# Activities of sensory and frontal cortices in sequence learning in Wild Type and FMR1 KO mice

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## Introduction

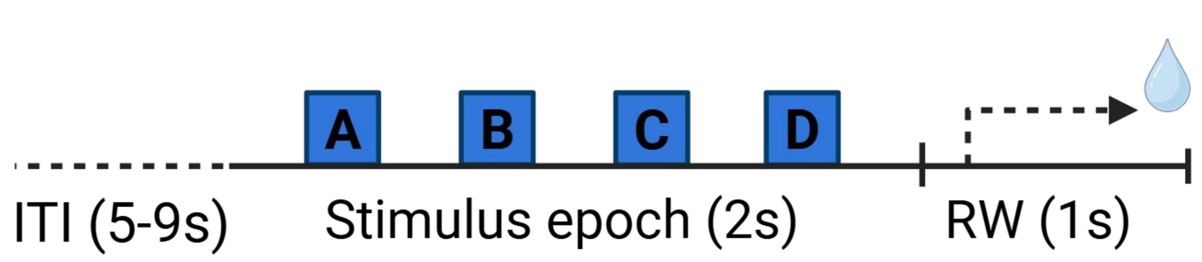
Learning to recognize and predict temporal sequences is fundamental to sensory perception and is impaired in several neurodevelopmental disorders, but little is known about where and how this occurs in the brain. This study investigates how mice learn and respond to repeated sensory sequences, focusing on the activities within frontal cortices (FC) and sensory cortex (S1). Furthermore, we compare behavioral and neural measures in Wild Type (WT) and FMR1 KO mice, a mouse model of Fragile X Syndrome (FXS). We train WT and FMR1 KO mice in a sequence learning operant task while measuring FC and S1 population activities using widefield $Ca^{2+}$ imaging. We find notable differences in WT vs FMR1 KO behavioral strategies. Neural analyses are aimed at testing sensory encoding vs frontal cortex modulation as potential contributors to the differential behavioral strategies. Our findings have implications for understanding sequence learning deficits in FXS and other autism spectrum disorders.

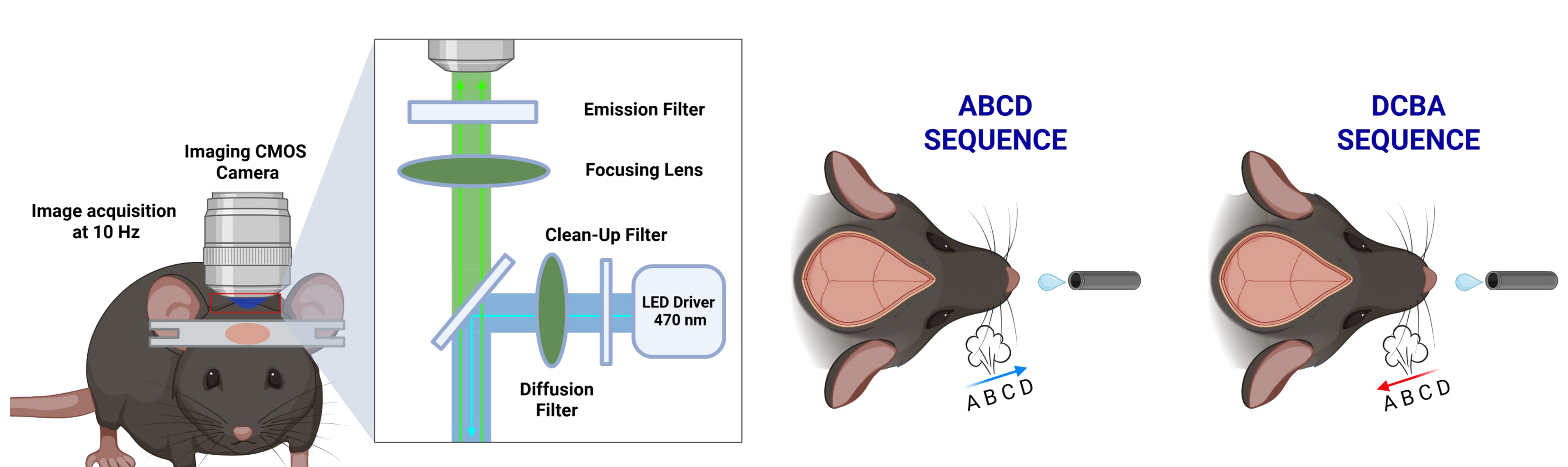
## Methods

**Subjects & Preparation:**
Adult mice, both WT and FMR1 KO (8 weeks–8 months) expressing the calcium indicator *Snap25-2A-GCaMP6s-D.* Mice were water restricted throughout behavioral training and testing.

