Adult neurogenesis, cell cycle and drug discovery in psychiatry

Robert N Pechnick[1,2,3] and Vera Chesnokova[4,5]

[1]Department of Psychiatry and Behavioral Neurosciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA;
[2]Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, CA, USA;
[3]Brain Research Institute, University of California, Los Angeles, CA, USA;
[4]Division of Endocrinology, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA;
[5]Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
E-mail: pechnickr@cshs.org

For many years the production of new neurons in mammalian brain was thought to be restricted to development. It is now clear that neurogenesis does occur in adult mammals, including humans (Eriksson *et al*, 1998). Antidepressant drugs and procedures that reduce depression, such as electroconvulsive shock and exercise, increase neurogenesis. The relationships among adult neurogenesis, antidepressant drugs, and depression have generated considerable interest and controversy (Duman, 2004; Scharfman and Hen, 2007).

p21Cip1, a cyclin-dependent kinase inhibitor, restrains cell-cycle progression and proliferation. It is found in neuroblasts and newly developing neurons in the subgranular zone of the hippocampus (Pechnick *et al*, 2008). Chronic treatment with the tricyclic antidepressant imipramine decreases p21Cip1 transcript and protein levels and stimulates neurogenesis in this region. Moreover, mice lacking p21Cip1 have increased rates of hippocampal neurogenesis. Thus, p21Cip1 restrains neurogenesis in the hippocampus, and antidepressant-induced stimulation of neurogenesis might be due to decreased p21Cip1 expression. Cell-cycle regulation occurs downstream from the primary site of action of antidepressants, suggesting that new therapeutic strategies might directly target cell-cycle proteins.

Currently, neurogenesis is a phenomenon in search of a function. There are four key questions that must be answered prior to the implementation of effective treatment strategies directed at altering neurogenesis. First, what is the role of adult neurogenesis in normal brain function? In humans, neurogenesis occurs in the hippocampus and olfactory bulb (Gould, 2007). Advances in imaging technology would help establish the conditions and pathological states under which neurogenesis is altered and whether neurogenesis is a latent process in other brain regions. This information is important because drug-induced stimulation of neurogenesis could disrupt fundamental neurobiological processes. Second, are changes in behavior and/or functional deficits in any disease state due to decreased (or increased) neurogenesis? Excessive neurogenesis could result in inappropriate integration into existing neural networks and could underlie pathological conditions such as epilepsy (Scharfman and Hen, 2007). Drug-induced stimulation of neurogenesis might have unforeseen adverse consequences.

Third, are basal and drug-induced neurogenesis age-dependent in humans? In rodents, the rate of neurogenesis decreases from adolescence to adulthood, and the decline is very steep (Abrous *et al*, 2008). If the rate of neurogenesis is profoundly decreased in older humans, then drugs targeted at stimulating neurogenesis might have limited efficacy in that population. Fourth, are there adverse consequences associated with long-term stimulation of neurogenesis? Long-term and unrelenting stimulation of mitosis without appropriate differentiation and migration could lead to unexpected problems. In addition, it is possible that adult neural stem cells have finite proliferation potentials. Long-term stimulation of neurogenesis might eventually produce premature exhaustion of neuronal precursors, the subsequent loss of therapeutic efficacy and premature 'aging' in the system.

There is a growing list of drugs and behavioral procedures that can stimulate or decrease neurogenesis. Modulating neurogenesis could be a new therapeutic target for the treatment of psychiatric disorders; however, more fundamental information on neurogenesis in humans needs to be obtained to design rational therapeutic strategies and avoid unforeseen adverse consequences.

DISCLOSURE/CONFLICT OF INTEREST
In addition to income received from his primary employer, Dr Robert Pechnick has received financial support from Sepracor Inc and Forest Laboratories Inc. Except for income received from her primary employer, Dr Vera Chesnokova declares that no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service. For either investigator there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. This work was partially support by a NARSAD Young Investigator Award (VC), National Institutes of Health Grants MH079988 (VC), MH078037 (RNP) and MH079370 (RNP), and the Levine Family Fund Research Endowment (RNP).

Abrous DN, Koehl M, Le Moal M (2008). Adult neurogenesis: from precursors to network and physiology. *Physiol Rev* **85**: 523–569.
Duman RS (2004). Depression: a case of neuronal life and death? *Biol Psychiatry* **56**: 140–145.
Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA *et al* (1998). Neurogenesis in the adult human hippocampus. *Nat Med* **4**: 1313–1317.
Gould E (2007). How widespread is adult neurogenesis in mammals? *Nat Rev Neurosci* **8**: 481–488.
Pechnick RN, Zonis S, Wawrowsky K, Pourmorady J, Chesnokova V (2008). p21Cip1 restricts neuronal proliferation in the subgranular zone of the dentate gyrus of the hippocampus. *Proc Natl Acad Sci USA* **105**: 1358–1363.
Scharfman HE, Hen R (2007). Is more neurogenesis always better? *Science* **315**: 336–338.

