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Hedgehog-interacting protein in habenula regulates nicotine intake

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Background: The US is in the midst of an opioid abuse and overdose epidemic, with over 115 people dying each day from opioid overdose; this has been declared a public health emergency. Rationale: The vast majority of research on opioid addiction has focused on a small number of circuits, primarily the mesocorticolimbic circuit. We used iDISCO+ tissue clearing and c-Fos staining to create a whole-brain map of transcriptionally responsive neurons following acute oxycodone (5 mg/kg) or saline injection. Results: This revealed 39 regions with significant oxycodone-induced alterations in c-Fos expression. The dorsal peduncular nucleus (DP) is positioned in the ventral-most component of medial prefrontal cortex (mPFC). Single-cell RNA-sequencing, qPCR, and RNAscope revealed unique properties of opioid-responsive DP cells relative to surrounding mPFC. The DP is enriched in *Oprm1* and *Slc17a6* (vGlut2) relative to neighboring infralimbic cortex. Surprisingly, *Oprm1* and *Slc17a6* are co-expressed in DP neurons in layer 5 that show robust transcriptional responses to oxycodone. Using FosTRAP mice, we 'tagged' opioid-responsive neurons DP, and optogenetic stimulation of this DP ensemble produced aversion-related behaviors that were blocked by oxycodone. Further, optical stimulation of DP in opioid-dependent mice enhanced naloxone-precipitated withdrawal symptoms. Whole-brain projection mapping revealed opioid-responsive DP neurons project to parabrachial nucleus (PBN), and optical stimulation of DP-PBN circuit was aversive. Discussion: The DP is a major prefrontal site that regulates opioids reward and dependence.

Background: Allelic variation in *HHIP*, which encodes Hedgehog-interacting protein, confers risk for chronic obstructive pulmonary disease (COPD) and lung cancer, the major causes of which is tobacco smoking. However, underlying mechanisms of HHIP-regulated disease vulnerability are unclear. Medial habenular (mHb) cholinergic neurons that project to the interpeduncular nucleus (IPn) regulate aversive behavioral responses to nicotine that protect against tobacco addiction. Little is known about the nicotine-evoked cellular or molecular adaptations in these neurons that influence the development of the smoking habit. Rationale: We hypothesized that nicotine-responsive genes in mHb cholinergic neurons may regulate nicotine reinforcement and vulnerability to tobacco addiction and smoking-related diseases. Results: Using *in vivo* calcium

imaging and single-cell RNA sequencing, we found that a dose of nicotine that stimulates mHb neural activity evokes robust transcriptional plasticity in neuronal and non-neuronal cells in mHb, including upregulated expression of *Hhip* in putative cholinergic neurons. Using Translating Ribosome Affinity Purification (TRAP) sequencing and RNAscope, we confirmed that *Hhip* transcripts are highly enriched in mHb cholinergic neurons. Moreover, *in vivo* CRISPR/Cas9-mediated genomic cleavage of *Hhip* in the mHb reduced the activity of the mHb-IPn circuit and attenuated noxious responses to nicotine. Habenular *Hhip* cleavage also increased intravenous nicotine self-administration behavior in mice. Discussion: These findings suggest that *HHIP* may act in the habenula to regulate sensitivity to the addiction-relevant behavioral actions of nicotine and that *HHIP* alleles may increase vulnerability to smoking-related diseases by enhancing the addictive properties of tobacco.