

Ventral tegmental area GABA neurons integrate positive and negative valence

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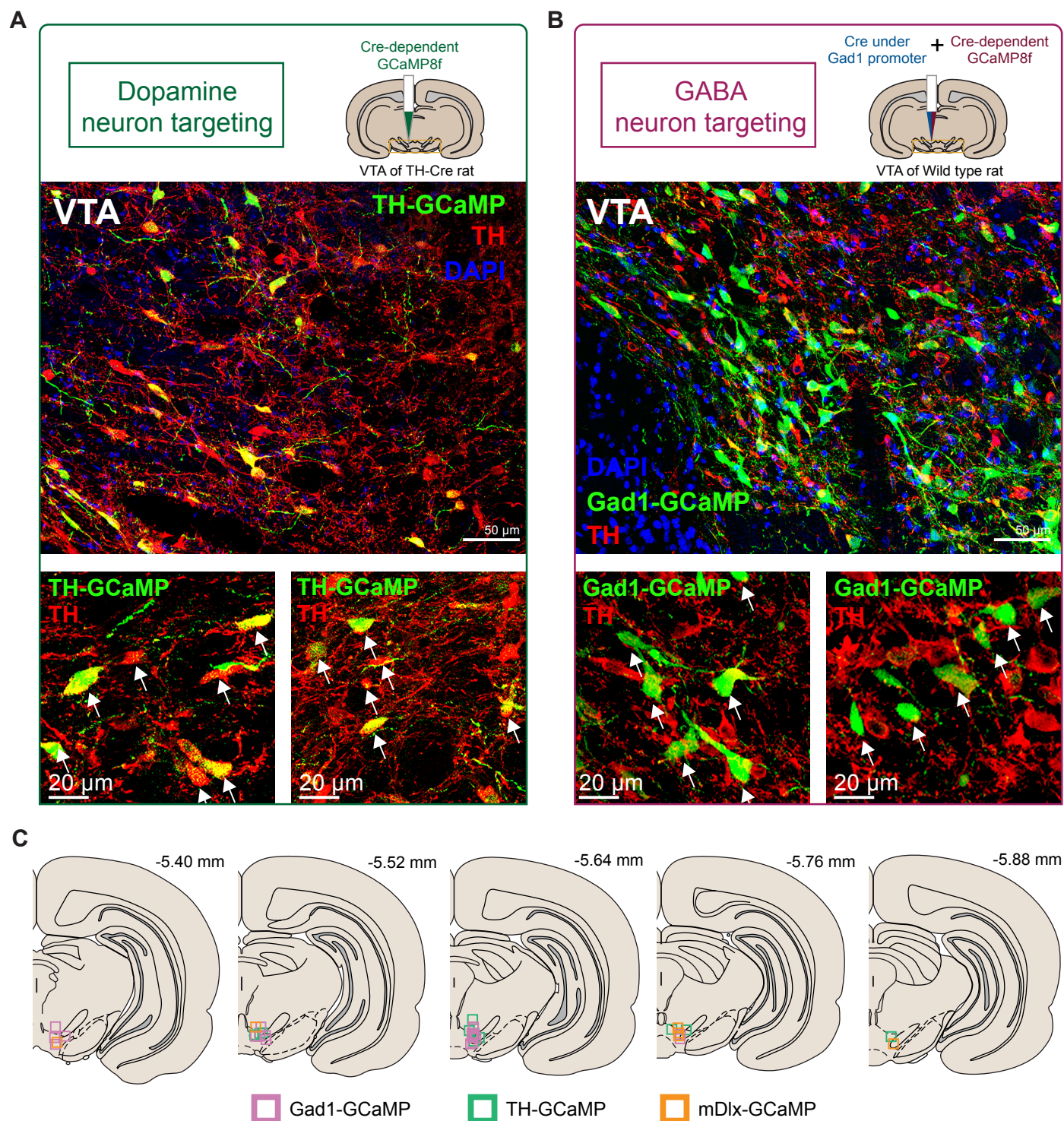
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## **Supplementary Materials**