Neuropsychopharmacology Reviews (2009) **34,** 244; doi:10.1038/npp.2008.164

Targeting nicotinic receptor antagonists as novel pharmacotherapies for tobacco dependence and relapse

Tobacco dependence is a significant health concern and the most preven-

table cause of death in the United States. Nicotine, the principal tobacco alkaloid, activates nicotinic receptors (nAChRs) on dopamine terminals in the mesolimbic and nigrostriatal systems to evoke dopamine release, leading to reward and tobacco dependence. Bupropion, which inhibits both neurotransmitter transporters and acts as a nAChR antagonist, has benefit as a smoking cessation agent. In addition, mecamylamine, a noncompetitive antagonist at both central and peripheral nAChRs, has shown benefit in clinical trials, but is limited by anticholinergic side effects because of its lack of nAChR selectivity. Bupropion and mecamylamine provide proof of the concept that nAChR antagonists have efficacy in treating nicotine addiction; however, high relapse rates indicate a continuing need for alternative pharmacotherapies.

Our hypothesis is that discovery of selective antagonists targeted at neuronal nAChRs mediating nicotine-evoked neurotransmitter release, which mediates the reinforcing effects of nicotine, will provide clinically effective smoking cessation agents, circumventing unwanted side effects. The current pharmacological approach using a subtype-selective nAChR antagonist to block reversibly the specific nAChR subtype mediating the reinforcing effects of nicotine is similar to employing nAChR subunit deletion to prevent the expression of these nAChRs. In this regard, landmark work has shown that in β-2 subunit knockout mice, targeted injection into the ventral tegmental area of a lentiviral vector that efficiently expresses β-2 subunit protein, restores both nicotine-evoked dopamine release in the nucleus accumbens and nicotine reinforcement, providing convincing evidence that β-2-containing nAChRs expressed specifically in the ventral tegmental area are important in the reinforcing effects of nicotine (Molles *et al*, 2006).

Because nicotine interacts with all nAChR subtypes, discovery of subtype-selective nAChR antagonists that inhibit nicotine-evoked dopamine release was initiated using nicotine as the structural scaffold. Simple addition of an *N*-*n*-alkyl group converted nicotine from an agonist to an antagonist, and subtype selectivity began to emerge depending on the number of methylene groups in the *n*-alkyl chain (Dwoskin *et al*, 2004). The classic discovery that the bis-tri-alkylammonium nAChR channel blockers, hexamethonium and decamethonium, exhibit subtype selectivity between ganglionic and muscle-type nAChRs led us to use a similar approach by generating a sub-library of small molecules consisting of a bis-nicotinium analog structure, incorporating a variety of head groups and diverse linkers varying in length, unsaturation, and polarity. From this novel sub-library, a new lead compound, *N,N′*-dodecyl-1, 12-diyl-bis-3-picolinium dibromide (bPiDDB), emerged. bPiDDB potently inhibited nicotine-evoked dopamine release from superfused rat striatal slices (Dwoskin *et al*, 2008a). Using microdialysis, systemically administered bPiDDB also inhibited the nicotine-induced increase in extracellular dopamine in nucleus accumbens (Rahman *et al*, 2007). Thus, following *in vitro* and *in vivo* peripheral administration, bPiDDB decreased nicotine-evoked dopamine release. Utilizing radiolabeled bPiDDB, we also demonstrated its brain bioavailability by the blood–brain barrier choline transporter (Albayati *et al*, 2008).

Investigation of the behavioral pharmacology of bPiDDB revealed that this compound decreases nicotine-induced hyperactivity in nicotine-sensitized rats, a response associated previously with enhanced nicotine-evoked dopamine release in nucleus accumbens. Since bPiDDB did not reduce activity when administered alone in nicotine-sensitized rats, the decrease in nicotine-induced hyperactivity is not due to nonspecific motor impairment, but rather likely reflects inhibition of nicotine-evoked dopamine release. Moreover, bPiDDB decreases intravenous nicotine self-administration in rats (Neugebauer *et al*, 2006). Surprisingly, bPiDDB did not block the discriminative stimulus effects of nicotine, indicating that bPiDDB dissociates the rewarding and discriminative stimulus properties of nicotine (Dwoskin *et al*, 2008a). Following extinction of nicotine self-administration, bPiDDB also attenuated nicotine-induced reinstatement of nicotine-seeking behavior in rats (Dwoskin *et al*, 2008b). Taken together, the effectiveness of bPiDDB in decreasing both nicotine self-administration and reinstatement designates bPiDDB as a lead in our search for nAChR antagonists that may be a useful as treatments for tobacco dependence and relapse.

