

Effects of Hyperexcitation of Layer V Neocortical Pyramidal Neurons during early Postnatal Development on Adult Behavior

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Introduction

- Patterns of neuronal activity during early development are believed to guide the assembly of neural circuits.
- Thus, alterations of neuronal activity in this time window may cause structural and functional changes that persist into adulthood.
- Specific alterations may lead to unique patterns of aberrant circuit formation, a mechanism which has been implicated in neurodevelopmental disorders such as autism.
- Findings from human and animal studies suggest that multiple etiologically distinct forms of autism alter the physiology of specific deep-layer (layer V) prefrontal cortical neurons that project to subcortical targets.
- These neural populations play an important role in both normal and abnormal social behavior.

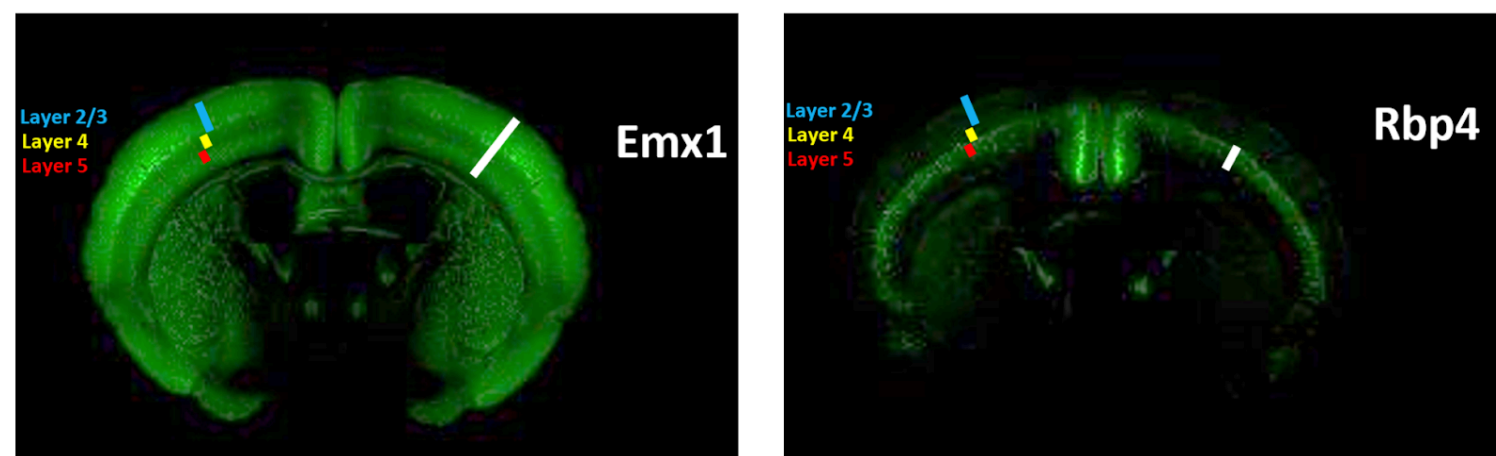


Figure 1: Emx1 and Rbp4 cortical expression

Activating Emx1 positive neurons in development

- Decreased social interaction & increased grooming activity in adult animals

Rbp4 ?

- Role of layer V pathway in cortical & thalamic computations is not well understood
- Layers V & VI originating outputs represent the only means by which the cortex can influence subcortical processes, thereby influencing behavior.
- To probe a potential functional link between development of cortical circuitry and adult behavior, we hyperexcited layer V cortical neurons during early postnatal development and assessed the effects on adult behavior.

