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Supplemental information

Ventral pallidum neurons projecting to the ventral tegmental area reinforce but do not invigorate reward-seeking behavior

Dakota Palmer, Christelle A. Cayton, Alexandra Scott, Iris Lin, Bailey Newell, Anika Paulson, Morgan Weberg, and Jocelyn M. Richard

SUPPLEMENTAL INFORMATION

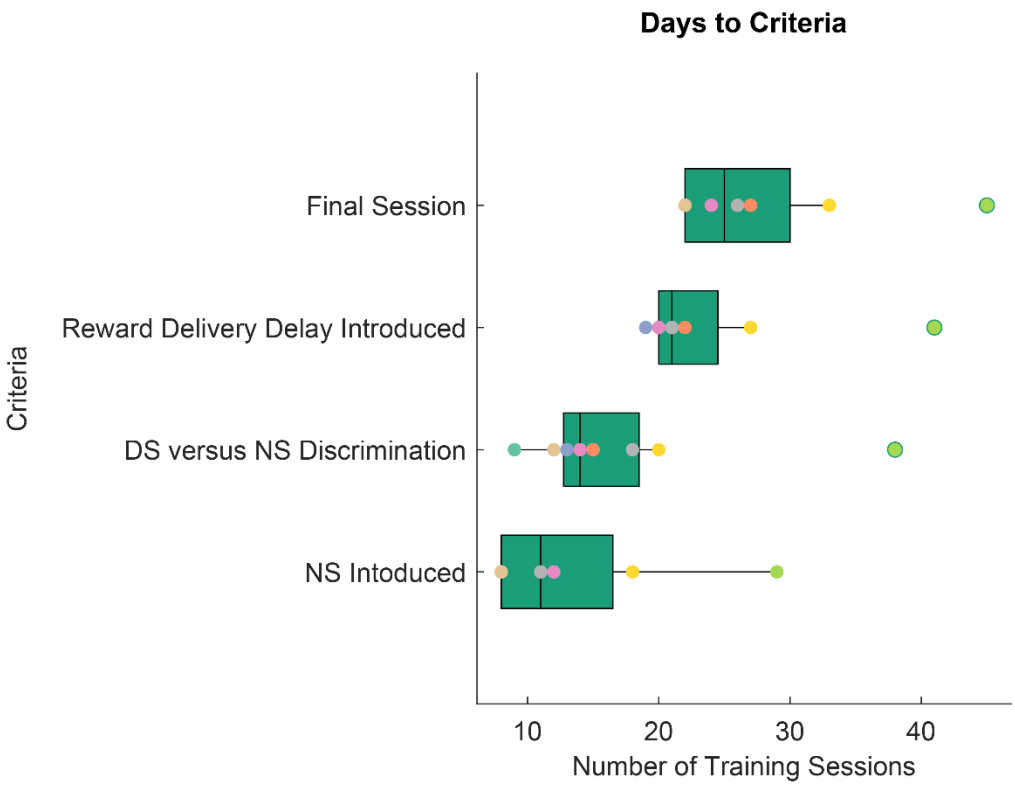

Figure S1. Days to behavioral criteria at each stage of DS task training. Boxplots show the number of training sessions completed prior to the NS introduction, completion of DS versus NS discrimination criteria, the introduction of the reward delivery delay, and the final testing session. Circles represent individual rats. Related to Figure 1.