ACKNOWLEDGEMENTS

This work was supported by NIH U19 DA017548, K02 DA00399, T32 DA007304.

Linda P Dwoskin[1] and Michael T Bardo[1]

[1]University of Kentucky National Cooperative Drug Discovery Group, Lexington, KY, USA
E-mail: ldwoskin@email.uky.edu

DISCLOSURE/CONFLICT OF INTEREST

The University of Kentucky holds patents on *N,N′*-dodecyl-1,12-diyl-bis-3-picolinium dibromide. A potential royalty stream to LPD may occur consistent with University of Kentucky policy. The authors declare that over the past three years LD has received compensation from Boston University, Emory University, Meharry Medical College, the Universities of Arkansas, Florida, Indiana, Mississippi, Michigan, Nebraska, New Mexico as well as Yaupon Therapeutics Inc., and MB received compensation from Oregon Health Sciences University, University of Nebraska, University of Minnesota, Duke University, Morehead State University, Rutgers University, Medical College of Georgia, Centre College, Concordia University, Kansas University, University of Cincinnati, Yaupon Therapeutics, Inc., US World Meds and Targacept Inc.

Albayati ZF, Dwoskin LP, Crooks PA (2008). Pharmacokinetics of the novel nicotinic receptor antagonist *N,N′*-dodecane-1,12-diyl-bis-3-picolinium dibromide (bPiDDB). *Drug Metab Dispos*, PMID:18617603 [E-pub ahead of print, 10 July].

Dwoskin LP, Pivavarchyk M, Joyce BM, Neugebauer NM, Zheng G, Zhang Z *et al* (2008b). Targeting reward-relevant nicotinic receptors in the discovery of novel pharmacotherapeutic agents to treat tobacco dependence. 55th Annual Nebraska Symposium on Motivation: The Motivational Impact of Nicotine and its Role in Tobacco Use. In: RA Bevins and AR Caggiula (eds) Springer, in press.

Dwoskin LP, Sumithran SP, Zhu J, Deaciuc AG, Ayers JT, Crooks PA (2004). Subtype-selective nicotinic receptor antagonists: potential as tobacco use cessation agents. *Bioorg Med Chem Lett* **14**: 1863–1867.

Dwoskin LP, Wooters TE, Sumithran SP, Siripurapu KB, Joyce BM, Lockman PR *et al* (2008a). *N,N′*-Alkane-diyl-bis-3-picoliniums as nicotinic receptor antagonists: inhibition of nicotine-induced dopamine release and hyperactivity. *J Pharmacol Exp Ther* **326**: 563–576.

Molles BE, Maskos U, Pons S, Besson M, Guiard P, Guilloux JP *et al* (2006). Targeted *in vivo* expression of nicotinic receptors in mouse brain using lentiviral expression vectors. *J Mol Neurosci* **30**: 105–106.

Neugebauer NM, Zhang Z, Crooks PA, Dwoskin LP, Bardo MT (2006). Effect of a novel nicotinic receptor antagonist, *N,N′*-dodecane-1,12-diyl-bis-3-picolinium dibromide, on nicotine self-administration and hyperactivity in rats. *Psychopharmacology* **184**:426–434.

Rahman S, Neugebauer NM, Zhang Z, Crooks PA, Dwoskin LP, Bardo MT (2007). The effects of a novel nicotinic receptor antagonist *N,N*-dodecane1,12-diyl-bis-3-picolinium dibromide (bPiDDB) on acute and repeated nicotine-induced increases in extracellular dopamine in rat nucleus accumbens. *Neuropharmacology* **52**: 755–763.

Neuropsychopharmacology Reviews (2009) **34,** 244–246; doi:10.1038/npp.2008.157

RNA editing as a therapeutic target for CNS disorders

The conversion of adenosine to inosine (A-to-I) by RNA editing is a widespread RNA processing event by which genomically encoded sequences are altered through site-specific deamination of adenosine residue(s) by a family of enzymes referred to as adenosine deaminases that act on RNA (ADARs). Notable targets of RNA editing in the CNS include transcripts encoding subunits of the AMPA and kainate (KA) subtypes of glutamate receptor, the α3 subunit of the $GABA_A$ receptor, the serotonin 2C (5 HT_{2C}) receptor, and the K_v1.1 voltage-gated potassium channel. RNA editing can modulate the functional properties of the encoded protein products and variations in the editing of glutamate receptor subunit and 5 HT_{2C} mRNAs have been observed in several CNS disorders (Rula and Emeson, 2007), suggesting that modulation of RNA editing in the nervous system could represent a therapeutic strategy for the treatment of nervous system dysfunction.

