PERSPECTIVE

Nicotine-induced dopamine plasticity: a gateway to neurotransmitter replacement?

Nicotine, the principal neuroactive component of tobacco, acts on the reward systems of smokers, inducing addiction. Experimental evidence indicates that nicotine-induced addiction alters the activity of dopaminergic neurons within reward-processing brain centers. The effect of developmental nicotine exposure on neuroplasticity of identified reward neurocircuitry in the adult is finally emerging and begins to be understood at the molecular, cellular, and behavioral level (Romoli et al., 2019).

For many decades, neurons of the adult central nervous system have been assumed to lack the ability to change their neurotransmitter expression since this is specified early during development. However, an increase in the number of dopamine-expressing neurons in the ventral tegmental area of adult mice has been observed following chronic exposure to methamphetamines (Kesby et al., 2017). Since maternal nicotine exposure during late gestation and lactation has been shown to elicit long-term plasticity altering the dopaminergic reward system in rat offspring (Pinheiro et al., 2015), the long-term effects of neonatal circuit activation leading to neurotransmitter plasticity in adults represent a fundamental question for translational research.

Few studies have reported the effects of developmental nicotine exposure on enhanced susceptibility to substance abuse in adulthood (Heath and Picciotto, 2009; Zhu et al., 2012). Romoli et al. (2019) provided strong evidence that neonatal nicotine exposure greatly increases nicotine preference in adult mice through an activity-dependent mechanism of dopamine plasticity also known as neurotransmitter switching. The characterization of the molecular adaptations that paralleled changes in reward-seeking behaviors revealed the key cellular players associated with dopamine plasticity induced by developmental exposure to substances of abuse. The mouse model system offered cutting-edge genetic tools to identify the specific neuronal pool recruited for this nicotine-induced form of neuroplasticity in the ventral tegmental area. We found that developmental nicotine exposure primes the reward center to become "hyperplastic" at the dopaminergic level. Such neuroplasticity mediates an increase in the number of neurons expressing the dopaminergic marker, tyrosine hydroxylase (TH), following a secondary nicotine exposure in adulthood.

Several new insights emerged from our recent report. We highlight them here for the scientific community.

Through the discovery of the link between nicotine-induced neurotransmitter plasticity in the reward centers and enhanced consumption of nicotine and other substances of abuse, our study is a reminder that the brain is plastic and malleable during the earliest phase of life and external interference affecting normal development can elicit long-lasting effects, even when they are not immediately detectable after exposure to altered environmental stimuli. Neurons of the ventral tegmental area acquired a "molecular memory" of neonatal nicotine exposure. By increasing the expression of nicotine receptors and boosting calcium signaling coupled with ectopic transcriptional regulation of the molecular marker Nurr1, a protein normally found only in dopaminergic differentiated neurons, non-dopaminergic neurons acquire a dormant "readiness" to switch to a dopaminergic program when properly motivated by nicotine re-exposure in adulthood.

This pre-clinical work (Romoli et al., 2019) identified new cellular and molecular targets that may guide future clinical studies to redefine treatment strategies. Because we found that this form of nicotine-induced neuroplasticity facilitates addiction to other addictive substances, such as ethanol in adults, uncovering the mechanism contributing to increased addiction susceptibility opens the gate to the discovery of new ways to interfere with the mechanism of drug-mediated plasticity and prevent the negative consequences on reward-seeking behavior in adulthood.

Finally, our research showed that neonatal nicotine exposure through lactation has direct translational implications since the nicotine concentrations used in our studies are comparable to the concentrations human newborns are exposed to when lactating from smokers. However, tobacco-use by pregnant smokers and nursing moms, including nicotine patches which are occasionally used during pregnancy to protect smokers and their babies from the deleterious effects of smoke compounds, is not carefully regulated. For instance, nicotine patches deliver a high concentration of nicotine in the blood stream of pregnant women and nursing moms. Because post-mortem studies in humans (Aumann et al., 2016) have shown that dopamine plasticity discovered in rodents (Aumann et al., 2011; Dulcis et al., 2013) can also occur in the human dopaminergic system, our findings on the deleterious effects of neonatal nicotine exposure on normal physiology of the reward system might have implications applicable to human physiology and drug addiction. This knowledge should have a profound impact on state policies that fails to regulate the use of substitutive nicotine therapies by pregnant and postpartum women via nicotine patches or electronic cigarettes.