Methods and Materials

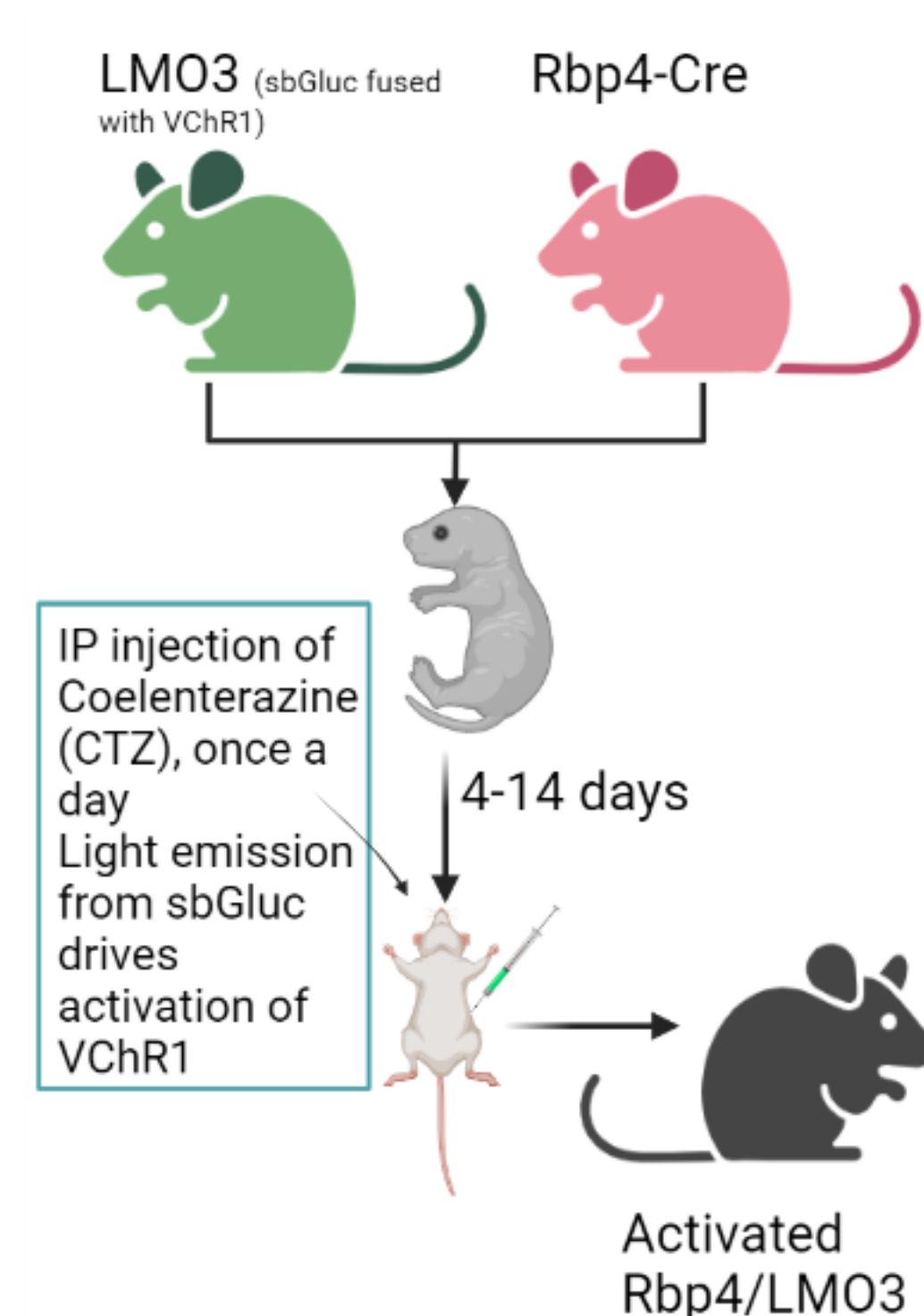


Figure 2: Activation of Rbp4/LMO3 in pups

- Postnatal day 60 onwards behavior of all mice were tested with open field, water T-maze, sociability, novel object, and rotarod tests.

