



**Supplementary information, Fig. 4 Retrograde labeling of D1<sup>NAc-VM</sup> and D1<sup>NAc-VP</sup> neurons in the NAc by CTB.**

**a**, Schematic of CTB labeling in *D1-tdTomato* and *D2-eGFP* mice. **b**, Schematic of sites imaged for quantification across the rostro-caudal gradient of NAc. **c,g**, Confocal images for injections of CTB647 into the VM and CTB488 into the VP of *D1-tdTomato* mice, and injections of CTB647 into the VM and CTB555 into the VP of *D2-eGFP* mice. **d,h**, Confocal images for CTB labeled neurons in the NAc of *D1-tdTomato* (**d**) and *D2-eGFP* mice (**h**). Scale bar: 100  $\mu$ m. **e,i**, Bar graph shows average proportion of CTB647 and CTB488/555 neurons relative to total tdTomato<sup>+</sup> or eGFP<sup>+</sup> retrograde labeling neurons in the NAc [tdTomato<sup>+</sup>: VM: 58.7%  $\pm$  4.1%, VP: 48.0%  $\pm$  4.4%, Dual: 6.7%  $\pm$  0.4%, n = 6 mice; eGFP<sup>+</sup>: VM: 55.7%  $\pm$  2.2%, VP: 50.2%  $\pm$  2.8%, Dual: 5.9%  $\pm$  0.8%, n = 5 mice; **h**, CTB647: 789  $\pm$  77 cells, CTB555: 700  $\pm$  45 cells of 4 slices per mouse]. **f,j**, Distribution of CTB-labeled tdTomato<sup>+</sup> or eGFP<sup>+</sup> neurons in the whole NAc, NAc core, dmShell, and vmShell correspondingly across NAc rostro-caudal gradient. **k**, Schematic of viral strategy for dual-projection labeling of VP projecting and VM projecting NAc neurons in the same animal. **l**, Confocal images for *drd1* expression, tdTomato and eGFP labeled neurons in the NAc. Scale bar: 100  $\mu$ m. **m**, Left: quantification of *drd1*<sup>+</sup> tdTomato<sup>+</sup> and *drd1*<sup>+</sup> eGFP<sup>+</sup> cell counts in the NAc. Right: summary of the number of tdTomato<sup>+</sup> D1-MSNs, eGFP<sup>+</sup> D1-MSNs and double positive D1-MSN in the NAc [VM = 406  $\pm$  72 cells, VP = 420  $\pm$  45 cells, Dual = 45  $\pm$  8 cells of 4 slices per mouse]. \**p* < 0.05. **n**, Bar graph shows proportion of *drd1*<sup>+</sup> tdTomato<sup>+</sup> and *drd1*<sup>+</sup> eGFP<sup>+</sup> neurons relative to total retrograde labeling D1-MSNs in the NAc [VM: 49.1%  $\pm$  1.8%, VP: 56.8%  $\pm$  2.2%, Dual: 5.8%  $\pm$  1.1%, n = 5 mice]. **o**, Distribution of retrograde labeling D1-MSNs across

NAc rostro-caudal gradient. Numbers represent number of labeled cells per mouse. Related to Figure 4.