



Response to “Fallacies of Mice Experiments”

Zhenyu Gao¹ · Alyse M. Thomas² · Michael N. Economo^{3,4} · Amada M. Abrego² · Karel Svoboda³ · Chris I. De Zeeuw^{1,5} · Nuo Li²

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Abstract

In a recent Editorial, De Schutter commented on our recent study on the roles of a cortico-cerebellar loop in motor planning in mice (De Schutter 2019, *Neuroinformatics*, 17, 181–183, Gao et al. 2018, *Nature*, 563, 113–116). Two issues were raised. First, De Schutter questions the involvement of the fastigial nucleus in motor planning, rather than the dentate nucleus, given previous anatomical studies in non-human primates. Second, De Schutter suggests that our study design did not delineate different components of the behavior and the fastigial nucleus might play roles in sensory discrimination rather than motor planning. These comments are based on anatomical studies in other species and homology-based arguments and ignore key anatomical data and neurophysiological experiments from our study. Here we outline our interpretation of existing data and point out gaps in knowledge where future studies are needed.

Main Text

Anatomical studies in macaques suggest a prominent connection between primate frontal cortex and the dentate nucleus via thalamus.¹ In contrast, projections from the fastigial nucleus to the thalamus are thought to be relatively weak.² Therefore, the dentate nucleus is often associated with cognitive functions. However, the degree of homology across species and the strength of projections assessed anatomically do not necessarily correspond to their importance functionally.^{3,4,5}

We began our analysis in the mouse anterior lateral motor cortex (ALM), a brain region that is critically involved in planning of directional tongue movements.^{6,7,8} ALM projects to the cerebellum via the basal pontine nucleus,^{9,10} which sends widespread projections to the cerebellum.^{11,12} We

¹ Middleton, F. A., & Strick, P. L. (2001). Cerebellar projections to the prefrontal cortex of the primate. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, 21, 700–712.

² Thach, W. T., Goodkin, H. P., & Keating, J. G. (1992). The cerebellum and the adaptive coordination of movement. *Annual Review of Neuroscience*, 15, 403–442.

³ Katz, P. S. (2016). Phylogenetic plasticity in the evolution of molluscan neural circuits. *Current Opinion in Neurobiology*, 41, 8–16. <https://doi.org/10.1016/j.conb.2016.07.004>.

⁴ Finlay, B. L., & Darlington, R. B. (1995). Linked regularities in the development and evolution of mammalian brains. *Science*, 268, 1578–1584.

⁵ Barton, R. A., & Harvey, P. H. (2000). Mosaic evolution of brain structure in mammals. *Nature*, 405, 1055–1058. <https://doi.org/10.1038/35016580>.

⁶ Guo, Z. V., et al. (2014). Flow of cortical activity underlying a tactile decision in mice. *Neuron*, 81, 179–194.

⁷ Chen, T. W., Li, N., Daie, K., & Svoboda, K. (2017). A map of anticipatory activity in mouse motor cortex. *Neuron*, 94, 866–879 e864. <https://doi.org/10.1016/j.neuron.2017.05.005>.

⁸ Inagaki, H. K., Inagaki, M., Romani, S., & Svoboda, K. (2018). Low-dimensional and monotonic preparatory activity in mouse anterior lateral motor cortex. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, 38, 4163–4185. <https://doi.org/10.1523/JNEUROSCI.3152-17.2018>.

⁹ Li, N., Chen, T. W., Guo, Z. V., Gerfen, C. R., & Svoboda, K. (2015). A motor cortex circuit for motor planning and movement. *Nature*, 519, 51–56.

¹⁰ Economo, M. N., et al. (2018). Distinct descending motor cortex pathways and their roles in movement. *Nature*, 563, 79–84. <https://doi.org/10.1038/s41586-018-0642-9>.

¹¹ Suzuki, L., Coulon, P., Sabel-Goedknecht, E. H., & Ruigrok, T. J. (2012). Organization of cerebral projections to identified cerebellar zones in the posterior cerebellum of the rat. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, 32, 10854–10869. <https://doi.org/10.1523/JNEUROSCI.0857-12.2012>.

¹² Mihailoff, G. A., Lee, H., Watt, C. B., & Yates, R. (1985). Projections to the basilar pontine nuclei from face sensory and motor regions of the cerebral cortex in the rat. *The Journal of Comparative Neurology*, 237, 251–263. <https://doi.org/10.1002/cne.902370209>.