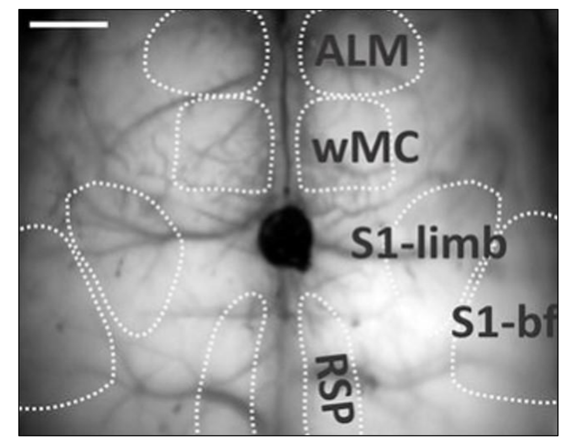
**Behavioral Task:**
Head-fixed mice received air puff sequences directed at their whiskers: A–B–C–D, delivered from caudal to rostral. Following the stimulus sequence, mice were required to lick to a central lickspout to trigger a water reward. On testing day, mice were challenged with additional sequences: A-A-A-A (oddball) and D-C-B-A (reversal).

**Imaging & Data Collection:**
Neural activity across the dorsal cortex was recorded using wide-field calcium imaging.



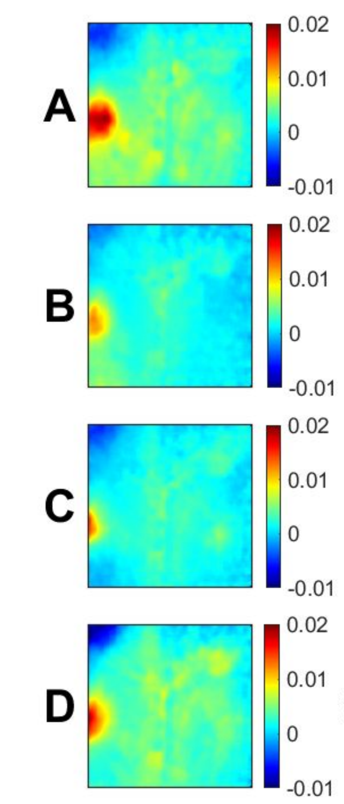




## Results

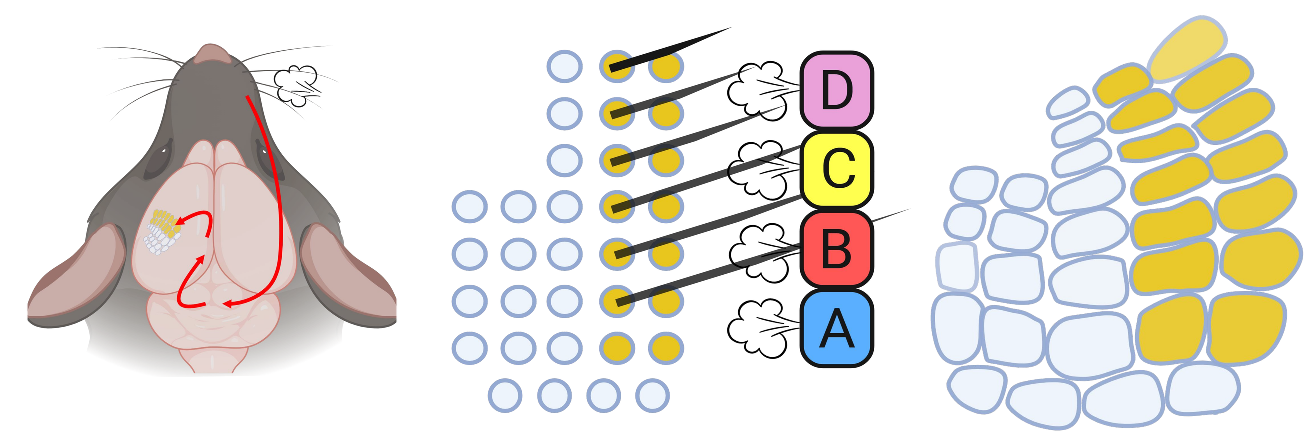



**Left:** Dorsal view of mouse neocortex as captured by widefield imaging. Regions of interest include anterolateral motor cortex (ALM), whisker motor cortex (wMC), and primary sensory cortex (S1).

