Opposing Roles of Rapid Dopamine Signaling Across the Rostral-Caudal Axis of the Nucleus Accumbens Shell in Drug-Induced Negative Affect

Supplemental Information

Supplemental Methods and Materials

Histology Methods

Rats were heavily anesthetized with ketamine/xylazine and transcardially perfused with phosphate-buffered saline followed by 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde for 6 hours at room temperature or 20 hours at 4℃. Next, brains were stored in 20% sucrose dissolved in 0.1M phosphate buffer for 24 hours at 4℃ and then frozen and sectioned at 40 µm on a cryostat. Tissue was either immediately mounted onto slides or stored in cryoprotectant at -20℃ for later mounting. A subset of sections were stained for TH immunoreactivity. Briefly, free-floating sections were exposed to a TH antibody (Abcam ab112, 1:1000) in 0.3% triton-x phosphate buffered saline for 20 hours at room temperature. Afterwards, sections were washed and incubated with an Alexa Fluor 594 secondary antibody (Jackson Immunoresearch, 1:200) in 0.3% triton-x phosphate buffered saline for 1 hour. Finally, tissue was washed and then stained with 2% NeuroTrace (435/455) for 1 hour at room temperature. Fluorescence was visualized using a Leiss LSM 800 confocal microscope. Tracts from the optical fiber implants were examined to determine the location of the optical fiber tips along the rostral-caudal extent of the NAc shell based upon stereotaxic coordinates from Paxinos and Watson (1). In some subjects, the optical fibers implanted across the left and right hemisphere of the shell were found to be along different rostral-caudal coordinates. Specifically, one rat in the TG Cocaine Caudal group had optical fiber placements that were placed 0.5 mm apart from each

other while all other rats had placements that were ≤ 0.14 mm apart. In cases where rostral-caudal placement differed across the hemispheres, an average of the coordinates of the two placements was calculated.

Supplemental Results

Histology

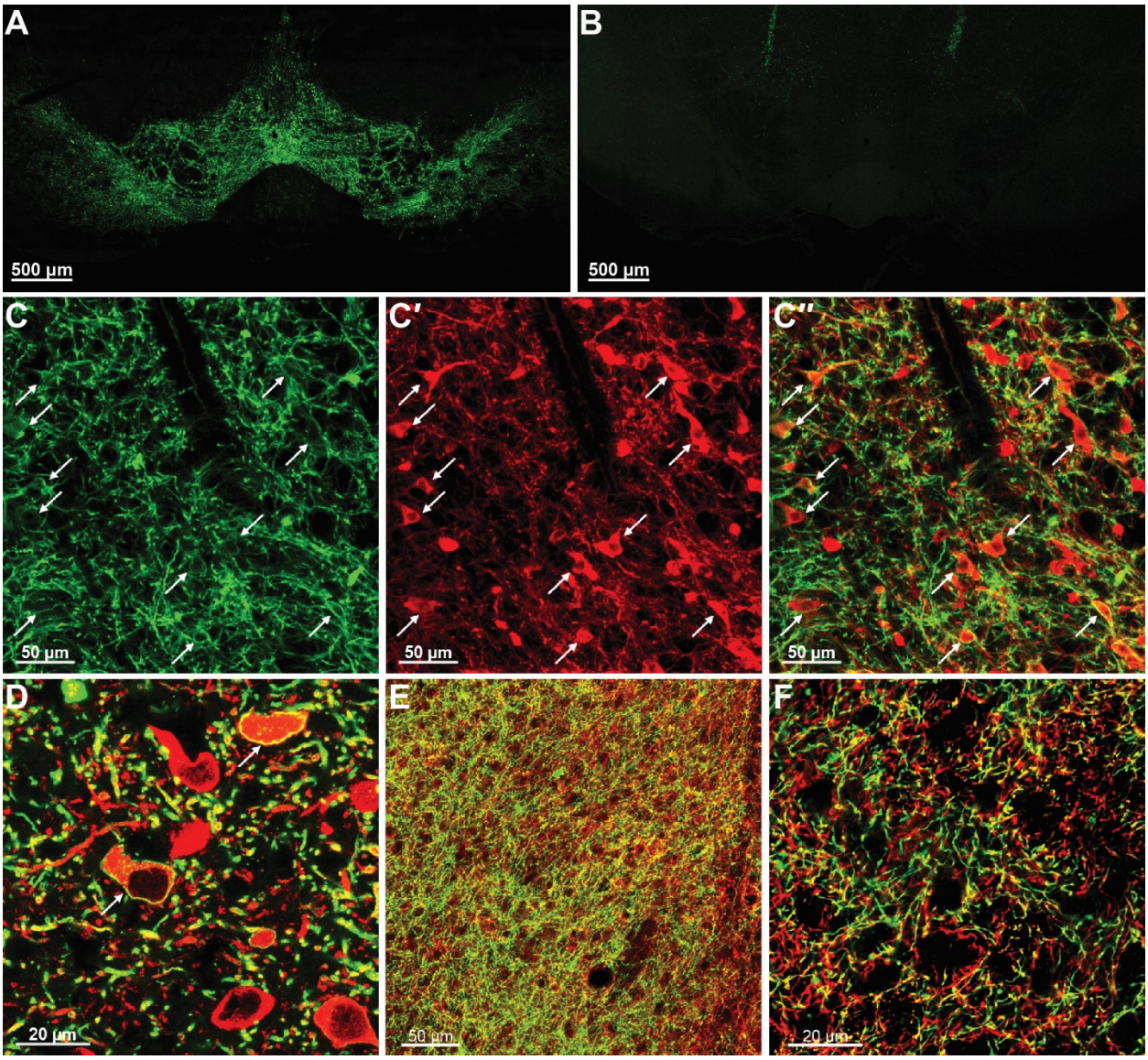
ChR2 expression was observed in both VTA DA cell bodies and terminals in the NAc shell in all TG rats (Supplemental Figure S1). Expression of ChR2 was observed on the membrane of TH positive neurons in the VTA (Supplemental Figure S1A, C-C″, and D) and projections in the NAc shell (Supplemental Figure S1E and SF). ChR2 was not observed in Non-TG rats (Supplemental Figure S1B). Optical fiber tip placements were in the rostral (between +1.9 to +2.4 AP from bregma) or caudal NAc shell (between +0.5 to +1.8 AP from bregma).