A failure to edit GluR2 transcripts results in Ca^{2+}-permeable AMPA receptors that can lead to excitotoxicity. Recent studies have observed a significant reduction in Q/R site editing for affected motor neurons isolated from patients with amyotrophic lateral sclerosis, suggesting that glutamatergic excitotoxicity may underlie the selective neuronal death of these motor neurons (Kwak and Kawahara, 2005). RNA editing of another site within GluR2 transcripts (R/G site) alters both the recovery rate and desensitization kinetics of AMPA receptors containing edited subunits (Rula and Emeson, 2007) and increased editing has been observed in the hippocampus of patients with temporal lobe epilepsies (Vollmar *et al*, 2004). Significant alterations in KA receptor editing and ADAR2 protein expression also have been seen following seizures in humans and rats, and genetically modified animals solely expressing a nonedited GluR6(Q) subunit demonstrate increased susceptibility to KA-induced seizures (Vissel *et al*, 2001), suggesting KA receptor editing may modulate seizure vulnerability.

Editing of 5 HT_{2C} receptor transcripts can generate up to 24 receptor isoforms that not only have distinct functional properties affecting constitutive activity and receptor/G-protein coupling, but also differ in their pattern of CNS expression (Rula and Emeson, 2007). Altered editing patterns have been observed in suicide victims with a history of major depression and in response to antidepressant treatment (Niswender *et al*, 2001; Gurevich *et al*, 2002), suggesting that editing may be involved in affective disorders and the maintenance of appropriate serotonergic neurotransmission.

Although no conclusive data have demonstrated that changes in RNA editing are causal for human brain disorders, the role of ADARs in the function of numerous receptors/channels highlights the potential for ADAR activity as a future target for the treatment of CNS dysfunction. Therapeutic modulation of A-to-I editing patterns for specific substrates is complicated by the fact that only two ADAR proteins have been shown to catalyze all known editing events in mammals, predicting that broad alterations in ADAR activity could result in untoward effects. Observations that RNA editing changes in CNS disorders may occur in specific brain regions, or even subpopulations of neurons, suggest that substrate- or region-selective approaches to modify editing represent more promising areas of research for the treatment of CNS disorders.

Michael V Morabito[1,2] and Ronald B Emeson[1,2]

[1]Department of Pharmacology, Vanderbilt University, Nashville, TN, USA and
[2]Center for Molecular Neuroscience, Vanderbilt University, Nashville, TN, USA
E-mail: ron.emeson@vanderbilt.edu

DISCLOSURE/CONFLICT OF INTEREST

The authors declare that except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

Gurevich I, Tamir H, Arango V, Dwork AJ, Mann JJ, Schmauss C (2002). Altered editing of serotonin 2C receptor pre-mRNA in the prefrontal cortex of depressed suicide victims. *Neuron* **34**: 349–356.

Kwak S, Kawahara Y (2005). Deficient RNA editing of GluR2 and neuronal death in amyotropic lateral sclerosis. *J Mol Med* **83**: 110–120.

Niswender CM, Herrick-Davis K, Dilley GE, Meltzer HY, Overholser JC, Stockmeier CA *et al* (2001). RNA editing of the human serotonin 5-HT2C receptor: alterations in suicide and implications for serotonergic pharmacotherapy. *Neuropsychopharmacology* **24**: 478–491.

Rula EY, Emeson RB (2007). Mouse models to elucidate the functional roles of adenosine-to-inosine editing. *Methods Enzymol* **424**: 333–367.

Vissel B, Royle GA, Christie BR, Schiffer HH, Ghetti A, Tritto T *et al* (2001). The role of RNA editing of kainate receptors in synaptic plasticity and seizures. *Neuron* **29**: 217–227.

Vollmar W, Gloger J, Berger E, Kortenbruck G, Kohling R, Speckmann EJ *et al* (2004). RNA editing (R/G site) and flip-flop splicing of the AMPA receptor subunit GluR2 in nervous tissue of epilepsy patients. *Neurobiol Dis* **15**: 371–379.

Neuropsychopharmacology Reviews (2009) **34,** 246; doi:10.1038/npp.2008.159