**Below:** Whisker-related primary somatosensory cortex (wS1) contains anatomically distinct structures called 'barrels,' each corresponding to an individual facial whisker. These barrels are organized in a somatotopic map, and targeted air puffs to specific whiskers selectively activate their associated barrels.

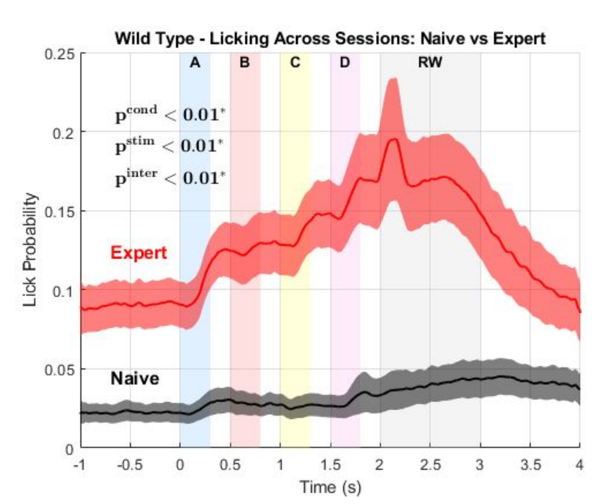



**Above:** Mean S1 responses to A-B-C-D stimuli from an example session, matching the known whisker/barrel somatotopy.



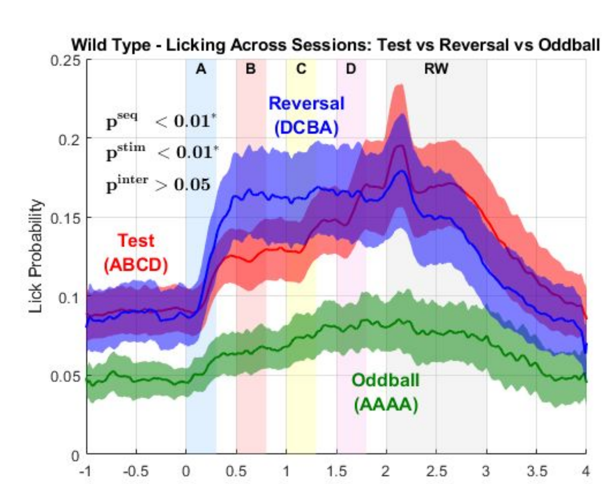

### Comparison of behavioral activity between WT and FMR1 KO

WT mice increase licking to each subsequent stimulus element, and display altered licking to oddball and reversal sequences.

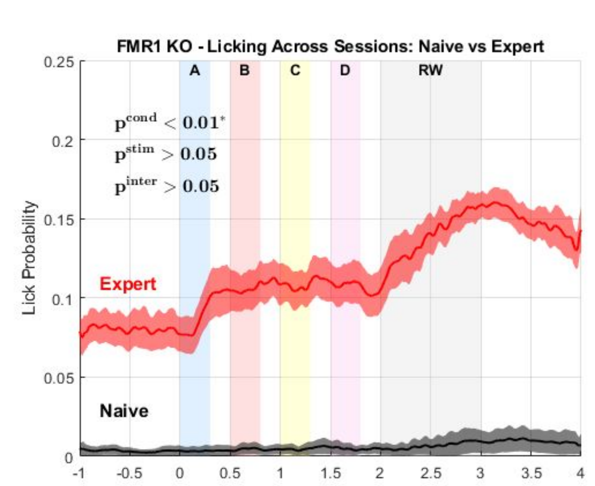



**Left:** Mean licking probabilities over time between naive sessions (black) and expert (red) sessions for ABCD sequences in WT mice. Note in expert mice the ramping licking behavior during the stimulus window.