✉ Nuo Li
nuoli@bcm.edu

Extended author information available on the last page of the article

examined ALM-cerebellar connections using triple injections in the same brain. A retrograde tracer was injected into ALM to label the ALM-projecting thalamus; two separate anterograde tracers were injected into the fastigial and dentate nucleus to map their projections to thalamus. The anatomical data show clearly that fastigial projections selectively target the ALM-projecting thalamus (primarily VM), whereas the dentate neurons project mostly to regions of VAL that do not project to ALM (Gao et al. Extended Data Fig. 8).

Electrophysiological data further support the anatomical data. Inactivation of ALM abolished movement-selective preparatory activity in the fastigial nucleus. Thus ALM drives preparatory activity in the fastigial nucleus. Conversely, photoactivation of the fastigial nucleus, but not the dentate nucleus, destroyed preparatory activity in ALM. Therefore, ALM and the fastigial nucleus, form a selective cortico-cerebellar loop that is segregated from loops in which the dentate nucleus participates.

Furthermore, these findings may not be at odds with the primate studies cited by De Schutter. A recent study in non-human primates found that the cerebellar vermis, which provides input to the fastigial nucleus, receive input from the motor cortex via the pons.¹³ In addition, anterograde tracing studies have identified fastigial projections to the thalamus.¹⁴ But which part of the frontal cortex the fastigial output targets via thalamus remains unclear. In rodents¹⁵ and dogs,¹⁶ the fastigial nucleus projects to parts of VM, consistent with the results in Gao et al. More quantitative comparative anatomy is needed to bridge the anatomical organizations of cortico-cerebellar connectivity in rodent and primate brains.

With regard to the fastigial function, citing fastigial roles in axial and proximal motor control, De Schutter argues that the involvement of the fastigial nucleus in the behavioral task used in Gao et al. is primarily in tactile discrimination rather than motor planning.

We do not find support in our data for this claim. To investigate brain regions involved in motor planning we used a standard delayed response task modified from primate studies.^{17,18,19,20,21,22} Mice judge the location of a tactile stimulus using their whiskers and report their choice using directional licking. Importantly, an intervening delay period separates the sensory stimulus and the time when mice are instructed to respond. The intervening delay thus separates sensation and motor response in time and provides a window of time during which neural activity related to motor planning may be examined.^{8,11,23} In Gao et al., fastigial involvement in the behavior was probed using temporally specific optogenetic manipulations during individual epochs of the behavior. Optogenetic manipulation experiments, including both neuronal activation and inactivation, produced the largest effects when perturbing the fastigial activity during the delay and response epochs, as compared to the sample epoch (see Gao et al. Figs. 1 h and 1 k, Extended Data Figs. 1e and 2 g). Importantly, the same manipulations in the dentate nucleus did not produce any detectable effect in the delayed response task. These results are consistent with the view that the fastigial nucleus plays a role in preparing and initiating directional tongue movement.

Electrophysiological data further support this interpretation: movement-selective preparatory activity is observed in the fastigial nucleus and is strongest during the delay and response epochs. The time course of the optogenetic manipulation behavioral effect size matched the time course of neuronal selectivity (Compare Gao et al. Figs. 1 and 2).

Our studies do not exclude potential involvements of the fastigial nucleus in other aspects of the behavior, including tactile discrimination. We note that some behavioral effect was induced by perturbing fastigial activity during the late sample epoch. The fastigial

¹³ Coffman, K. A., Dum, R. P., & Strick, P. L. (2011). Cerebellar vermis is a target of projections from the motor areas in the cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 16068–16073. <https://doi.org/10.1073/pnas.1107904108>.

¹⁴ Asanuma, C., Thach, W. R., & Jones, E. G. (1983). Anatomical evidence for segregated focal groupings of efferent cells and their terminal ramifications in the cerebellothalamic pathway of the monkey. *Brain Research*, *286*, 267–297.

¹⁵ Hintzen, A., Pelzer, E. A., & Tittgemeyer, M. (2018). Thalamic interactions of cerebellum and basal ganglia. *Brain Structure & Function*, *223*, 569–587. <https://doi.org/10.1007/s00429-017-1584-y>.

¹⁶ Sakai, S. T., & Patton, K. (1993). Distribution of cerebellothalamic and nigrothalamic projections in the dog: A double anterograde tracing study. *The Journal of Comparative Neurology*, *330*, 183–194. <https://doi.org/10.1002/cne.903300204>.

¹⁷ Tanji, J., & Evarts, E. V. (1976). Anticipatory activity of motor cortex neurons in relation to direction of an intended movement. *Journal of Neurophysiology*, *39*, 1062–1068.