No rostral-caudal differences in aversion or self-stimulation in TG Saline rats

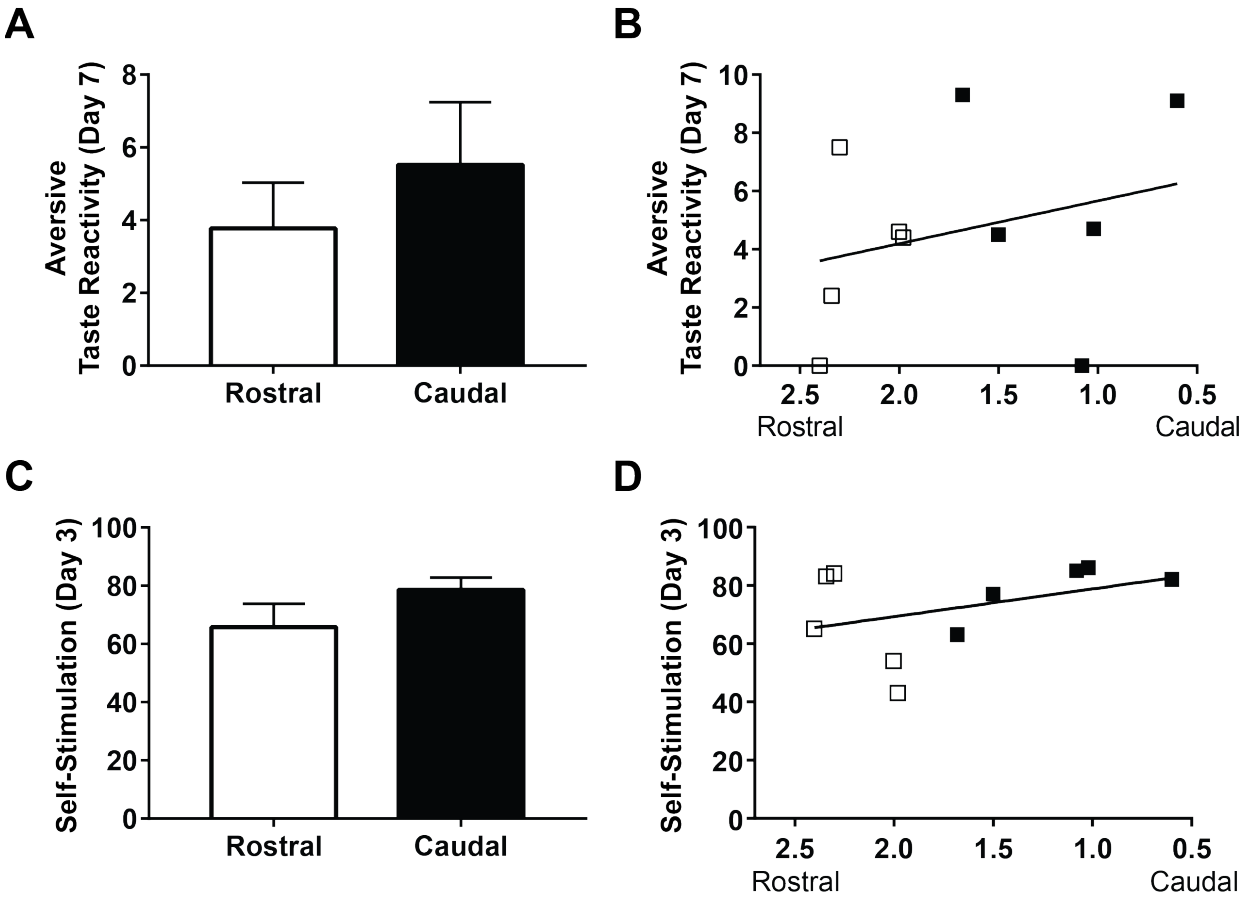
No significant differences were observed in aversion or self-stimulation responding between rats with stimulated DA release in the rostral or caudal shell. That is, TG Saline rats with stimulation in the rostral or caudal shell exhibited identical aversive taste reactivity on day 7 of stimulation [$t(8) = 0.8185$, $p > 0.4$; Supplemental Figure S2A]. Furthermore, no correlation was found between rostral-caudal placement and the degree of aversive taste reactivity [$r(9) = -0.27$, $p > 0.4$; Supplemental Figure S2B]. Similarly, when examining self-stimulation on day 3, we did not find rostral-caudal differences in self-stimulation rates [$t(8) = 1.414$, $p > 0.19$; Supplemental Figure 2C] or a significant correlation between rostral-caudal placement and self-stimulation [$r(9) = -0.39$, $p > 0.25$; Supplemental Figure S2D].

