also involved in sleep, aggression, reward, and appetite. However, the role DA plays during development of the central nervous system has not been fully elucidated. The arrival of DA fibers in the cortex is concurrent with the development of cortical projections and axonal pathfinding of cortical efferents. Recently DA has been shown to affect the migration of interneurons to the cerebral cortex. Animals treated with drugs that increase dopaminergic tone upregulate expression of the axon guidance factor receptors DCC and Unc5c and show neuroanatomical changes in the prefrontal cortex (PFC), a brain region adversely affected in schizophrenia. In addition, DA receptor activation triggers downstream effectors that influence cellular levels of cyclic nucleotides and PKA activity, both of which play a role in growth cone steering and cytoskeletal reformation. Understanding the role of DA receptor activation during development is relevant to the field of psychiatry as schizophrenia is typically first seen in late adolescence and pharmacological treatments for the disorder target D2 DA receptors. This review will examine data that address the role of DA in cortical development, specifically axon guidance. Understanding how DA affects the formation of cortical circuits may shed light on how the DA system functions in diseased brains.

Dopamine (DA). A modulatory neurotransmitter involved in motor function and emotion. DA also contributes to the establishment of cortical circuitry and brain development.

Frontal Cortex
A region of the brain that plays a role in executive functions, working memory, and attention; the frontal cortex is adversely affected in many psychiatric conditions.

*Neuroscience Graduate Program, Vanderbilt University Medical School, U1205 Medical Center North, Nashville, TN 37232, USA.

§Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA. Correspondence to S.B. e-mail: stephanie.e.bronson@vanderbilt.edu

Dopaminergic neurons originate mainly from two midbrain regions, the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc). SNc neurons project to the dorsal caudate nuclei of the striatum, forming the nigrostriatal pathway. The striatum participates in extrapyramidal motor circuits involving the thalamus and motor cortex. Altered dopaminergic tone from the SNc to the striatum can result in hypo- or hyper-kinesic movement disorders such as Parkinson’s Disease (PD) and Huntington’s Disease. VTA neurons send dopaminergic projections to the prefrontal cortex (PFC), forming the mesocortical pathway, and to the nucleus accumbens (NAcc), amygdala, and hippocampus to form the mesolimbic pathway. The mesolimbic system mediates pleasure seeking, reward, and addictive behavior. Decreases in PFC gray matter and reduced PFC activation during cognitive tasks have been seen consistently in schizophrenic patients, making mesocortical dopamine (DA) signaling an area of interest in the field of psychiatry.

Schizophrenia is a devastating and debilitating mental disorder that affects approximately 1% of the world population. The disease is characterized by positive symptoms (hallucinations, psychosis, delusions), negative symptoms (withdrawal, avolition, anhedonia), and cognitive deficits. Weinberger has postulated that schizophrenic patients suffer from an imbalance of DA innervation—an overactive mesolimbic system causes the positive symptoms while an underactive mesocortical system causes negative and cognitive symptoms. Postmortem analysis of schizophrenic brains reveals a decrease in tyrosine hydroxylase (TH)+ and dopamine transporter (DAT)+ axons innervating the PFC. The PFC mediates executive function, decision-making, working memory tasks, and critical thinking skills. Individuals with schizophrenia perform poorly on tests that evaluate these skills.

DA receptors have long been the target for pharmacological treatment of psychotic disorders. All antipsychotic drugs (APDs) antagonize the D2 DA receptor, essentially decreasing dopaminergic signaling in patients. APDs relieve positive symptoms of the disease but do little to improve cognitive deficits and negative symptoms. Overexpression of striatal D2 receptors in animal models results in decreased DA turnover in the PFC and impaired performance on PFC-mediated working memory tasks. The imbalance of DA circuitry in schizophrenia may underlie PFC dysfunction and involve mechanisms that are not alleviated with current pharmacological therapies. Early life insults, especially those involving the DA system, may profoundly contribute to the pathophysiology of schizophrenia and alter nervous system development.
in such a way that it cannot be corrected later in life. Understanding how the DA system affects development of the PFC, as well as how DA circuits mature in patients with psychiatric disorders, is crucial to developing treatments for these conditions.