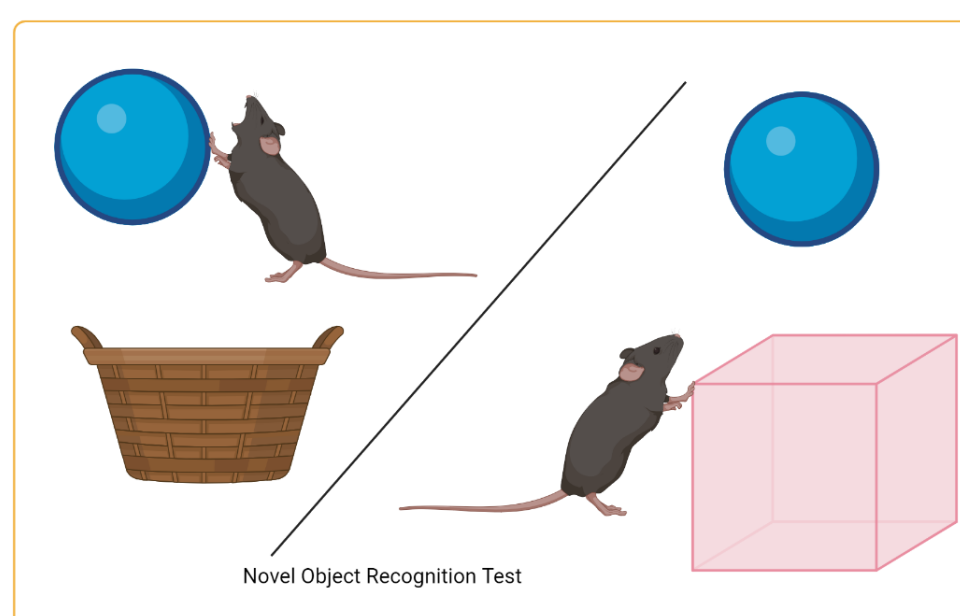


Figure 3: Novel Object Recognition Test

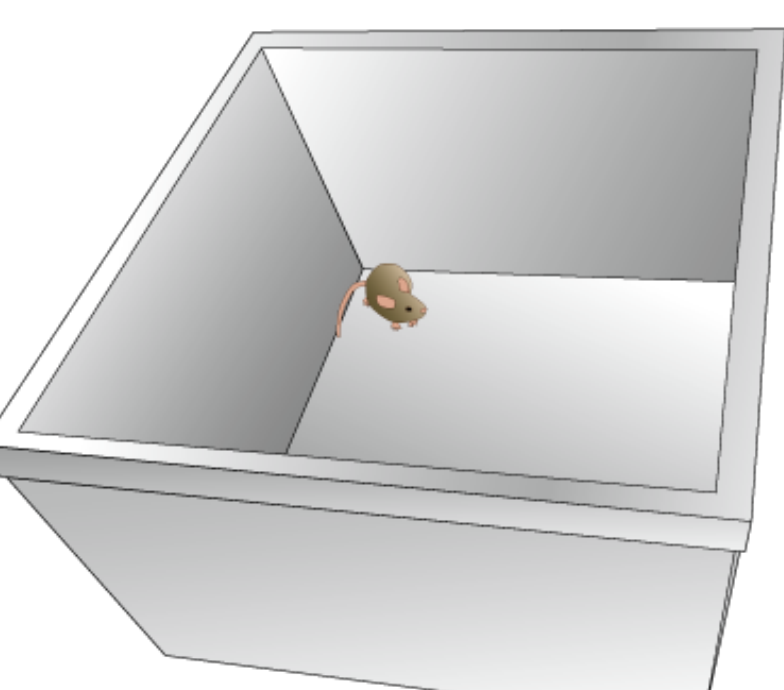


Figure 4: Open Field

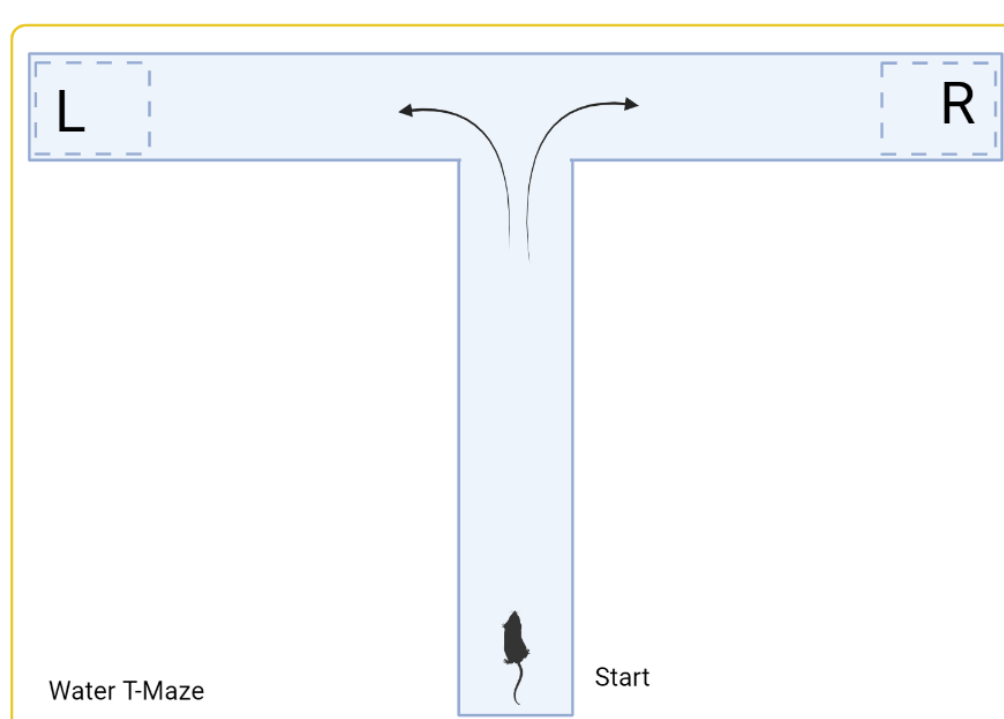


Figure 5: Water T-Maze