Relationship between negative affect and days 1 and 2 of self-stimulation in TG Cocaine rats

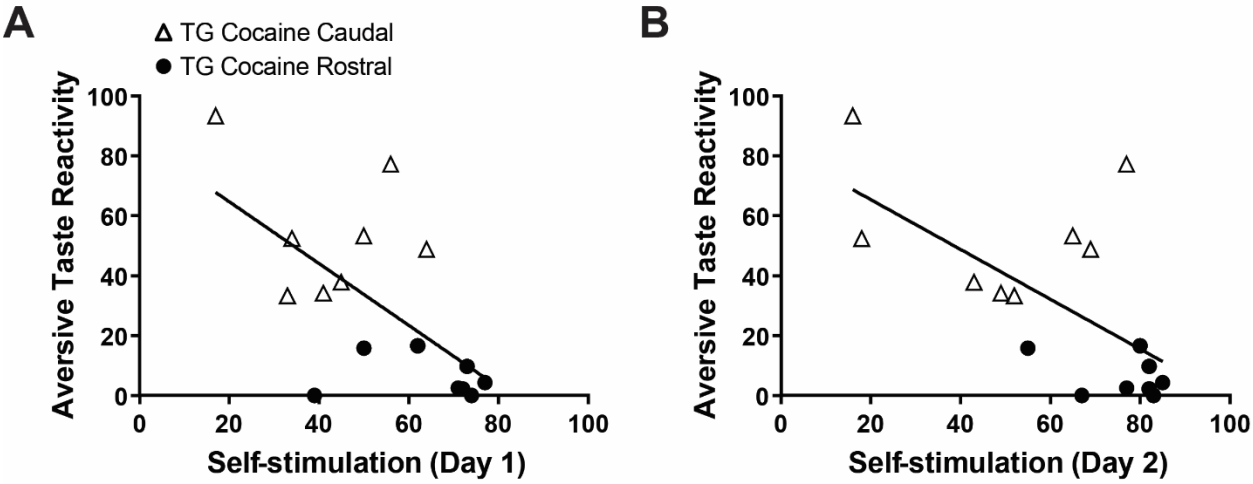
Aversion exhibited by TG Cocaine rats (rostral and caudal) during training day 7 negatively correlated with self-stimulation responding on both days 1 [$r(15) = -0.64$, $p < 0.01$; Supplemental Figure S3A] and 2 [$r(15) = -0.64$, $p < 0.01$; Supplemental Figure S3B].



Supplemental Figure S1. TH::CRE$^{(+/-)}$ rats express ChR2 in VTA DA neurons. Fluorescent labels represent tyrosine hydroxylase (red) and ChR2 (green). Expression of ChR2 was observed in the VTA of TG rats (**A**) and was absent in non-TG controls (**B**). ChR2 was expressed along the membrane of TH positive neurons in the VTA (**C-C′′**, arrows indicate TH positive neurons that express ChR2; a 63x magnification is shown in panel **D**) and projections in the NAc shell (**E & F**).



Supplemental Figure S2. No significant differences were found between rostral-caudal placement and aversion or self-stimulation in TG Saline rats. A comparison of the degree of aversive responses on day 7 in TG saline rats with rostral or caudal stimulation is presented in **A**. A correlation between rostral placement (open squares) and caudal placements (closed squares) in TG saline rats is displayed in **B**. No differences in self-stimulation between rats with rostral and caudal placements was observed on day 3 of self-stimulation (**C**), nor was a correlation found between self-stimulation and rostral-caudal placement (rostral placements – open squares, caudal placements – closed squares, **D**).



Supplemental Figure S3. The degree of aversion exhibited on day 7 of saccharin-cocaine pairings inversely correlated with self-stimulation responding on days 1 and 2. Open triangles represent TG Cocaine Caudal rats and closed circles indicate TG Cocaine Rostral rats.

Supplemental Reference

1. Paxinos G, Watson C (2007): The Rat Brain in Stereotaxic Coordinates. London: Academic Press.