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Peer Review

Review of: "GABAergic Neurons from the Ventral Tegmental Area Represent and Regulate Force Vectors"

Karolina Farrell[1]

1. University College London, University of London, United Kingdom

The authors present a series of experiments that examine the role of VTA GABA neurons in mice in encoding and modulating forward and backwards head movements while headfixed via a force sensor. They use a moveable reward spout in a Pavlovian conditioning paradigm to induce the mice to move their head forwards and backwards. They show how VTA GABA neurons show differential responses to forward and backward head motion, which they classify into four groups: forward+, backward+, bidirectional+, and bidirectional-. These groups reflect neurons' positive or negative responses to either forward or backward motion or both. This tuning remains consistent when the mice are instead presented with an airpuff to the face or during spontaneous movement outside of the conditioned task. Optogenetic activation of these neurons stops force exertion and licking and does not appear to impact performance on the subsequent trial. These data are convincing for the argument that VTA GABA neurons encode force exertion, however, I have concerns about some of the interpretations regarding VTA GABA neurons' role in encoding force *generation* and *not* reward prediction. I offer suggestions for further clarity and discussion of limitations could help improve the manuscript.

Major

- I am concerned about the efficiency of the optotagging procedure. So few neurons are effectively optotagged, and the majority of the manuscript seems to focus on putative GABAergic neurons rather than optotagged neurons. I also wonder how this impacts the optogenetic experiments, which the authors rely on heavily to make their argument. Relatedly, it would be good to clarify exactly which neurons are being examined in each figure and why the optotagged neurons are not shown except for an example neuron for each cluster in figS3. The rasters are hard to read and appear less convincingly tuned than the putative GABA neurons.

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- In my opinion, the experiments are not designed to distinguish between reward prediction and force encoding. The authors assume that reward prediction is a static, perhaps binary, variable that is read out in licking behaviour, while not directly testing for reward prediction in their experiments. It is also argued that because there are different clusters of responses that correlate with force direction, this is evidence against reward prediction. Given that heterogeneity in neural responses is seen across many brain regions including VTA, this seems a weak argument against reward prediction coding.
- I'm unclear exactly how the reward omission experiments were done as the main text and methods say different proportions of omission trials. It is also unclear whether the reward expectation has been extinguished or not in the omission trials being considered or whether they have all been pooled together – reduced force exertion in figS8 suggests some loss of reward expectation but licking remains in fig7 - a better experiment would have used a separate CS for the omission trials to ensure that there was no reward prediction during these trials. I would be cautious about interpreting the results from these experiments without clarifying which trials are being analysed and how the authors know whether the mice are still expecting reward (indeed, if they are overtrained there may be habitual responses?)
- The discussion argues that some neurons' responses leading the force exertion suggests involvement in force *generation*. The authors then say that their optogenetic experiment directly shows this role, but they only show cessation of force exertion and licking i.e. necessity rather than sufficiency. I think it is very interesting that they do not show any immediate learning effect on the subsequent trial, but I would caveat that this doesn't exclude the potential for build-up of effect across trials or compensation within trial once the laser is turned off through rebound activity. This type of stimulation is also not physiological and it would be good to discuss this.

Minor

- VTA GABA neurons encompass different kinds of neurons, some being local interneurons, some being projection neurons, and others being glutamate/GABA co-expressing, as the authors note in their Discussion. There is no investigation of which neurons have been targeted here, which could result in heterogeneous neuronal representations being pooled. This is related to the optotagging issues and the targeting in S12 also appears to be quite variable with some recording sites being on the border of VTA or potentially in the decussation/rostral linear nucleus/ventral tegmental relay zone/interfascicular nucleus.

- Fig 2 legend is missing panel K
- Fig S4 shows the variability in force exertion within a session – it would be interesting to see if this correlates with time in session (do the mice get tired? Or is it correlated with attentiveness?) It's also interesting that the bidirectional- neurons that are inhibited by force exertion do not show much change in firing rate, perhaps suggesting a floor effect.
- The fact that the tuning appears preserved in spontaneous movement and with airpuff is very interesting – it might be good to clarify that these are the same neurons in each cluster (I assume?). In my opinion, this is the strongest argument for force exertion representation
- I'm curious why the blanket optogenetic stimulation of VTA GABA neurons stops force exertion and licking when there are the differently tuned neuron clusters – it would be good of the authors to discuss this in more detail. Is it that this stimulation strongly inhibits VTA dopamine neurons, producing an aversive behavioural response? It would also be good in general to discuss how their results match up to VTA dopamine neuron activity beyond force or kinematics encoding.
- I think the conclusions in the discussion are reaching beyond what the data shows and could use some caveats and further discussion of the limitations in this study.

Declarations

Potential competing interests: No potential competing interests to declare.