Journal Club

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Potential Involvement of a Substantia Nigra Circuit in Opioid Reinforcement

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Review of Galaj et al.

A long-held tenet in the pharmacology of drug abuse is that, despite marked differences in cellular targets, addictive drugs reinforce drug taking by increasing dopamine (DA) transmission in the Nucleus accumbens (NAc; Di Chiara and Imperato, 1988). Opioid-evoked DA release in the NAc has been proposed to require activation of inhibitory μ opioid receptors (MORs) on VTA GABAergic interneurons that tonically inhibit VTA DA neurons that project to the NAc. Thus, opioids are thought to disinhibit VTA DA neurons, leading to increased DA release in the NAc (Gysling and Wang, 1983; Johnson and North, 1992). A major problem with this hypothesis, however, is that DA release in the NAc is not required for opioid reinforcement (Badiani et al., 2011). This not only contradicts the hypothesis for a universal mechanism of action for drugs of abuse (Nutt et al., 2015), but also implies that alternative neural circuits or neurotransmitters drive opioid intake. Recent work in rats and mice (Galaj et al., 2020) describes such a VTA-independent pathway for opioid

reinforcement, involving disinhibition of GABAergic neurons in the SNr that innervate DA neurons in the SNc. This work indicates that DA signaling supports opioid reinforcement, but the VTA to NAc circuit may not be required.

First, Galaj et al. (2020) examined MOR mRNA (*OPRM1*) expression in the midbrain. *OPRM1* mRNA was expressed in GABAergic interneurons in the VTA (their Fig. 2D), rostromedial tegmental nucleus (RMTg) (their Fig. 2E), and SNr (their Fig. 2C), but not in DA neurons in the VTA or SNc (their Fig. 2B,F). Although there was dense MOR mRNA expression in the RMTg and prior work demonstrating dense RMTg projections to VTA DA neurons (Jalabert et al., 2011; Matsui et al., 2014), Galaj et al. (2020) focused on GABAergic neurons in SNr and VTA.

To investigate whether MOR signaling in the SNr and VTA influences intravenous heroin self-administration, the authors infused the MOR antagonist naloxonazine into these midbrain regions. Intra-VTA naloxonazine infusion increased responding (e.g., active lever pressing) for a low dose of heroin, but not for higher doses, suggesting that VTA MOR blockade diminished heroin potency, requiring animals to respond more to reach preferred drug levels (their Fig. 3). In contrast, micro-infusions of naloxonazine into the SNr increased heroin responding at all doses (their Fig. 4), suggesting that SNr

MORs exert greater control over opioid reinforcement than VTA MORs.

Galaj et al. (2020) next asked whether SNr MORs are involved in relapse to drug seeking after abstinence. In humans, drug craving and relapse during abstinence are often triggered by reexposure to the drug or drug-associated cues. This phenomenon can be modeled in rodents using reinstatement procedures. After animals have developed reliable responding for drug access, they undergo a period of forced abstinence in which the active response does not provide drug delivery. Animals progressively decrease their responding over the abstinence period. However, when reexposed to the drug or drug-paired cues, animals will reinstate their responding behavior in an attempt to gain access to the drug. Cue-primed reinstatement tests whether particular stimuli (e.g., lights or tones) that have been paired with drug administration can reinstate responding. In contrast, drug-primed reinstatement relies on the primary reinforcing qualities of the drug to reinvigorate responding. Galaj et al. (2020) found that MOR blockade within the SNr diminished heroin-primed reinstatement without affecting cue-primed reinstatement (their Fig. 5). This indicates that MOR signaling within the SNr is integral to the primary reinforcing properties of heroin, but not to learned associations between heroin and associated cues.

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To verify this, the authors mimicked the MOR-induced inhibition of SNr GABAergic neurons using optogenetics. They found that mice would lever press at high rates (~300 responses in the last 2 h session) to receive brief optical inhibition of SNr GABA neurons (their Fig. 9G).