**CORTICAL DEVELOPMENT AND DOPAMINE SIGNALING PATHWAYS**

During development of the cerebral cortex, neural progenitor cells proliferate in a region bordering the lateral ventricle of the forebrain called the ventricular zone (VZ)\(^{17-18}\). Neurons born in the VZ then migrate along radial glial columns to the 6 layers of the cortex in an inside-out fashion, such that deep-layer 6 forms first and more superficial layers form last\(^{17}\). Once they have reached their laminar position, neurons extend axonal processes and their growth cones begin the course of axon pathfinding\(^{1-2}\). Growth factors and chemical cues present in the neuronal environment guide axons to their targets where synapse formation will occur\(^{19}\). An overabundance of synapses is produced during nervous system development and axonal “pruning” occurs in childhood to remove unnecessary synapses\(^{20}\). The remaining synaptic connections strengthen, axons become myelinated, and the brain volume increases\(^{20}\). The pruning process occurs until late adolescence, commencing with the PFC\(^{7,20}\). The overabundance of synapses in the PFC during youth may “mask” the phenotype of schizophrenia until early adulthood, when the first psychotic episode is typically seen and the PFC undergoes reorganization and maturation, resulting in the drastic behavioral changes seen in patients with psychosis\(^{7,11,20}\). Postmortem studies in human schizophrenic subjects reveal PFC-specific decreases in neuropil and synaptic protein content, as well as decreased mRNA expression of genes involved in synaptic activity\(^{21-22}\). Determining the role DA plays in PFC axon guidance and synapse formation could enhance our understanding of the neuropathological changes seen in psychiatric patients.

DA receptor stimulation has been shown to affect crucial developmental events\(^{17,18,23-25}\). Five types of DA receptors exist: D\(_1\) and D\(_2\) are considered “D\(_1\)-like” and couple to G\(_{i/o}\) to activate adenylyl cyclase, increasing cyclic nucleotide levels; D\(_3\), D\(_4\), and D\(_5\) are “D\(_2\)-like” and couple to G\(_{i/o}\), inhibiting the activation of adenylyl cyclase\(^{26-28}\). D\(_1\) and D\(_2\) show temporal and spatial differences in their expression patterns\(^{29}\). Both have been detected in the frontal cortex and stratum of rodents as early as E12, despite the fact that VTA fibers don’t begin to reach the cortex until E16\(^{29}\). Because second messenger activity of G-protein coupled receptors (GPRC) can influence transcriptional activity, the ratio of D\(_1\)/D\(_2\) receptors in a given cell or circuit can have both immediate and long-lasting consequences\(^{30-31}\). G-protein mediated second messenger pathways for D\(_1\) and D\(_2\) are differentially affected by cocaine treatment during critical periods of DA system development in animal models\(^{16,27,32}\). Drugs of abuse such as amphetamine and cocaine target the DAT and can elicit psychotic symptoms resembling paranoid schizophrenia\(^{17}\). These drugs trigger DAT-mediated DA efflux and elevate levels of synaptic DA\(^{33}\). Chronic cocaine treatment of pregnant rabbits during critical periods of cortical development (E16-E25) affects dendrite length and D\(_1\) surface density in both the PFC and striatum of offspring\(^{16,27}\). D\(_1\)-G\(_{i/o}\) coupling was reduced, while D\(_2\)-G\(_{i/o}\) coupling remained unchanged\(^{16,27}\). The surface density of DA receptors and their trafficking patterns following activation is important to study in a developmental context, as they may signal to molecules that regulate the outgrowth and path of PFC axons. Treatment of animals with specific D\(_1\) or D\(_2\) agonists in utero and examination of PFC function with cognitive behavioral tasks could reveal an important role of the DA system in the proper assembly of PFC architecture during development.

Cortical circuitry is tightly regulated by a balance of glutamatergic excitation and GABAergic inhibition\(^{23}\). The majority of cortical GABA interneurons originate from the ganglionic eminences (GE) of the forebrain and migrate up to the cortex\(^{23}\). The GE later develops into the striatum, a region rich in DA receptors\(^{17,23}\). Stimulation of DA receptors in forebrain slices from E15 mice affects interneuron migration to the cerebral cortex\(^{23}\). D\(_1\) agonists increase migration of neurons from the GE to the cortex, while D\(_2\) agonists have the opposite effect\(^{23}\). CDHC, a motor protein that regulates cytoskeleton organization and plays a role in neuron migration, localizes to neurites in D\(_1\) stimulated cultures but is retained in the nucleus of D\(_2\) treated cultures\(^{23,34}\). This suggests that the balance of D\(_1\) versus D\(_2\) receptor stimulation is crucial for the formation of inhibitory and excitatory cortical circuitry and that DA receptor signaling might communicate with proteins involved in cytoskeletal reorganization, a key component of axon guidance\(^{23}\).

**THE ROLE OF NEUROTRANSMITTERS DURING NETRIN-1 MEDIATED AXON GUIDANCE**

Axon guidance factors influence axon pathfinding throughout the entire nervous system\(^{12,19}\). Of the four major families of axon guidance cues, netrin-1 and its two receptors, Deleted in Colorectal Cancer (DCC) and Unc5c, play an important role in the pathfinding of cortical efferent axons\(^{35}\). Netrin-1 is a secreted guidance cue that is heavily expressed in the area surrounding the striatum. Netrin can cue attraction or repulsion, as well as axon outgrowth\(^{19,36-39}\). In the
presence of netrin-1, DCC homodimers signal for attraction, while Unc5c-DCC heterodimers cause repulsion19,40-42.