**Right:** Mean licking probabilities in expert WT mice for standard A-B-C-D (red), reversal D-C-B-A (blue), and oddball A-A-A-A (green) sequences. Note the differences in licking responses to each sequence.

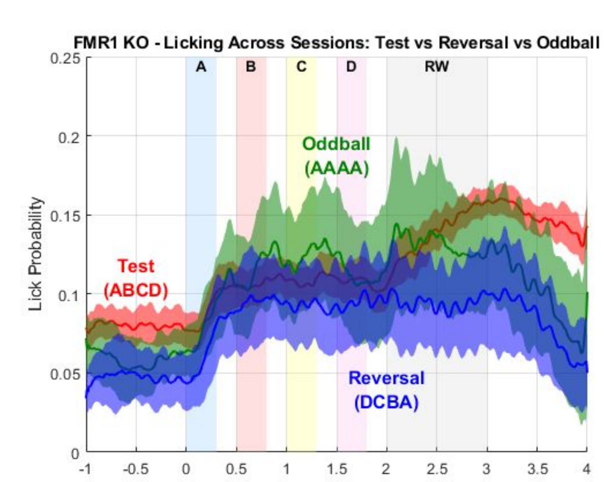



FMR1 KO mice lick robustly to the first stimulus element, regardless of the sequence.

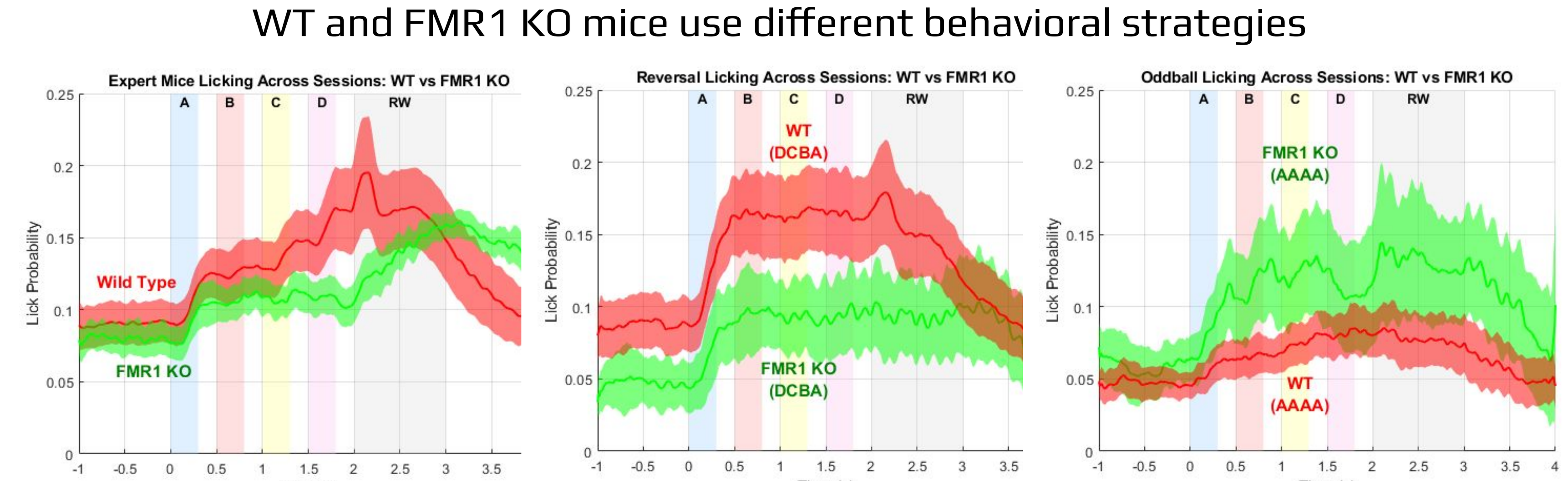



**Left:** Mean licking probabilities for naive (black) and expert (red) sessions in FMR1 KO mice. Note in expert mice, there is a lack of ramping during stimulus window, suggesting impaired sequence anticipation

**Right:** Mean licking probabilities in expert FMR1 KO mice for standard A-B-C-D (red), reversal D-C-B-A (blue), and oddball A-A-A-A (green) sequences. Note the lack of a difference in licking responses to each sequence.



