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Dorsal Peduncular Prefrontal Cortex contains cells uniquely sensitive to opioids: Relevance to opioid reward and addiction.

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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. We chose to further examine the dorsal peduncular area (DP), the ventral-most component of the medial prefrontal cortex (mPFC). Single-cell RNA-sequencing, qPCR, and RNAscope revealed unique properties of opioid-responsive DP neurons relative to surrounding mPFC. The DP is enriched in *Oprm1* (μ opioid receptor) 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 several mid- and hindbrain sites with known involvement in aversive behaviors, including the parabrachial nucleus (PBN). Optical stimulation of DP-PBN circuit recapitulated the aversive phenotype seen during DP somatic stimulation. Discussion: The DP is a major prefrontal site that regulates opioids reward and dependence.