

## Supplementary Discussion

In this paper, we detail a glutamatergic pathway between the dorsopeduncular nucleus and the medial subdivision of the central amygdala (CeM) that is vital for top-down control of conditioned flight behavior. Interestingly, we found that this pathway is comprised of two populations of glutamatergic neurons, one expressing Vglut1 and the other expressing Vglut2. Optogenetic stimulation of Vglut1+ terminals in the CeM induced real-time place preference and reduced conditioned freezing, suggesting a positive valence function while optogenetic stimulation of Vglut2+ terminals in the CeM induced real-time place avoidance and increased conditioned flight responses, demonstrating a role in negative valence processing.

These findings add to a growing literature that is investigating the role of the central amygdala in affective valence processing, both positive and negative. Through valence processing, central amygdala circuits can drive appropriate approach or avoidance behaviors that promote survival. The CeM is viewed as the major output of the central amygdala that drives behavioral, physiological, and endocrine responses to stimuli of either valence. While extensive work has identified genetically defined neuronal populations encoding valence in other subdivisions of the central amygdala, the neuronal substrates in the CeM that control approach or avoidance are largely unknown. One cell type important for avoidance may be CeM neurons expressing tachykinin 2/neurokinin B, because these neurons are vital for fear memory consolidation.<sup>1</sup> Approach could be mediated by CeM neurons expressing neurotensin, somatostatin, or tachykinin2, as mice will nose poke for optogenetic self-stimulation of these neuronal populations.<sup>2</sup> The CeM also mediates various aspects of predatory hunting behavior through distinct output pathways,<sup>3</sup> raising the possibility that approach behavior is controlled through an intersection of genetic identity and projection target. Interestingly, in the current manuscript, we identify numerous projection targets of CeM neurons innervated by the DP (see Extended Data Figure 10). It is likely that the decision to approach or avoid is dependent on activation of CeM neurons defined by their genetic makeup and projection targets.

### References:

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