Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

CNTF: A Putative Link between Dopamine D_2 Receptors and Neurogenesis

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Review of Yang et al. (http://www.jneurosci.org/cgi/content/full/28/9/2231)

Despite a preponderance of evidence highlighting the involvement of dopamine in the regulation of neurogenesis in the adult mammalian brain, the mechanism by which this occurs remains unknown. Previous results demonstrated that dopaminergic nigrostriatal projections regulate neural precursor cell proliferation in the subventricular zone of the lateral ventricles and that dopamine depletion and/or denervation impairs neural precursor cell proliferation. These observations have been confirmed in Parkinson disease patients. Previous reports also showed that the cytokine ciliary neurotrophic factor (CNTF) enhances forebrain neurogenesis in adult mice and is expressed in astrocytes in the subventricular zone. Together, these results suggest a link between dopamine, CNTF, and neurogenesis. Further evidence for this link appeared in a recent issue of *The Journal of Neuroscience*, in which Yang et al. (2008) presented a series of *in vivo* and *in vitro* experiments that implicate CNTF as an endogenous regulatory component of dopamine D_2-receptor-dependent neurogenesis in the subventricular zone and the dentate gyrus of the hippocampus. Because an imbalance in dopaminergic signaling is a pathological hallmark of several neurological diseases, such as Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis, pharmacologically modulating CNTF may be an attractive therapeutic strategy for normalizing dopaminergic and neurogenic deficits.

The majority of adult neurogenesis occurs in two brain regions, the subventricular zone and the subgranular zone of the dentate gyrus (for review, see Zhao et al., 2008). It is of considerable interest that a variety of external and internal factors including environment, exercise, hormones, neurotransmitters, and growth factors influence the proliferation and survival of neural precursor cells and their functional insertion into existing neuronal circuitry. Understanding the key regulators of neurogenesis may hold therapeutic potential for a number of neurological disorders.

When studying neurogenesis, acute administration and exposure of the thymidine analog bromodeoxyuridine (BrdU) is usually used to investigate cell proliferation and nuclear markers such as proliferating cell nuclear antigen and Ki67 are used to identify actively dividing cells. To study long-term survival, cells are monitored for BrdU retention over long periods of time (28 d), and the expression of specific molecular markers in these BrdU-positive cells helps to identify stages of neuronal maturation (for review, see Zhao et al., 2008).

Using acute BrdU incorporation to label proliferating neural progenitor cells, Yang et al. (2008) demonstrated an increase in cell proliferation within the subventricular and subgranular zones during dopaminergic activation by the selective D_2 receptor agonist quinpirole [Yang et al. (2008), their Fig. 4 (http://www.jneurosci.org/cgi/content/full/28/9/2231/F4) and their Fig. 8 (http://www.jneurosci.org/cgi/content/full/28/9/2231/F8)]. This effect depended on CNTF, because it was abolished in mutant mice lacking CNTF [Yang et al. (2008), their Fig. 5 (http://www.jneurosci.org/cgi/content/full/28/9/2231/F5)]. A CNTF-dependent increase in doublecortin-positive neuroblasts was also observed after quinpirole injection [Yang et al. (2008), their Fig. 6 (http://www.jneurosci.org/cgi/content/full/28/9/2231/F6)], leading the authors to suggest that dopaminergic activation of CNTF causes an increase in proliferation and neuroblast formation.

The authors then used a neurotoxin, 6-hydroxydopamine, to selectively eliminate dopaminergic projections from the substantia nigra in *CNTF*-null and wild-type mice. Subsequent treatment with the D_2 receptor agonist increased the number of BrdU-positive cells in injured wild-type but not in injured *CNTF*-null mice [Yang et al. (2008), their Fig. 7 (http://www.jneurosci.org/cgi/content/full/28/9/2231/F7)]. Together, these key findings led the

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authors to suggest that activation of postsynaptic dopamine D_2 receptors regulates subventricular zone neurogenesis via CNTF (Fig. 1).

Although the above finding that CNTF mediates dopaminergic-induced neurogenesis is intriguing, the role of dopamine D_2 receptors in neurogenesis remains uncertain. For example, Kippin et al. (2005) found that chronic dopamine D_2 receptor antagonism using haloperidol increased both the rate at which neural stem cells proliferate and the long-term retention of BrdU. One may ask how both dopamine antagonism (Kippin et al., 2005) and agonism (Yang et al., 2008) can increase progenitor cell proliferation.

The discrepancies between these (and other) studies are likely attributable to several factors. First, perhaps acute stimulation (Yang et al., 2008) and chronic blockade (Kippin et al., 2005) of D_2 receptors increase neurogenesis resulting from the ability of the brain to upregulate or downregulate receptor expression. In this regard, it is known that postsynaptic dopamine receptors exhibit adaptive changes after chronic exposure to both dopamine agonists and antagonists (Cooper et al., 2003). Second, given differences in experimental methodologies related to treatment delivery (osmotic pumps vs intraperitoneal injections), dose (10–100 nM vs 2 mg/kg quinpirole), duration of exposure (chronic 30 d haloperidol vs acute 3 d quinpirole treatments), and detection of neurogenesis (BrdU incorporation vs neurosphere formation), it is not surprising that a discord exists between the findings of these two groups. Whereas Kippin et al. (2005) monitored the long-term retention of BrdU in proliferating neural precursor cells after haloperidol treatment, Yang et al. (2008) did not report the survival and integration of BrdU-labeled cells. Therefore, it remains unclear whether D_2–CNTF signaling affects the survival and integration of adult neuronal stem cell progeny.