¹⁸ Riehle, A., & Requin, J. (1989). Monkey primary motor and premotor cortex: Single-cell activity related to prior information about direction and extent of an intended movement. *Journal of Neurophysiology*, *61*, 534–549.

¹⁹ Seidemann, E., Zohary, E., & Newsome, W. T. (1998). Temporal gating of neural signals during performance of a visual discrimination task. *Nature*, *394*, 72–75. <https://doi.org/10.1038/27906>.

²⁰ Bisley, J. W., Zaksas, D., & Pasternak, T. (2001). Microstimulation of cortical area MT affects performance on a visual working memory task. *Journal of neurophysiology*, *85*, 187–196.

²¹ Afshar, A., et al. (2011). Single-trial neural correlates of arm movement preparation. *Neuron*, *71*, 555–564. <https://doi.org/10.1016/j.neuron.2011.05.047>.

²² de Lafuente, V., & Romo, R. (2005). Neuronal correlates of subjective sensory experience. *Nature Neuroscience*, *8*, 1698–1703.

²³ Svoboda, K., & Li, N. (2017) Neural mechanisms of movement planning: Motor cortex and beyond. *Current opinion in neurobiology* In press.

nucleus is known to control proximal body parts, including eye movement^{24,25} and licking.²⁶ The fastigial nucleus also receives input from Purkinje cells that respond to whisking and touch.^{27,28,29} It is conceivable that the fastigial nucleus could play roles in integrating whisker touch into motor plans that instruct proximal body parts.^{30,31} The dentate nucleus on the other hand, may be recruited during motor planning in other motor behaviors.

Citing a recent study on the involvement of the dentate nucleus in a visually-guided behavior,³² De Schutter suggests that changing the modality of the sensory stimulus (e.g., from tactile to visual) would cease the involvement of the fastigial nucleus in the delayed response task, and instead cause the dentate nucleus to be involved. We find this unlikely for two reasons. First, the involvement of the dentate nucleus in the delayed response task was clearly ruled out by multiple experiments (e.g., lesion, optogenetic photoactivation). Second, important task differences between Chabrol et al. and Gao et al. should be taken into consideration. In Chabrol et al., mice ran through a virtual corridor, and based on visual stimulus mice stopped running and licked for anticipated reward. Licking and locomotion were strongly anti-correlated. Mice therefore likely planned and coordinated running and licking. In Gao et al., mice sat in a tube and they were primarily

concerned with licking. The fastigial nucleus forms a cortico-cerebellar loop with ALM through VM thalamus, whereas the dentate nucleus targets somatic M1 through VAL thalamus. Rather than a single deep cerebellar nucleus performing motor planning, it is more likely that different regions of the cerebellum interact with distinct regions of the frontal cortex during different motor behaviors through parallel cortico-cerebellar loops.³³

De Schutter acknowledges that the findings in Gao et al. are in line with a growing body of literature that supports the role of the cerebellum beyond online motor control.^{33,34,35,34,35,36,37,38,39,40,41,42,43,44,45} The functions of the cerebellum in nonmotor behaviors are just beginning to be elucidated. Resolving the functional organization of cortico-cerebellar loop underlying different forms of cognitive behaviors will be an important area of future research.

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²⁴ Robinson, F. R., Straube, A., & Fuchs, A. F. (1993). Role of the caudal fastigial nucleus in saccade generation. II. Effects of muscimol inactivation. *Journal of Neurophysiology*, *70*, 1741–1758.

²⁵ Robinson, F. R., & Fuchs, A. F. (2001). The role of the cerebellum in voluntary eye movements. *Annual Review of Neuroscience*, *24*, 981–1004. <https://doi.org/10.1146/annurev.neuro.24.1.981>.

²⁶ Lu, L., Cao, Y., Tokita, K., Heck, D. H. & Boughter, J. D., Jr. (2013). Medial cerebellar nuclear projections and activity patterns link cerebellar output to orofacial and respiratory behavior. *Frontiers in Neural Circuits* *7*, 56. <https://doi.org/10.3389/fncir.2013.00056>.

²⁷ Bosman, L. W., et al. (2010). Encoding of whisker input by cerebellar Purkinje cells. *The Journal of Physiology*, *588*, 3757–3783. <https://doi.org/10.1113/jphysiol.2010.195180>.

²⁸ Loewenstein, Y., et al. (2005). Bistability of cerebellar Purkinje cells modulated by sensory stimulation. *Nature Neuroscience*, *8*, 202–211. <https://doi.org/10.1038/nm1393>.

²⁹ Chen, S., Augustine, G. J., & Chadderton, P. (2016). The cerebellum linearly encodes whisker position during voluntary movement. *eLife*, *5*, e10509. <https://doi.org/10.7554/eLife.10509>.