Current clinical treatments of neurotransmitter-associated disorders include: pharmacological agents developed to restore appropriate levels of neurotransmitters or modulate neurotransmitter receptor activation, embryonic stem cell transplantation to replace degenerated cells, surgical removal of brain nuclei to reduce excitation in epilepsy, electroconvulsive therapies, deep brain stimulation, and transcranial magnetic stimulation. Activity-dependent neurotransmitter plasticity induced by selective activation of brain circuits might provide spatial refinement of current clinical treatments. Recruitment and integration of neurons with the correct transmitter identity and connectivity into damaged circuits via induction of neurotransmitter plasticity could increase the precision and effectiveness of future clinical approaches. In the postembryonic nervous system, where neurogenesis is restricted to a few regions in the brain and the extracellular environment no longer provides appropriate cues to guide axonal pathfinding of ectopically and surgically implanted embryonic stem cells, the activity-mediated recruitment of reserve pool neurons (Dulcis and Spitzer, 2012) for neurotransmitter plasticity opens new hope for a functional rescue of damaged neural circuits.

What is in the horizon for our future research? Nicotine-mediated dopamine induction in the reward system affecting reward-seeking behavior (Romoli et al., 2019) is the first detailed example of activity-dependent neurotransmitter plasticity induced by a psychostimulant. The knowledge on the molecular and cellular signature required for neurons to switch to a dopaminergic phenotype could represent the gateway to recruitment of other neurocircuits and could prove to be of clinical impact on patients with neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease.

Because PD is a serious debilitating neurological disorder resulting from neurodegeneration of dopamine neurons in the substantia nigra (SN) and given that low concentrations of nicotine appear to mitigate and delay the degenerative effects of PD (Huang et al., 2009), our future research will focus on understanding the mechanism involved in this form of neuroprotection. To build upon our previous published work on activity-dependent dopamine plasticity (Dulcis and Spitzer, 2008; Dulcis et al., 2013, 2017) and recent findings on nicotine-induced dopamine expression (Romoli et al., 2019), we are currently investigating whether nicotine-mediated activation of SN circuits can convert non-dopaminergic neurons to a dopaminergic phenotype potentially enabling them to release *de-novo* synthesized dopamine in the caudate nucleus. This possibility is encouraged by the fact that when massive cell death occurs in the neuromelanin-pigmented and dopamine-containing SN, the dopaminergic neurons of the SN are not the only neurons projecting to the striatum (González-Hernández and Rodríguez, 2000). The SN is composed of the pars compacta (SNc), which is mainly dopaminergic, and the pars reticulata (SNr), organized in large clusters of GABAergic neurons with thin intermingled stripes of dopaminergic neurons (González-Hernández and Rodríguez, 2000). Remarkably, the SNr remains intact in PD animal models (González-Hernández and Rodríguez, 2000), and the combination of retrograde labeling and immunocytochemistry has shown that clusters of GABAergic neurons of the SNr already project to the caudate nucleus (González-Hernández and Rodríguez, 2000). The existence of this non-dopaminergic nigrostriatal pathway was elegantly confirmed by Rodriguez and Gonzalez-Hernandez (2000) by antidromic responses to striatal stimulation, retrograde labeling, and resistance to neurotoxin 6-OHDA. These GABAergic neurons represent more than 80% of nigrostriatal neurons in PD-animal models. Consistent with their potential role as a reserve pool (Dulcis and Spitzer, 2012) that could rescue the loss of dopaminergic neurons, non-dopaminergic nigrostriatal neurons share afferents with dopaminergic neurons in addition to sharing the same target, the striatum (Gerfen, 1984).