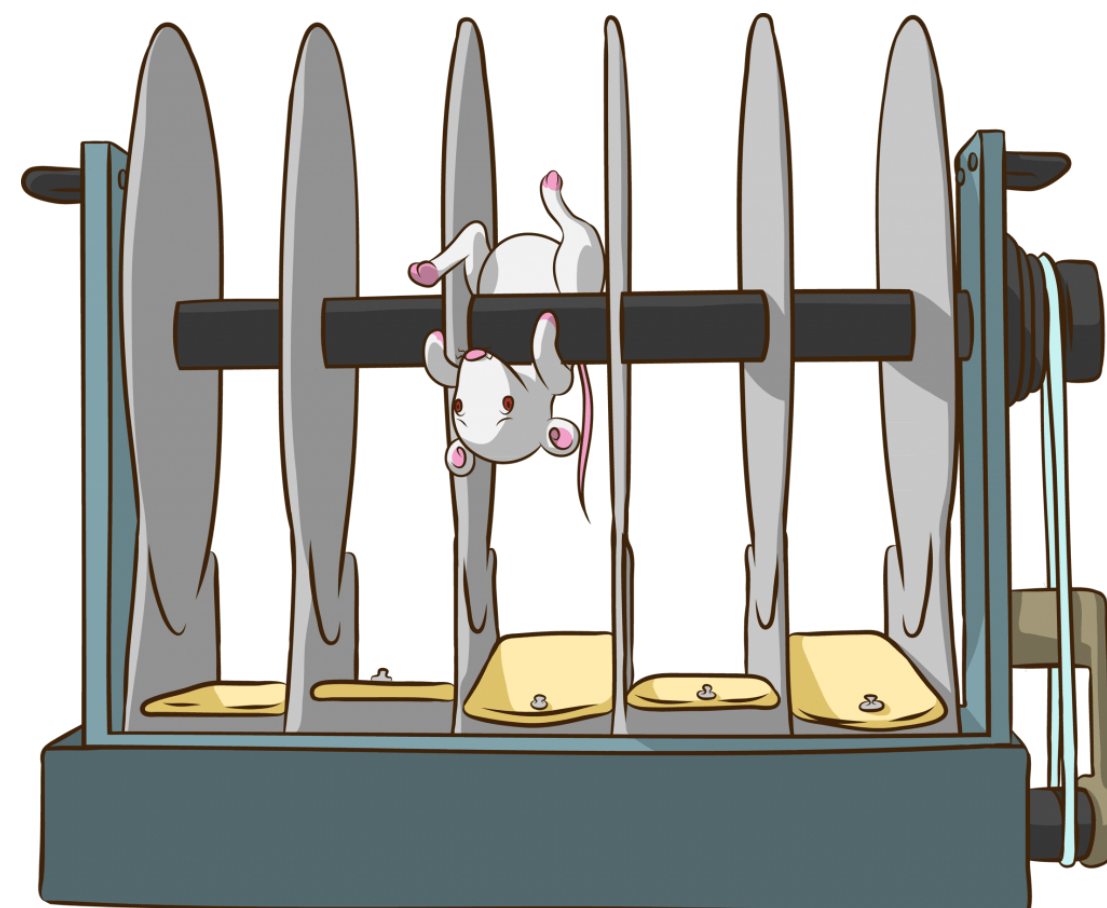


Figure 6: Rotarod Test

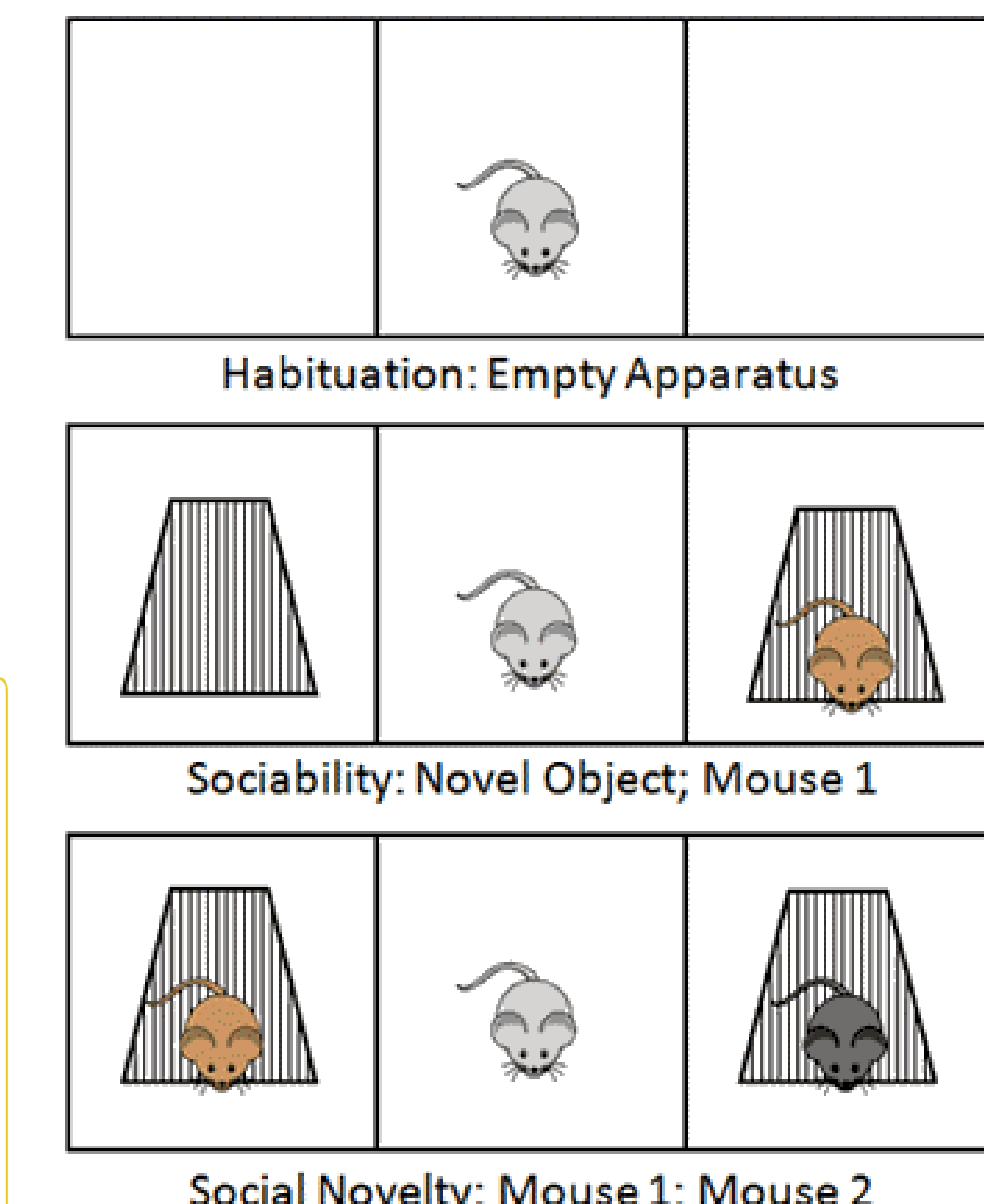


Figure 7: Social Interaction Test

Results

- We implemented DeepLabCut to standardize our quantification of behavioral data, which is an open-source