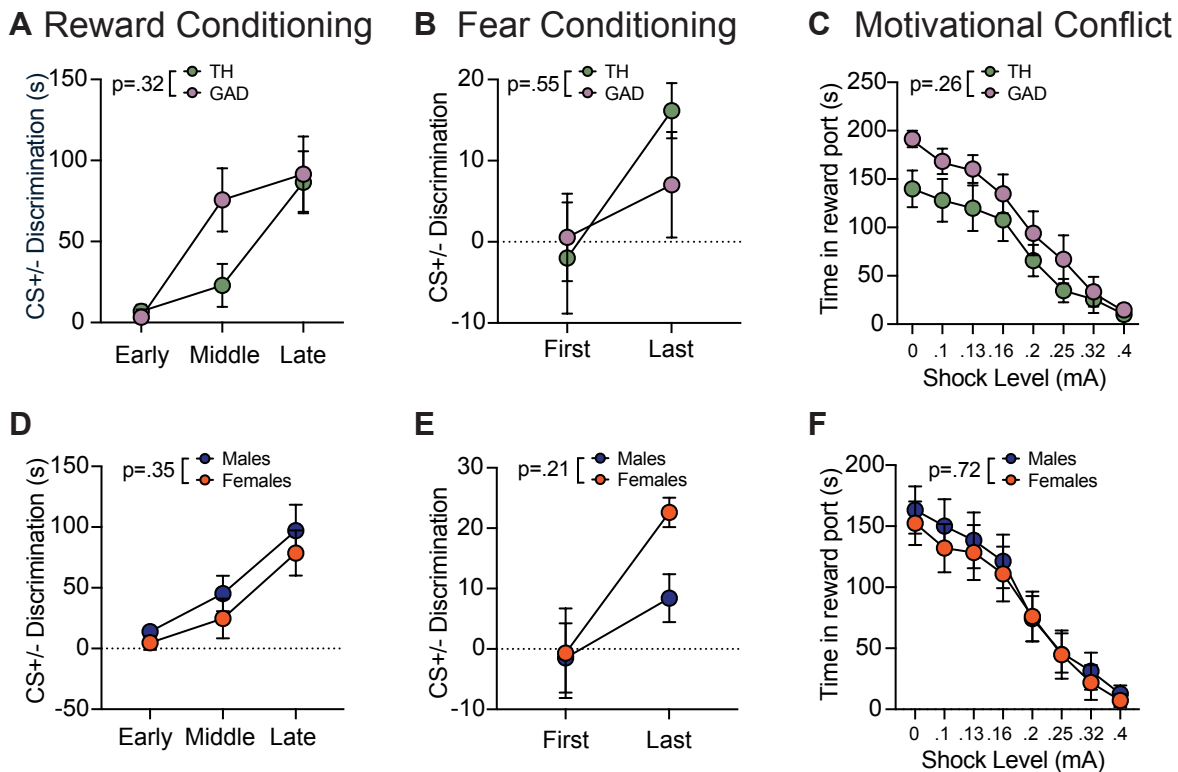
Supplementary Figures 1-5

Supplementary Figures Source Data are provided in the Source Data File



**Supplemental Figure 1. Histology and fiber placements in the VTA for dopamine and GABA targeted GCaMP recordings.**

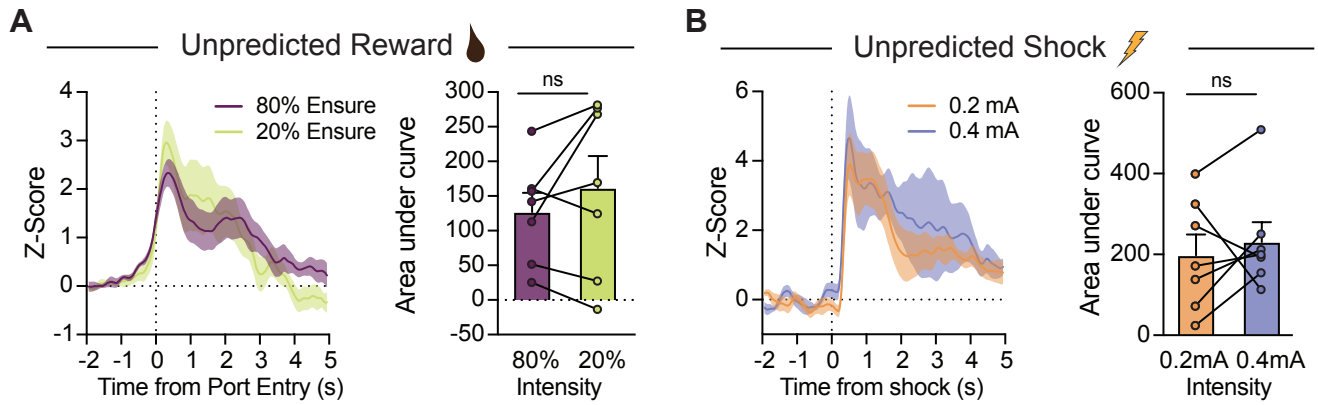
A) Dopamine/TH<sup>+</sup> neurons were targeted with a cre-dependent AAV in TH-cre rats, with good specificity. Bottom, zoom in on example brain images. White arrows highlight GCaMP expression in TH<sup>+</sup> neurons. B) Using a dual-virus approach, GAD1<sup>+</sup> neurons were targeted, resulting in minimal expression in TH<sup>+</sup> neurons in the VTA. Bottom, zoom in on example brain images. White arrows highlight GCaMP expression in neurons that are not TH<sup>+</sup>. C) Optic fiber placements for rats in recording experiments. Pink squares represent GAD1-targeted rats. Green squares represent TH-targeted rats. Orange squares represent mDlx-targeted rats.



**Supplemental Figure 2. Conditioned behavior did not significantly differ across cohorts or sexes.**

A) Gad and TH animals achieved the same level of cue discrimination during reward conditioning as measured by the difference in reward port duration between the CS+ and the CS-, indicating comparable learning. B) Gad and TH animals achieved the same level of discrimination during fear conditioning as measured by the difference in percent trials with freezing between the CS+ and the CS-. C) Gad and TH animals similarly decreased their cued responding during motivational conflict as shock intensity escalated, as measured by seconds in the reward port. D) Male and female animals achieved the same level of discrimination during reward conditioning as measured by the difference in reward port duration between the CS+ and the CS-. E) Male and female animals achieved the same level of discrimination during fear conditioning as measured by the difference in percent trials with freezing between the CS+ and the CS-. F) Male and female animals decreased responding similarly during motivational conflict as shock intensity escalated, as measured by seconds in the reward port. All tests are 2-way repeated measures ANOVAs. Data reflect subject mean +/- SEM.