To this aim, we investigated the effects of 2-week nicotine exposure via voluntary oral administration on dopamine expression in the SN of adult mice (P60). Experimental mice had access to a 50 mg/L nicotine in 1% saccharin solution while control animals to a 1% saccharin water solution. After 14 days of drinking nicotine solution, mice (P74) were sacrificed and their brains processed for diaminobenzidine immunohistochemistry to label the dopaminergic marker TH. TH-expressing dopaminergic cells were quantified with unbiased stereological counts of images obtained with a Leica DM4B microscope equipped with Stereologer2000 software. As shown in **Figure 1**, chronic nicotine treatment increased the number of TH-positive cells by 50% in SNr, but did not change TH expression in SNc. Chronic nicotine treatment did not affect the total number of neurons in the SNr (data not shown), indicating that the increase in TH-positive neurons was not due to an increase of neuroproliferation nor cell migration. Our findings suggest the presence of an endogenous reserve pool of neurons in the SNr that can be recruited to express a TH phenotype by chronic nicotine exposure. GABAergic neurons (González-Hernández and Rodríguez, 2000) of the SNr could represent a neuronal reserve pool available for recruitment to nicotine-mediated TH upregulation or responsive to other forms of targeted activity-dependent manipulations.

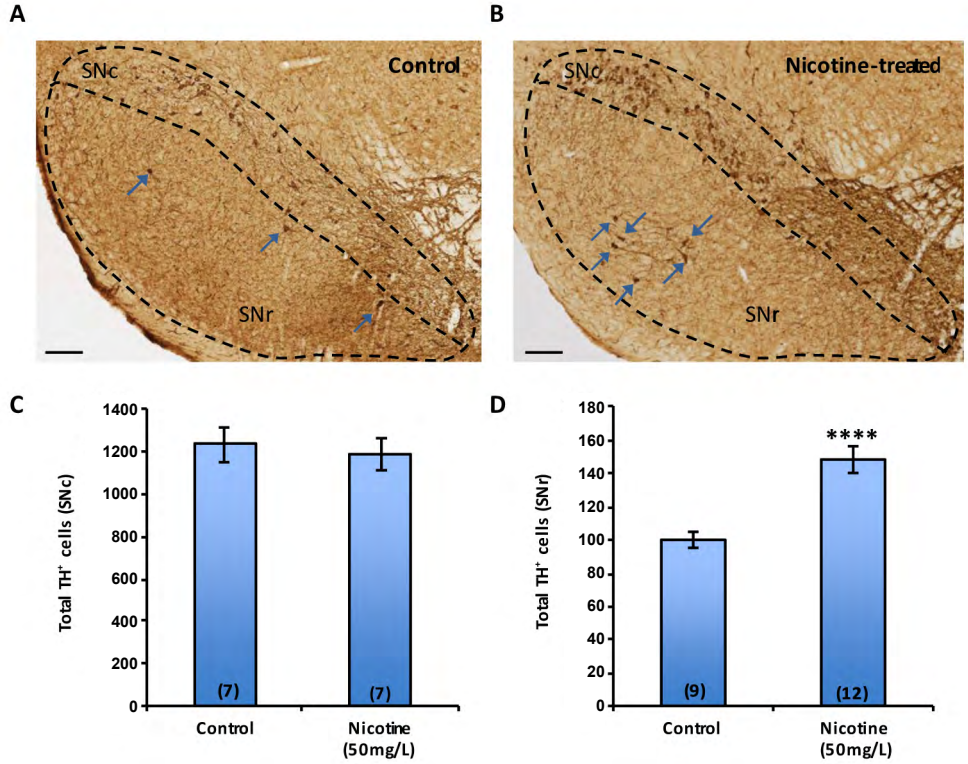

Figure 1 Effects of nicotine intake on TH expression in the substantia nigra (SN).
Bright-field image of coronal section (30 μm) through the SN of control (A) and nicotine-treated adult (P60) mice (B) immunostained for dopaminergic marker, tyrosine hydroxylase (TH). Dopaminergic neurons (arrows) are indicated in both compacta (SNc) and reticulata (SNr). Scale bars: 100 μm. Stereological quantification of the number of TH-expressing cells in the SNc (C) and SNr (D). ****$P < 0.0001$.

Investigating whether other forms of circuit activation, other than nicotine-mediated activity, could be sufficient to recruit non-dopaminergic nigrostriatal reserve pool to acquire a dopaminergic phenotype and reverse behavioral deficits in animal models for PD is part of our research mission. If successful, this approach could represent a paradigm shift in the way we intervene to restore loss of dopamine function in the future. We envision how induction of circuit-specific neurotransmitter plasticity might reveal an effective approach to accomplish neurotransmitter replenishment to treat neurodegenerative disorders as well as other neurological conditions associated with dopamine and other transmitter imbalance and dysfunction such as depression, schizophrenia, and drug addiction.

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