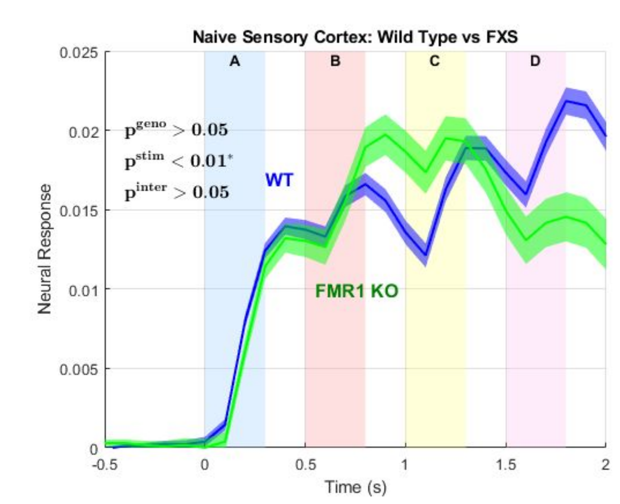

WT and FMR1 KO mice use different behavioral strategies




Comparison of licking probabilities between FMR1 KO expert mice and WT expert mice in response to the sequence ABCD, DCBA, and AAAA. In the FMR1 KO mice, the sustained licking activity could imply an anticipation of the water reward outcome. In the WT mice, there is an increase in licking after each air puff, which is the greatest after D.

## Cortical activity during task performance in WT and FMR1 KO

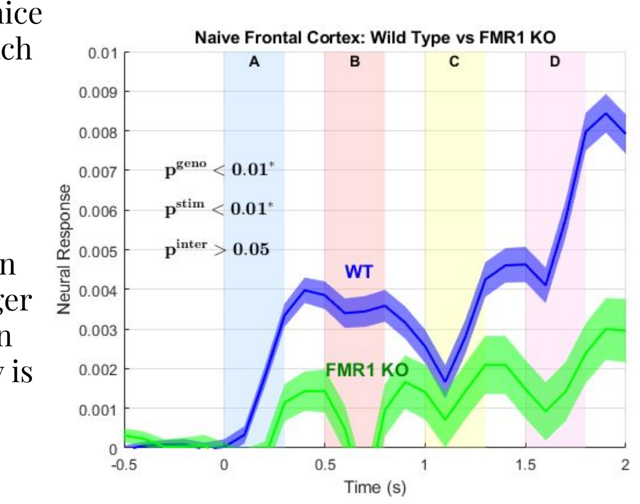
Naive
Sensory Cortex



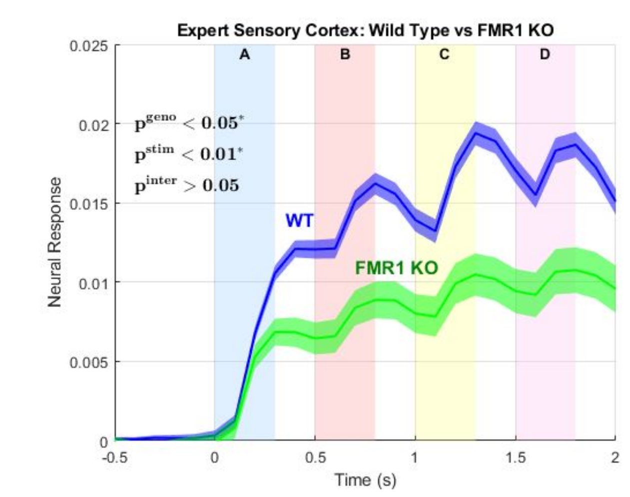

**Left:** Sensory cortex responses to the ABCD sequence in naive mice show ramping activity after each puff in WT (blue) mice, while FMR1 KO (green) mice display elevated responses primarily after puffs B and C.