Unlike VTA GABA neurons, the majority of SNr GABA neurons do not project to VTA DA neurons but instead innervate DA neurons in the SNc, as well as other targets throughout the brain (Tepper and Lee, 2007; Watabe-Uchida et al., 2012). Thus, optical inhibition of SNr GABA neurons is expected to disinhibit output targets and drive reinforcement by increasing the activity of SNc DA neurons. Consistent with this, Galaj et al. (2020) found that optogenetic activation of SNc DA neurons was reinforcing (their Fig. 6F; see also Rossi et al., 2013; Ilango et al., 2014).

Galaj et al. (2020) next assessed how GABAergic neurons in the SNr and VTA influence drug reinforcement by pairing each lever press for heroin or cocaine with brief, optical stimulation of GABAergic neurons in the SNr or VTA. In accordance with a primary role for VTA DA neurons in cocaine-seeking, optical activation of VTA GABAergic neurons, which presumably inhibits VTA DA neurons, reduced cocaine self-administration (their Fig. 7F). In contrast, optical activation of VTA GABAergic neurons did not alter heroin self-administration (their Fig. 7D), suggesting that GABAergic signaling in the VTA does not control heroin-seeking. Conversely, optical excitation of SNr GABAergic neurons increased heroin self-administration, suggesting that, similar to MOR blockade in the SNr, activation of SNr GABAergic neurons reduced heroin's reinforcing properties (their Fig. 8D,E). Consistent with the role of SNr GABAergic neurons in modulating the reinforcing properties of heroin, heroin-primed reinstatement was also reduced by optical activation of SNr GABAergic neurons (their Fig. 8G). While these findings indicate that GABAergic neurons in the VTA and SNr influence cocaine and heroin self-administration, respectively, it is difficult to interpret what these findings mean for drug reinforcement mechanisms, given the opposite effect of stimulation on drug intake. Nevertheless, SNr GABAergic neurons appear to serve a more integral role in opioid reinforcement than VTA GABAergic neurons.

The work by Galaj et al. (2020) provides convincing evidence that SNr

GABAergic neurons and SNc DA neurons are involved in heroin reinforcement, but it is unclear whether MOR activation in the SNr by heroin (1) stimulates striatal DA release and, if so, (2) whether heroin self-administration requires striatal DA release. Galaj et al. (2020) propose that SNr GABAergic inputs to SNc DA neurons form a potent inhibitory circuit that, when suppressed by receptor binding or optical manipulation, can drive robust reinforcement (their Figs. 6F, 9G; see also Rossi et al., 2013; Ilango et al., 2014). However, the necessity of SNc DA in opioid reinforcement is not entirely clear from this work since behavioral output (i.e., heroin self-administration or neuronal optical self-stimulation) is used as an indirect proxy of DA neurotransmission. Notably, a subclass of VTA DA neurons also corelease glutamate in the NAc (Wang et al., 2015). These glutamate coreleasing neurons support optical self-stimulation independently of DA release (Zell et al., 2020). Thus, nondopaminergic targets may support reinforcement following inhibition of SNr GABA neurons. One method to directly address whether heroin self-administration requires DA release induced by MOR agonism in the SNr-SNc circuit is to use cell type- and site-specific viral-genetic (e.g., Cre/loxP recombination, CRISPR/Cas9) ablation of MORs from SNr GABAergic neurons that project to SNc DA neurons (Zell et al., 2020). After establishing the role of the SNr-SNc inhibitory circuit in heroin reinforcement, DA receptor antagonist infused into SNc output regions during heroin self-administration could be used to demonstrate the necessity of DA in that particular region during heroin reinforcement (Hodebourg et al., 2019). Another strategy could be restoration of MOR expression in specific neuronal pathways in MOR systemic KO animals and then determining which circuits support systemic self-administration of opioids (Cui et al., 2014). If SNc DA is integral to opioid reinforcement, while VTA DA is not, then opioid reinforcement likely depends on distinct DA output targets. While SNc and VTA DA neurons have overlapping projection locations, including the NAc, the majority of SNc DA neurons project to dorsal striatal regions (Poulin et al., 2018). The SNc DA neuron projection profile, along with the finding that NAc DA is not integral to opioid reinforcement, suggests that future research should determine the necessity of DA signaling in subregions of the dorsal striatum (but see David and Cazala, 2000).