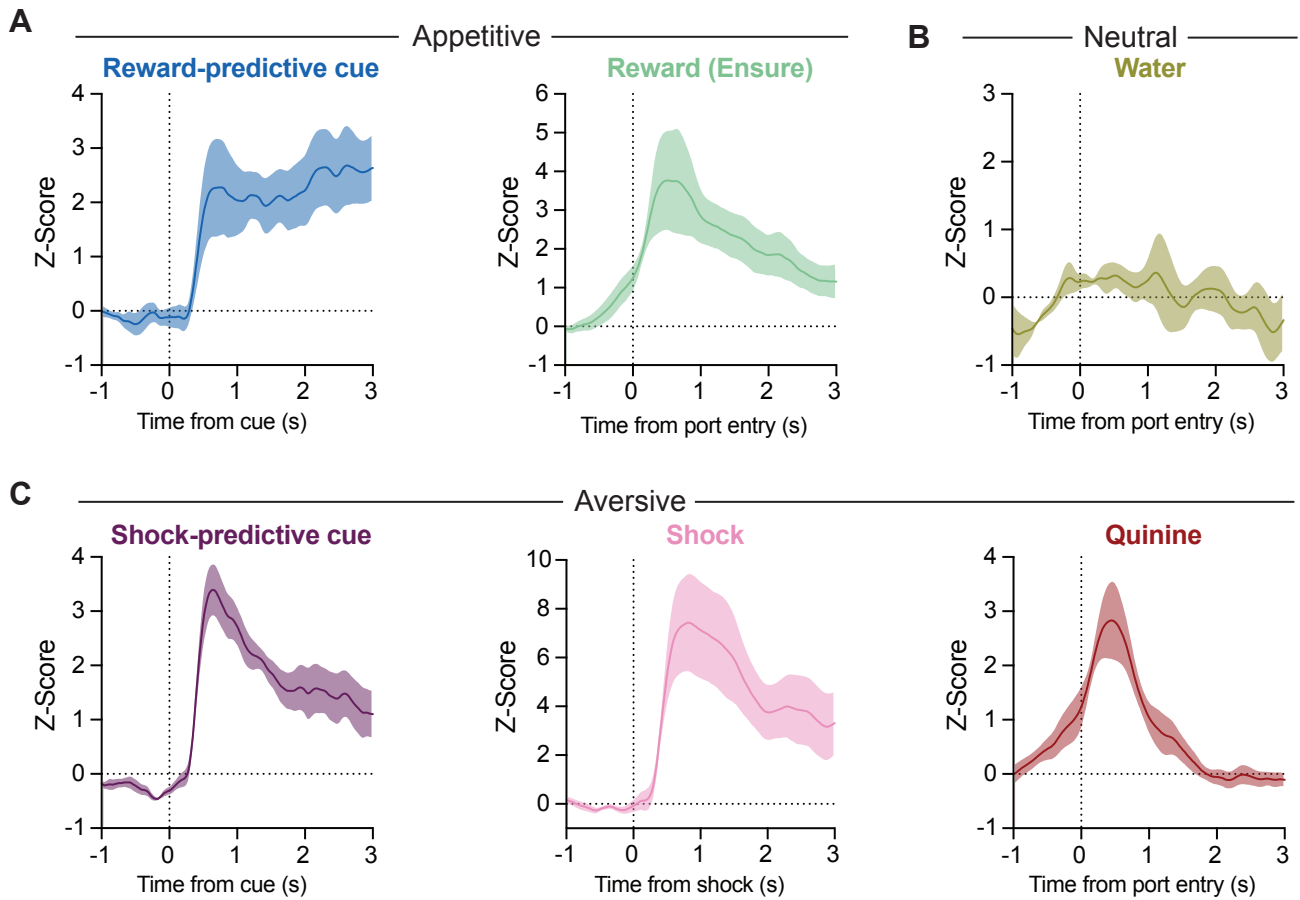
## GABA



**Supplemental Figure 3. VTA GABA neuron activity does not reliably scale with stimulus intensity.**

A) In GAD1+ VTA GABA neurons (n=7) reward consumption evoked similar VTA GABA neuron activity regardless of concentration as measured by AUC (paired t test,  $t(6)=1.18$ ,  $p=.281$ ). B) Footshock evoked similar VTA GABA neuron activity regardless of electrical charge as measured by AUC (paired t test,  $t(6)=0.65$ ,  $p=.542$ ). ns= non significant. Data reflect subjects mean  $\pm$  SEM.