machine learning tool that leverages recent advances in computer vision to allow accurate, markerless tracking of animals across behavior testing videos.
- SPSS was used to perform ANOVA and p-values less than 0.05 were considered to indicate significant differences between the groups.

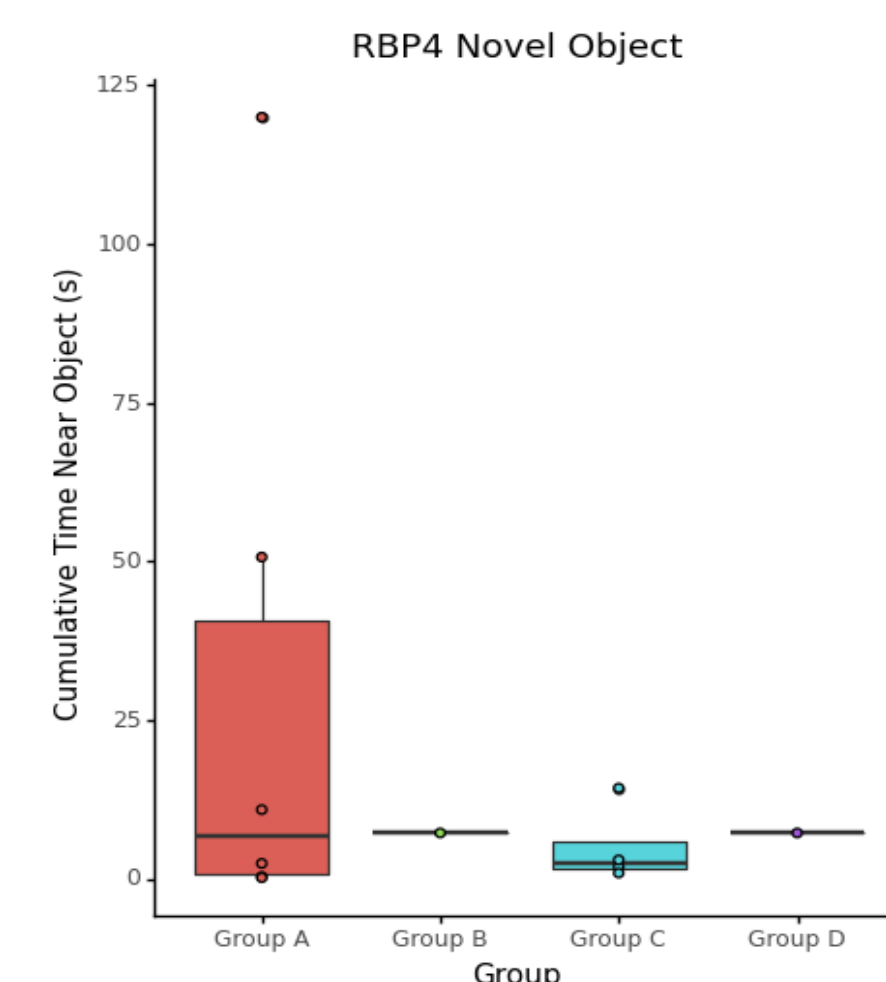


Figure 8: Novel Object recognition test showed Rbp4/LMO3 (group A) mice spend more time exploring the novel object than the control.