For newborn neurons to have functional relevance, they must differentiate, survive, integrate into, and make connections within the existing neuronal architecture. Thus, although effects on BrdU incorporation and neuroblast formation is indicative of acute cell proliferation, it would have been helpful to extend this investigation to determine whether BrdU was found in cells labeled with late markers of differentiation, including NeuN (neuronal nuclei, a neuronal-specific nuclear protein), calbindin, and/or Prox1, indicating that these cells had matured and integrated into the granule layer or olfactory bulbs. Furthermore, the conclusions would have been significantly strengthened if synaptic field responses and long-term plasticity in the dentate gyrus were measured. Such experiments using electrophysiology and multiphoton confocal imaging to look at the function of newborn cells could readily be performed in brain slices of *CNTF* knock-out and wild-type mice.

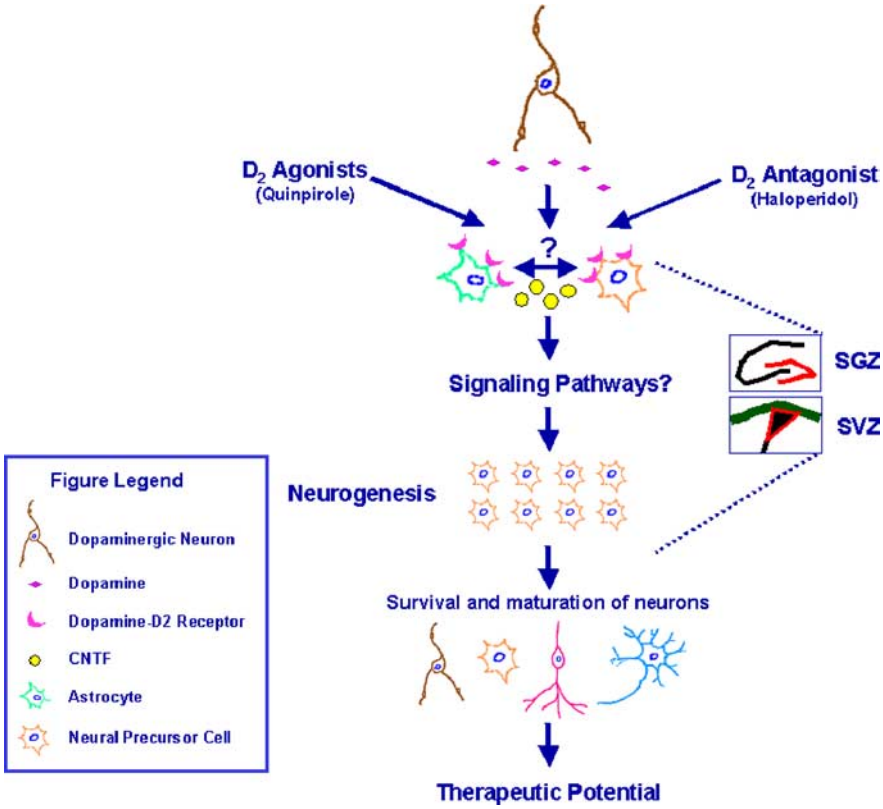

Figure 1. Dopamine D_2 receptors regulate adult neurogenesis via CNTF. Dopamine (purple), produced by dopaminergic neurons (brown) innervating the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus, activates D_2 receptors (pink) and stimulates CNTF expression in a subpopulation of GFAP-positive cells, presumably astrocytes (green) or neural precursor cells (orange). CNTF (yellow) is then released into neurogenic niches (SVZ and SGZ, red) and, through unknown cellular pathways, causes an increase in the proliferation of neural precursor cells. Survival and integration of these new neurons has therapeutic potential for a variety of diseases, including Parkinson's disease, major depressive disorder, and amyotrophic lateral sclerosis.

Determining the behavioral significance of D_2-dependent, CNTF-mediated neurogenesis would also prove interesting. For example, behavioral experiments could determine whether *CNTF*-null animals have motor deficiencies, cognitive impairments in learning and memory, or possess a differential susceptibility to depressive-like states (given the effects that antidepressants have on cell proliferation). Interestingly, it was reported that a null mutation in the human *CNTF* gene is not causally linked to neurological disease, thereby suggesting that additional mechanisms (for example, other cytokines) compensate for CNTF deficiency or that subtle deficits in the subjects were undetected during the previous investigation.

Finally, the localization of the CNTF receptor and the downstream signaling pathway through which CNTF mediates its effects on neurogenesis remain unclear (Fig. 1). One way CNTF could produce its effects is through interaction with a trimeric receptor complex and signaling through the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway (Stahl and Yancopoulos, 1994). It has also been shown that CNTF promotes self-renewal of neural precursor cells *in vitro* by increasing Notch1 expression. Yet it remains to be determined whether the same receptor complexes and signaling cascades are involved in dopamine–CNTF-dependent adult neurogenesis. Therefore, it will be interesting to look at the protein levels of JAK/STAT and/or Notch1 in future experiments.

The therapeutic potential of CNTF in neurodegenerative disorders has been suggested for some time based on several studies showing that CNTF promotes sur-

vival of neurons in both *in vitro* and *in vivo* models of neurodegeneration (Ip and Yancopoulos, 1996). However, previous attempts to use CNTF as a therapeutic agent in a variety of therapeutic areas yielded disappointing results. Systemic delivery of recombinant CNTF failed to achieve sufficient concentrations in the brain, leading to poor efficacy, whereas weight loss was an unwanted side effect accompanying CNTF treatment. Although initially discouraging, CNTF has shown some recent promise in early-stage clinical trials for Huntington's disease and neurodegenerative retinal diseases (Sieving et al., 2006) (for a complete list of studies, go to www.ClinicalTrials.gov). In light of these observations, the recent study by Yang et al. (2008) should rekindle drug discovery and development efforts focused on regulating signaling pathways that enhance endogenous CNTF for the treatment of neurodegenerative disorders.

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