## GABA

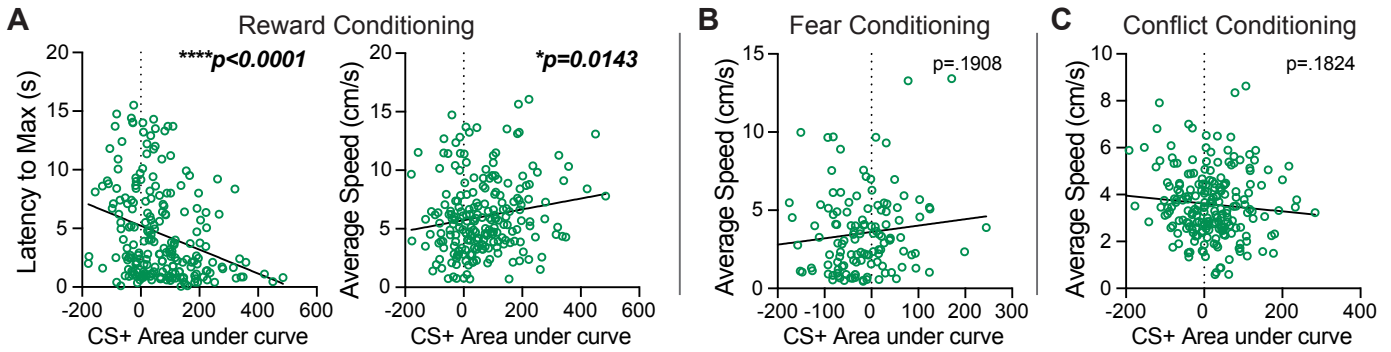


**Supplemental Figure 4. VTA mDlx GABA neurons are activated by emotionally salient stimuli of different valences and modalities.**

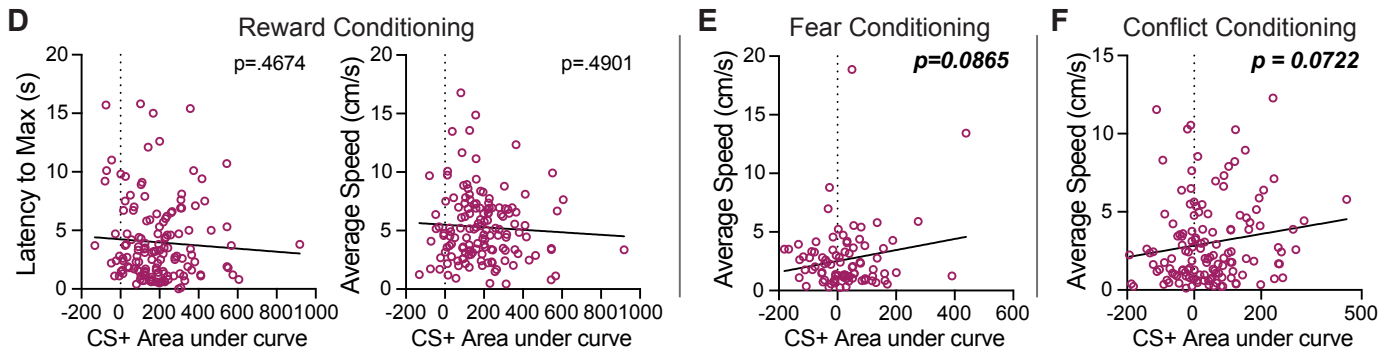
GCaMP was targeted to another VTA GABAergic population, mDlx+ neurons, followed by photometry recordings during and reward and fear conditioning (n=6). A) Reward and reward-predictive cues consistently activated mDlx+ GABA VTA neurons. B) Water consumption, in contrast, did not engage mDlx+ neurons. C) Shock and shock-predictive cues consistently activated mDlx+ neurons, and this population was also activated by bitter quinine drinking. Data reflect subjects mean  $\pm$  SEM.



## Dopamine



## GABA



**Supplemental Figure 5. Cue-evoked VTA dopamine and GABA neuron activity differentially predicts movement vigor across appetitive and aversive learning contexts.**

A-F) Trialwise Pearson correlations between the peak cue-evoked signal and speed for reward conditioning, fear conditioning, and conflict conditioning. A) Cue-evoked dopamine neuron activity was negatively correlated with the latency to reach maximum speed and positively correlated with average speed for reward conditioning, but was not correlated with speed for B) fear or C) conflict conditioning. D) Cue-evoked GABA neuron activity was not correlated with speed for reward conditioning, but there was a trend for positive correlations with speed for E) fear and F) conflict conditioning.