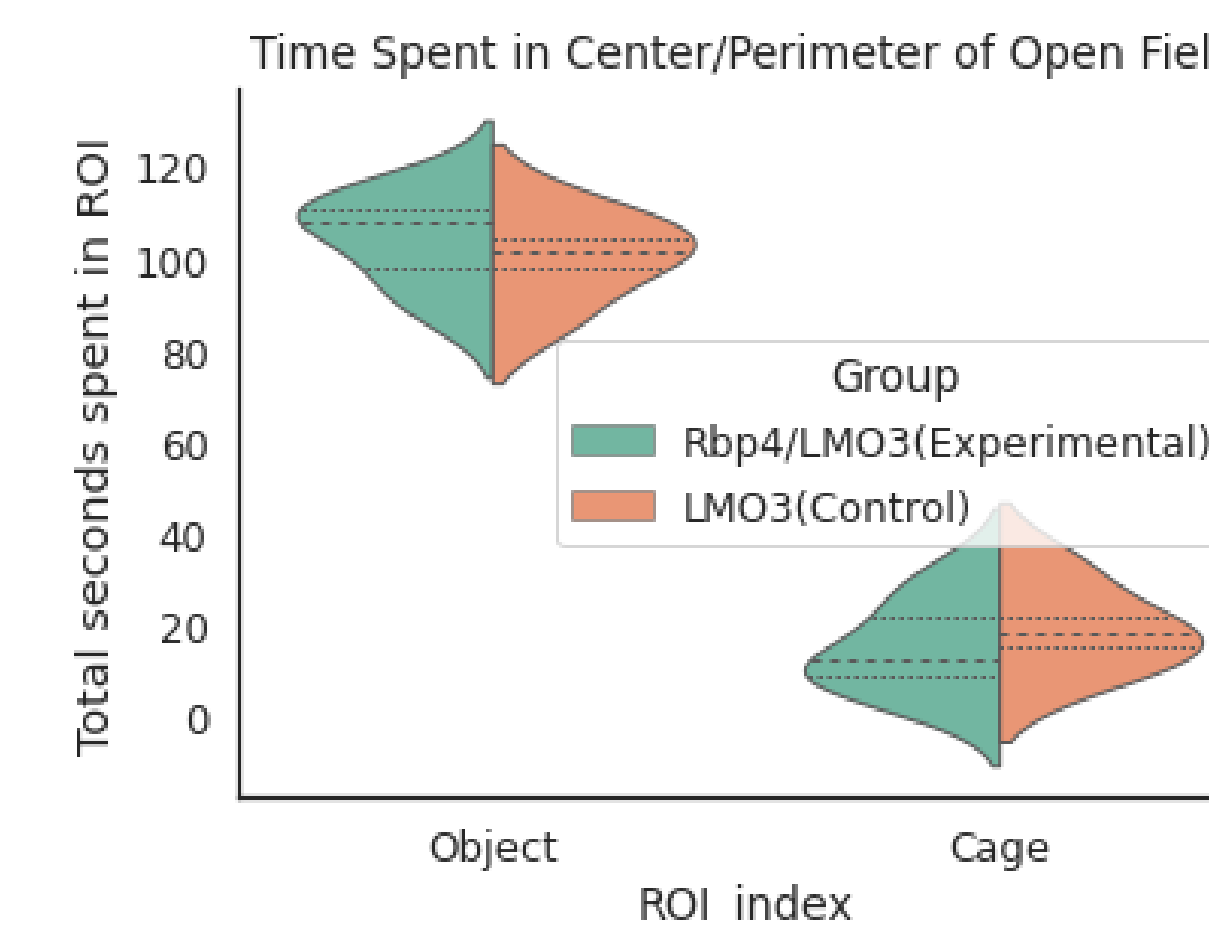


Figure 9: Open field test showed both Rbp4/LMO3 and control group preferred center of the field rather than the perimeter of the field.