In conclusion, this work suggests that a more generalized hypothesis focusing on the necessity of striatal DA neurotransmission, rather than the NAc specifically, may be required to highlight differences between opioids and other drug classes.

References

- Badiani A, Belin D, Epstein D, Calu D, Shaham Y (2011) Opiate versus psychostimulant addiction: the differences do matter. *Nat Rev Neurosci* 12:685–700.
- Cui Y, Ostlund SB, James AS, Park CS, Ge W, Roberts KW, Mittal N, Murphy NP, Cepeda C, Kieffer BL, Levine MS, Jentsch JD, Walwyn WM, Sun YE, Evans CJ, Maidment NT, Yang XW (2014) Targeted expression of μ -opioid receptors in a subset of striatal direct-pathway neurons restores opiate reward. *Nat Neurosci* 17:254–261.
- David V, Cazala P (2000) Anatomical and pharmacological specificity of the rewarding effect elicited by microinjections of morphine into the nucleus accumbens of mice. *Psychopharmacology* 150:24–34.
- Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* 85:5274–5278.
- Galaj E, Han X, Shen H, Jordan CJ, He Y, Humburg B, Bi GH, Xi ZX (2020) Dissecting the role of GABA neurons in the VTA versus SNr in opioid reward. *J Neurosci* 40:8853–8869.
- Gysling K, Wang RY (1983) Morphine-induced activation of A10 dopamine neurons in the rat. *Brain Res* 277:119–127.
- Hodebourg R, Murray JE, Fouyssa M, Puaud M, Everitt BJ, Belin D (2019) Heroin seeking becomes dependent on dorsal striatal dopaminergic mechanisms and can be decreased by N-acetylcysteine. *Eur J Neurosci* 50:2036–2044.
- Ilango A, Kesner AJ, Keller KL, Stuber GD, Bonci A, Ikemoto S (2014) Similar roles of substantia nigra and ventral tegmental dopamine neurons in reward and aversion. *J Neurosci* 34:817–822.
- Jalabert M, Bourdy R, Courtin J, Veinante P, Manzoni OJ, Barrot M, Georges F (2011) Neuronal circuits underlying acute morphine action on dopamine neurons. *Proc Natl Acad Sci USA* 108:16446–16450.
- Johnson SW, North RA (1992) Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J Neurosci* 12:483–488.
- Matsui A, Jarvie BC, Robinson BG, Hentges ST, Williams JT (2014) Separate GABA afferents to dopamine neurons mediate acute action of opioids, development of tolerance, and expression of withdrawal. *Neuron* 82:1346–1356.
- Nutt DJ, Lingford-Hughes A, Erritzoe D, Stokes PRA (2015) Opiate versus psychostimulant addiction: the differences do matter. *Nat Rev Neurosci* 16:305–312.
- Poulin JF, Caronia G, Hofer C, Cui Q, Helm B, Ramakrishnan C, Chan CS, Dombeck DA, Deisseroth K, Awatramani R (2018) Mapping projections of molecularly defined dopamine neuron subtypes using intersectional genetic approaches. *Nat Neurosci* 21:1260–1271.

- Rossi MA, Sukharnikova T, Hayrapetyan VY, Yang L, Yin HH (2013) Operant self-stimulation of dopamine neurons in the substantia nigra. *PLoS One* 8:e65799.
- Tepper JM, Lee CR (2007) GABAergic control of substantia nigra dopaminergic neurons. *Prog Brain Res* 160:189–208.
- Wang HL, Qi J, Zhang S, Wang H, Morales M (2015) Rewarding effects of optical stimulation of ventral tegmental area glutamatergic neurons. *J Neurosci* 35:15948–15954.
- Watabe-Uchida M, Zhu L, Ogawa SK, Vamanrao A, Uchida N (2012) Whole brain mapping of direct inputs to midbrain dopamine neurons. *Neuron* 74:858–873.
- Zell V, Steinkellner T, Hollon NG, Warlow SM, Souter E, Faget L, Hunker AC, Jin X, Zweifel LS, Hnasko TS (2020) VTA glutamate neuron activity drives positive reinforcement absent dopamine co-release. *Neuron* 107:864–873.