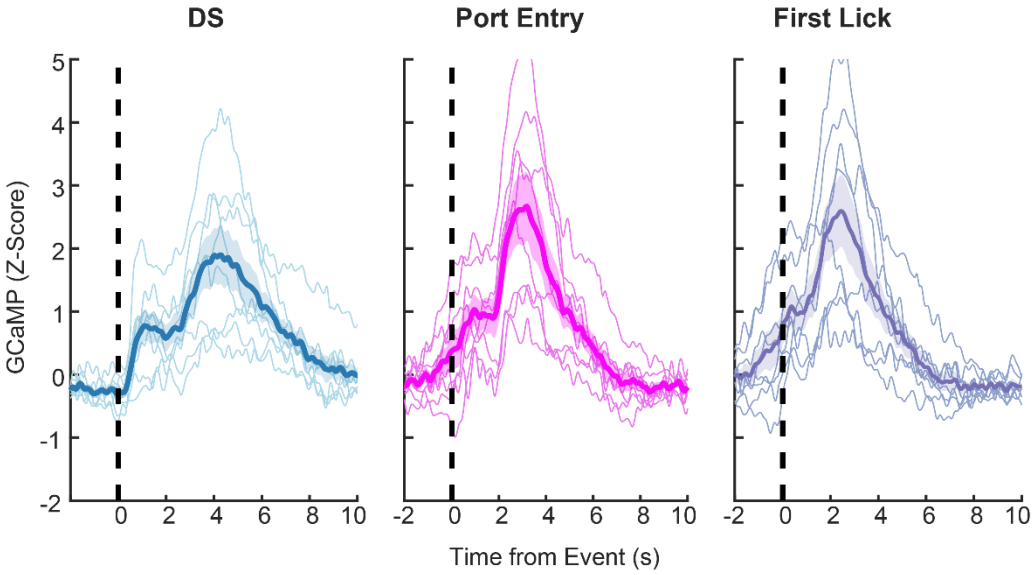

Figure S2. Peri-event VP→VTA calcium traces time-locked to distinct task events. Traces are shown time-locked to the DS (blue), first port entry (pink), and the first lick post-port entry (purple) from sessions with a 1s delay between port entry and reward delivery. Lines with shading indicate between-subjects mean +/- SEM (n=8 rats). Individual points and lines without shading indicate individual subject means. Related to Figure 3.

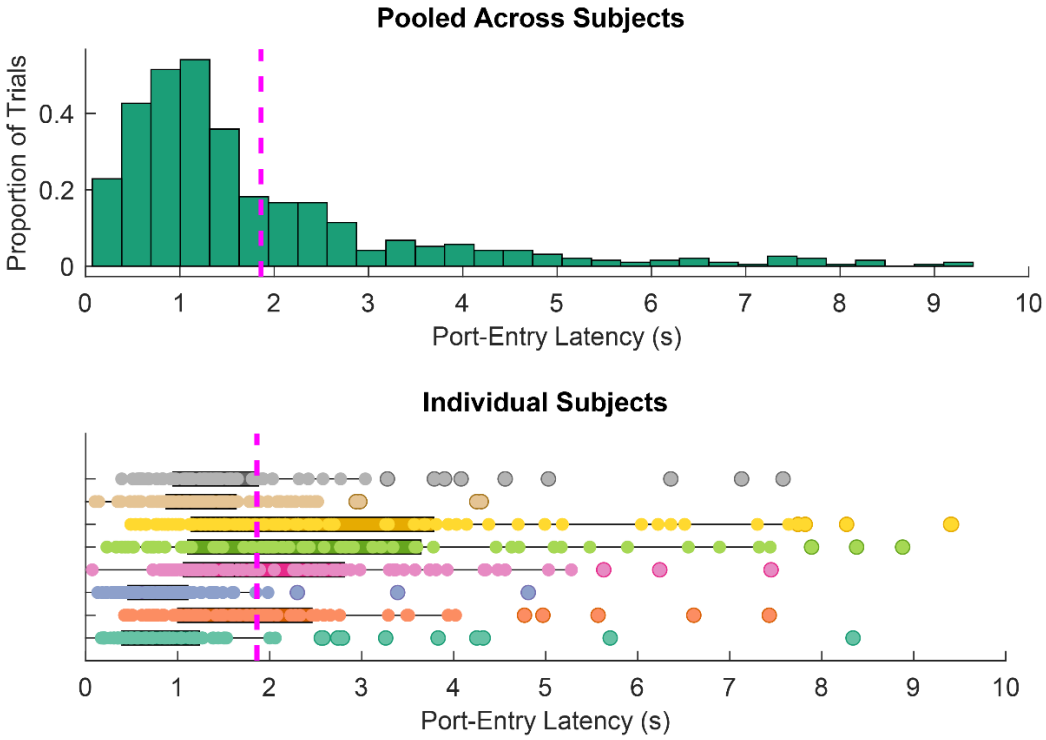

Figure S3. Distribution of port entry latencies. Histogram (top) shows the distribution of latencies pooled across subjects. Separate boxplots (bottom) show the distribution of latency values for individual subjects. Each color is a distinct subject. Circles represent individual trials. The dotted pink line shows the mean port entry latency. Related to Figure 3.