³⁰ Proville, R. D., et al. (2014). Cerebellum involvement in cortical sensorimotor circuits for the control of voluntary movements. *Nature Neuroscience*, *17*, 1233–1239. <https://doi.org/10.1038/nn.3773>.

³¹ Deverett, B., Koay, S. A., Oostland, M., & Wang, S. S. (2018). Cerebellar involvement in an evidence-accumulation decision-making task. *eLife*, *7*. <https://doi.org/10.7554/eLife.36781>.

³² Chabrol, F. P., Blot, A., & Mrcic-Flogel, T. D. (2019). Cerebellar contribution to preparatory activity in motor neocortex. *Neuron*, *103*, 1–14. <https://doi.org/10.1016/j.neuron.2019.05.022>.

³³ Strick, P. L., Dum, R. P., & Fiez, J. A. (2009). Cerebellum and nonmotor function. *Annual Review of Neuroscience*, *32*, 413–434. <https://doi.org/10.1146/annurev.neuro.31.060407.125606>.

³⁴ Schmahmann, J. D., & Sherman, J. C. (1998). The cerebellar cognitive affective syndrome. *Brain: a Journal of Neurology*, *121*(Pt 4), 561–579.

³⁵ Schmahmann, J. D., Guell, X., Stoodley, C. J., & Halko, M. A. (2019). The theory and neuroscience of cerebellar cognition. *Annual Review of Neuroscience*. <https://doi.org/10.1146/annurev-neuro-070918-050258>.

³⁶ Ito, M. (2008). Control of mental activities by internal models in the cerebellum. *Nature Reviews. Neuroscience*, *9*, 304–313. <https://doi.org/10.1038/nrn2332>.

³⁷ Ohmae, S., Uematsu, A., & Tanaka, M. (2013). Temporally specific sensory signals for the detection of stimulus omission in the primate deep cerebellar nuclei. *J Neurosci*, *33*, 15432–15441. <https://doi.org/10.1523/JNEUROSCI.1698-13.2013>.

³⁸ Heffley, W., et al. (2018). Coordinated cerebellar climbing fiber activity signals learned sensorimotor predictions. *Nature Neuroscience*, *21*, 1431–1441. <https://doi.org/10.1038/s41593-018-0228-8>.

³⁹ Wagner, M. J., Kim, T. H., Savall, J., Schnitzer, M. J., & Luo, L. (2017). Cerebellar granule cells encode the expectation of reward. *Nature*, *544*, 96–100. <https://doi.org/10.1038/nature21726>.

⁴⁰ Wagner, M. J., et al. (2019). Shared cortex-cerebellum dynamics in the execution and learning of a motor task. *Cell*, *177*, 669–682 e624. <https://doi.org/10.1016/j.cell.2019.02.019>.

⁴¹ Deverett, B., Kislin, M., Tank, D., & Wang, S. S. (2019). Cerebellar disruption impairs working memory during evidence accumulation. *BioRxiv*. <https://doi.org/10.1101/521849>.

⁴² Tsai, P. T., et al. (2012). Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature*, *488*, 647–651. <https://doi.org/10.1038/nature11310>.

⁴³ Kalmbach, B. E., Ohyama, T., Kreider, J. C., Riusech, F., & Mauk, M. D. (2009). Interactions between prefrontal cortex and cerebellum revealed by trace eyelid conditioning. *Learning & Memory*, *16*, 86–95. <https://doi.org/10.1101/lm.1178309>.

⁴⁴ Kostadinov, D., Beau, M., Pozo, M. B., & Hausser, M. (2019). Predictive and reactive reward signals conveyed by climbing fiber inputs to cerebellar Purkinje cells. *Nature Neuroscience*. <https://doi.org/10.1038/s41593-019-0381-8>.

⁴⁵ Carta, I., Chen, C. H., Schott, A. L., Dorizan, S., & Khodakhah, K. (2019). Cerebellar modulation of the reward circuitry and social behavior. *Science*, *363*. <https://doi.org/10.1126/science.aav0581>.

Affiliations

Zhenyu Gao¹ · Alyse M. Thomas² · Michael N. Economo^{3,4} · Amada M. Abrego² · Karel Svoboda³ · Chris I. De Zeeuw^{1,5} · Nuo Li² 

¹ Department of Neuroscience, Erasmus MC, Rotterdam, the Netherlands

² Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA

³ Janelia Research Campus, Ashburn, VA 20147, USA

⁴ Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA

⁵ Netherlands Institute for Neuroscience, Amsterdam, the Netherlands