Dopaminergic signaling has also been shown to modulate expression of netrin receptors6,43. Yetnikoff and colleagues showed that amphetamine treatment in adult rodents increased protein expression of both netrin receptors in the PFC as well as the VTA. The fact that adult animals continue to express netrin receptors could be a mechanism of plasticity following the drug treatment, emphasizing the importance of studying netrin-DA system interactions in development as well as adulthood6. Conversely, Jassen and colleagues treated neuroepithelial cell lines with D1 agonists and saw decreased DCC mRNA expression. However, these cell lines only contained D1 receptors and D1 agonists increase cyclic nucleotide levels, an event linked with increased DCC activation40,43. Evaluating the gene and protein expression of netrin receptors in young animals following drug treatment would be necessary to fully understand how netrin receptors and the DA system affect one another. D1 vs. D2 agonists might have opposite effects on netrin receptor expression because they activate different G-proteins and trafficking patterns of the DA receptors24,28,44-46. Expression of netrin receptors in the PFC could be important not only for establishing and maintaining DA circuitry in the PFC, but also for maintaining other glutamatergic or GABAergic PFC connections6.

In addition to DA, another monoamine neurotransmitter, serotonin (5-HT), has been shown to play a role in axon guidance during early brain development31. 5-HT receptors are also GPCRs and can affect cyclic nucleotide levels31. Stimulation of 5-HT1B and 5-HT1D receptors on thalamocortical axons converts attractive netrin cues to repulsive cues31. Both receptors couple to Gαi and inhibit the activation of adenylate cyclase31. Pharmacological agents that inhibit PKA have the same effect while 5-HT receptor antagonists or drugs that activate adenylate cyclase have the opposite effect19. In vivo data using in utero electroporation of 5-HT1B/1D siRNA in E14 mouse thalamocortical axons also revealed drastic changes in the trajectory of these axons, presumably due to the loss of 5-HT receptor stimulation31. This suggests that stimulation of a GPCR-mediated cascade that affects adenylate cyclase production or PKA activation, such as that of DA receptors, can alter the direction of axon growth and implies a role for DA in the pathfinding of axons40,47. If the amount of 5-HT receptors present on thalamocortical axons is vital to the development of typical thalamocortical connections, then the abundance and expression of DA receptors in the PFC may be crucial for the development of normal cortical and subcortical connections.

Axon guidance is a cAMP-dependent process and PKA activation triggers biochemical cascades involved in a number of cellular processes related to axon outgrowth and cytoskeleton remodeling40-41,47. PKA activation alone does not have the ability to mediate axon outgrowth or guidance but application of netrin and forskolin, a PKA activating drug, enhances axon outgrowth more than netrin alone in commissural neuron cultures40. Under basal conditions a small amount of DCC is present on the plasma membrane surface and vesicular stores of DCC are maintained near the growth cone40. Binding of netrin to a DCC receptor promotes the recruitment of additional DCC to the cell surface and PKA rapidly enhances the netrin-mediated insertion of DCC into the plasma membrane19,40. DCC homodimers are phosphorylated by Src/Fyn kinases that promote the recruitment of a protein complex to the cytoplasmic tail of the receptors19,41. Cdc42 and Rac1, members of the Rho family of GTPases, associate with N-WASP to signal changes in actin polymerization,
cytoskeleton reformation, and the formation of lamellipodia and filopodia on the growth cone\textsuperscript{41,48}. This results in axon movement and extension of the growth cone towards the source of netrin\textsuperscript{19}.

As described above, decreases in PKA have been linked to axon repulsion\textsuperscript{19,31}. It is not clear how the decrease in cyclic nucleotides affects netrin receptor density and the response of Unc5c to PKA has not been studied in great detail. One hypothesis is that decreases in cyclic nucleotides promote the insertion of Unc5c to the plasma membrane to form heterodimers with DCC, triggering signaling cascades that promote the reorganization of the growth cone cytoskeleton away from the source of netrin\textsuperscript{19}. One Unc5 vertebrate homolog, Unc5H2, has been shown to associate with the G\textsubscript{ai} protein in the presence of cAMP\textsuperscript{42}. Under conditions of netrin-mediated attraction, Unc5H2 might bind G\textsubscript{ai} to ensure attraction and not repulsion\textsuperscript{42}. Decreases in cAMP would release Unc5H2 from G\textsubscript{ai}, allowing G\textsubscript{ai} to inhibit adenyl cyclase production and decrease cyclic nucleotide levels\textsuperscript{42}. Stimulation of GPCRs that contain a G\textsubscript{ai} protein, such as D\textsubscript{2} and 5-HT\textsubscript{1B/1D}, would therefore promote a decrease in cAMP production and allow free Unc5c to traffic to the plasma membrane to dimerize with DCC\textsuperscript{31,42}. The interaction of G-proteins with netrin receptors represents a novel field of study that may explain how neurotransmitter receptors for DA and 5-HT could be affecting axon guidance during development.