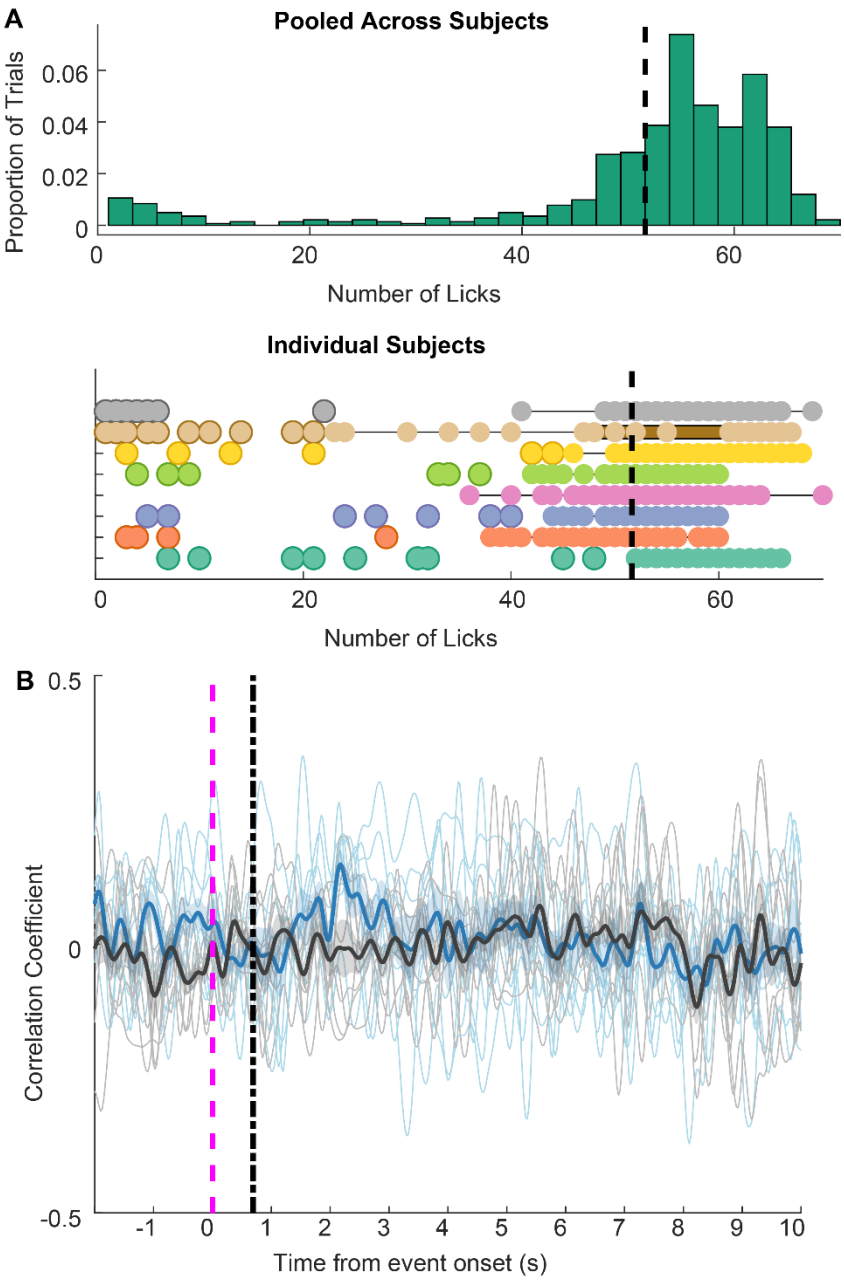

Figure S4. Relationship between post-port entry VP→VTA calcium activity and licking behavior. A) Histogram (top) shows the distribution of licks per trial pooled across subjects. Separate boxplots (bottom) show the distribution of latency values for individual subjects. Each color is a distinct subject. Circles represent individual trials. The dotted black line shows the mean number of licks per trial. B) Correlation between GCaMP signal at each 0.025s time bin sampled post port-entry and lick per trial (blue= true latency, gray= shuffled latency). Pink dashed line indicates port entry onset, and black dashed line represents mean lick latency. Lines with shading indicate between-subjects mean +/- SEM (n=8 rats). Individual points and lines without shading indicate individual subject means. Related to Figure 3.