**Right:** Frontal cortex activity in naive WT (blue) mice is stronger overall, peaking after puff D. In FMR1 KO (green) mice, activity is lower and shows only slight increases after each puff.

Naive
Frontal Cortex



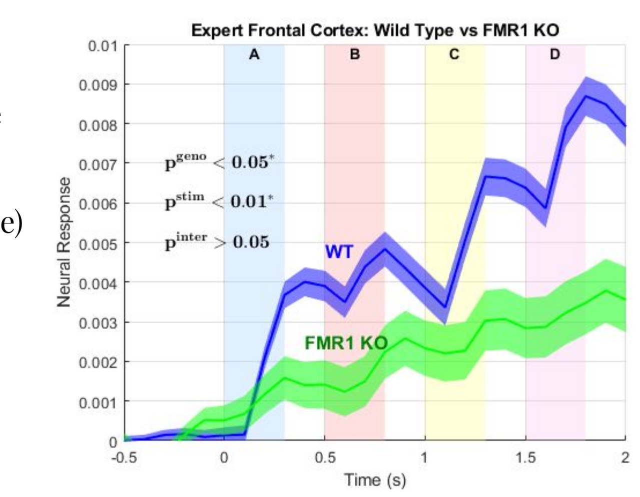

Expert
Sensory Cortex




**Left:** In expert mice, WT (blue) sensory cortex activity ramps up after A, B, and C, then slightly decreases after D. FMR1 KO (green) show modest, uniform increases after each puff. Note less activity in FMR1 KO mice.

**Right:** In expert mice, WT (blue) frontal cortex activity ramps consistently after each puff. FMR1 KO (green) mice show overall increased activity, but with less defined responses to individual puffs.

Expert
Frontal Cortex




## Conclusion

This study demonstrates that while both WT and FMR1 KO mice can learn a basic sensory sequence (ABCD), only WT mice adjust their behavior in response to altered sequences (DCBA, AAAA), indicating flexible sequence learning. In contrast, FMR1 KO mice fail to distinguish between sequence variations, reflecting impaired behavioral flexibility. Widefield calcium imaging reveals that sensory cortex activity remains consistent across genotypes, suggesting intact sensory encoding. However, WT mice exhibit increased frontal cortex activity aligned with sequence discrimination, whereas FMR1 KO mice show reduced frontal engagement. Typically, FMR1 KO mice display hypersensitivity but was not observed in trials. These results suggest that flexible sequence learning depends on frontal cortex modulation, and that deficits in top-down cortical communication may underlie impaired sequence processing in FXS and potentially other autism-related conditions.

## Future Directions

Future work will focus on disentangling sensory and motor-related signaling, including separating sensory vs. frontal cortical contributions. Ablation studies may help test whether specific regions are necessary for learning or task retention. We aim to improve stimulus encoding accuracy and explore how representations reflect stimulus order and value. A 4-way classifier with confusion matrix, combined with low-dimensional manifold analysis, mutual information, and effective dimensionality, will help quantify encoding quality. On the motor side, we plan to model high-dimensional behaviors by regressing neural activity into categorical licking and continuous face/body movements. Additionally, analyzing responses to oddball stimuli may reveal signs of predictive processing errors or behavioral hesitation.

## References

Audette NJ, & Schneider DM. Stimulus-Specific Prediction Error Neurons in Mouse Auditory Cortex. Journal of Neuroscience 25 October 2023, 43 (43) 7119-7129; DOI: 10.1523/JNEUROSCI.0512-23.2023
Gavornik, J., Bear, M. Learned spatiotemporal sequence recognition and prediction in primary visual cortex. Nat Neurosci 17, 732–737 (2014). https://doi.org/10.1038/nn.3683
Juczewski, K., von Richthofen, H., Bagni, C., Celikel, T., Fisone, G., & Krieger, P. (2016). Somatosensory map expansion and altered processing of tactile inputs in a mouse model of fragile X syndrome. *Neurobiology of disease, 96*, 201–215. https://doi.org/10.1016/j.nbd.2016.09.007