CONCLUSIONS

Axon pathfinding represents a fundamental period of nervous system development, as neurons establish synapses to communicate in circuits throughout the brain and the entire body. Evidence suggests that DA receptor stimulation communicates with the netrin family of receptors to contribute to these events. Other axon guidance families including the Ephrins, Semaphorins, and Slits contribute to the patterning of dopaminergic projections\textsuperscript{2,3,49-50}. DA receptors could be communicating with their receptors as well. Ephrins have been shown to guide SN neurons to the striatum and the Slit receptor ROBO must interact with DCC to mediate repulsive events\textsuperscript{3,4}. A detailed study of the expression and trafficking of netrin receptors following stimulation of dopamine receptors is necessary to address the role of the dopamine system in axon guidance. Mechanisms for axon guidance are different depending on the signal transduction cascade of a given receptor and different types of DA receptors could be important for different families of axon guidance factors. Importantly, expression levels of the membrane-bound guidance cues netrin-G1 and netrin-G2 were found to be decreased in post-mortem tissue from patients with schizophrenia and bipolar disorder\textsuperscript{51}.

The expression of DA and its receptors during early stages of development is necessary for interneuron migration, an event that ensures a balance of excitatory and inhibitory circuitry throughout the cerebral cortex\textsuperscript{23}. Activation of DA receptors triggers G-protein mediated cascades that control cAMP production, activation of kinases, and intracellular Ca\textsuperscript{2+} levels\textsuperscript{46,52}. These processes likely communicate with molecules poised to mediate neurite outgrowth, growth cone steering, and cytoskeletal reformation. Stimulation of the DA system in adolescent drug abuse studies reveals lasting neuroanatomical changes that reflect abnormal axon growth in cortical as well as striatal regions\textsuperscript{16,27,32}. The functions of the DA system in the PFC and striatum may share some common mechanisms in development. Additionally, some PD patients administered L-DOPA therapy experience psychotic symptoms such as hallucinations while a subset of schizophrenic patients receiving APDs develop extrapyramidal motor side effects\textsuperscript{5,37}. Knowledge of early dopamine systems has implications for PD research as the imbalance of excitation and inhibition of motor circuits involving the striatum and motor cortex underlies development of PD.

Understanding vertebrate brain development is crucial for interpreting and developing therapies for complex diseases of the human brain. Further studies must be done to understand how the neurotransmitters that contribute to the pathophysiology of psychiatric illnesses are functioning in embryonic and adolescent brains. The development of a psychiatric patient during childhood and early adolescence may seem fairly normal, but changes in brain chemistry have likely occurred much earlier to elicit such a drastic and enduring phenotype like schizophrenia\textsuperscript{11,20}. Other genetic and environmental factors contribute to the disease as well and may adversely affect brain development\textsuperscript{11}. Impairment could be permanent and result from alterations made to the cortical circuitry during a critical period of development. In addition, understanding the function of neurotransmitter systems during development has implications for ADHD, which is treated with amphetamines and is commonly seen in young children, as well as autism, a spectrum of developmental disorders in which 5-HT is implicated\textsuperscript{31,33}. Further knowledge of the developmental aspects of mental illness could facilitate the correct diagnosis of these disorders at earlier time-points when treatment intervention may be more beneficial, as well as the expansion of pharmacological therapies.

REFERENCES:

6. Yetnikoff L, Labelle-Dumais C and Flores C (2007). Regulation of netrin-1 receptors by amphetamine in the adult brain. Neuroscience. 150: 764-73. This is the first paper to examine the effects of dopamine receptor stimulation on the expression of netrin-1 receptors in the PFC.
17. This paper shows that stimulating the dopamine system during development has lasting effects on the behavior of dopamine receptors and their contribution to axon outgrowth.
family receptors converts netrin-induced growth cone attraction to repulsion. Cell. 97: 927-41.


This paper demonstrates how PKA, a molecule downstream of dopamine receptor activation, can mediate axon outgrowth and the surface density of the netrin-1 receptor DCC.


ACKNOWLEDGEMENTS: I would like to thank my advisor, Christine Konradi, for her continued support and guidance; Karoly Mirnics and the entire Mirnics lab; and Alex Bonnin.

FURTHER INFORMATION