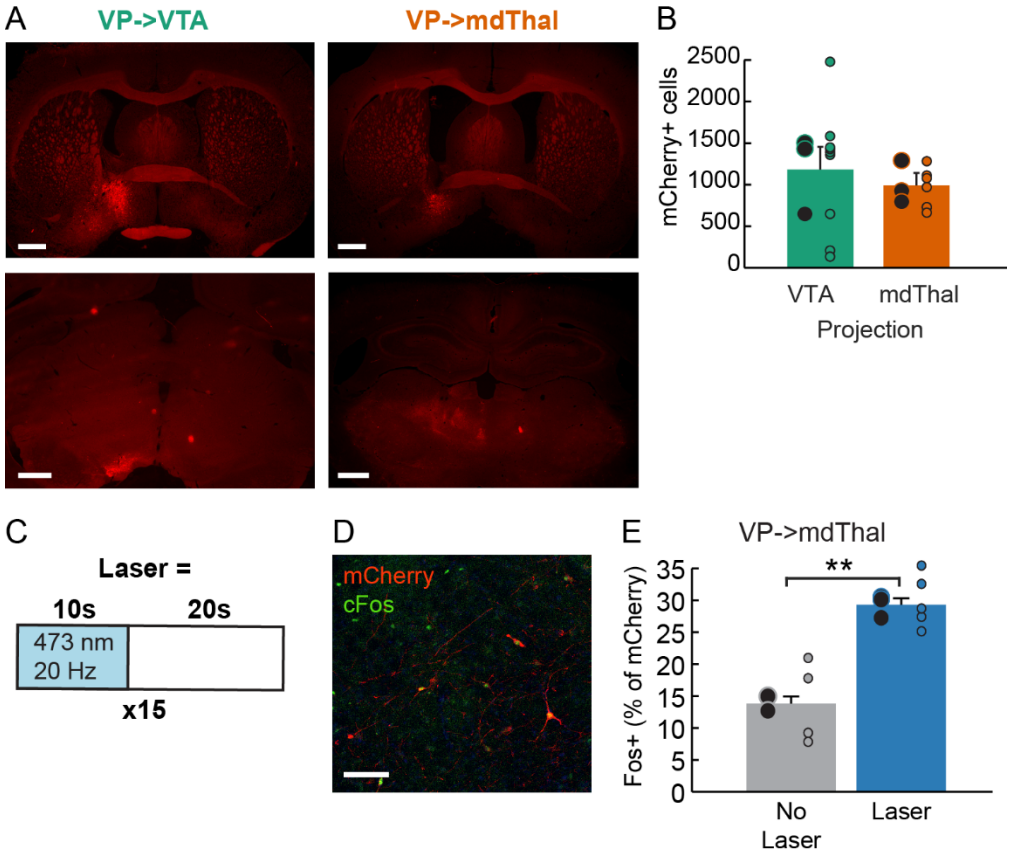

Figure S5. Cell counts and cFos assessment of VP→mdThal. A) Examples of ChR2 expression in VP (top) and terminal regions (bottom) following targeting of VP→VTA (left) and VP→mdThal (right). Scales bars = 1mm. B) Quantification of VP mCherry cells in VP->VTA (9 slices/3 rats; green) versus VP→mdThal (7 slices/3 rats; orange). Cells counts for the projections were not significantly different (unpaired t-tests: animal averages, $t(4) = 0.61$, $p = 0.58$; slices, $t(14) = 0.85$, $p = 0.41$). Data shown as mean +/- SEM; colored circles with black outlines counts from individual slices; black-filled circles are individual rat means. C) Laser stimulation protocol for assessment of cFos in VP→mdThal neurons. D) Example of dual mCherry and cFos immunostaining. Scale bar = 100 μm. E) Quantification of cFos-positive neurons as a % of mCherry-positive VP→mdThal neurons after no laser (4 slices/2 rats; grey) versus laser (5 slices/3 rats). Optogenetic stimulation significantly increased the % of Fos-positive cells (unpaired t-tests: animal averages, $t(3) = 9.85$, $p = 0.0022$; slices, $t(7) = 4.52$, $p = 0.0027$). Data shown as mean +/- SEM; colored circles with black outlines counts from individual slices; black-filled circles are individual rat means. Related to Figures 4, 5 and 6.