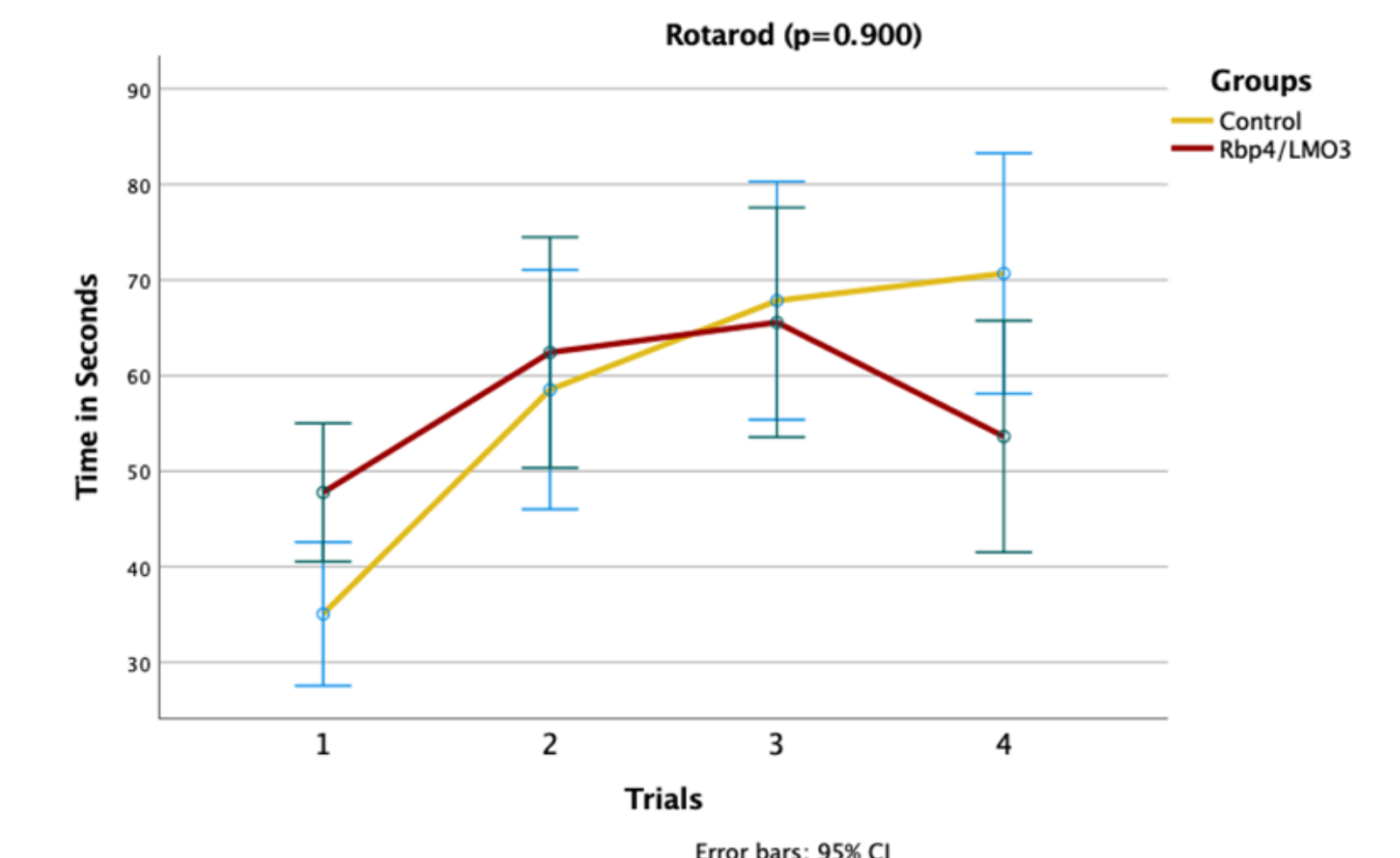


Figure 10: Rotarod test showed no differences between the Rbp4/LMO3 and the control group.

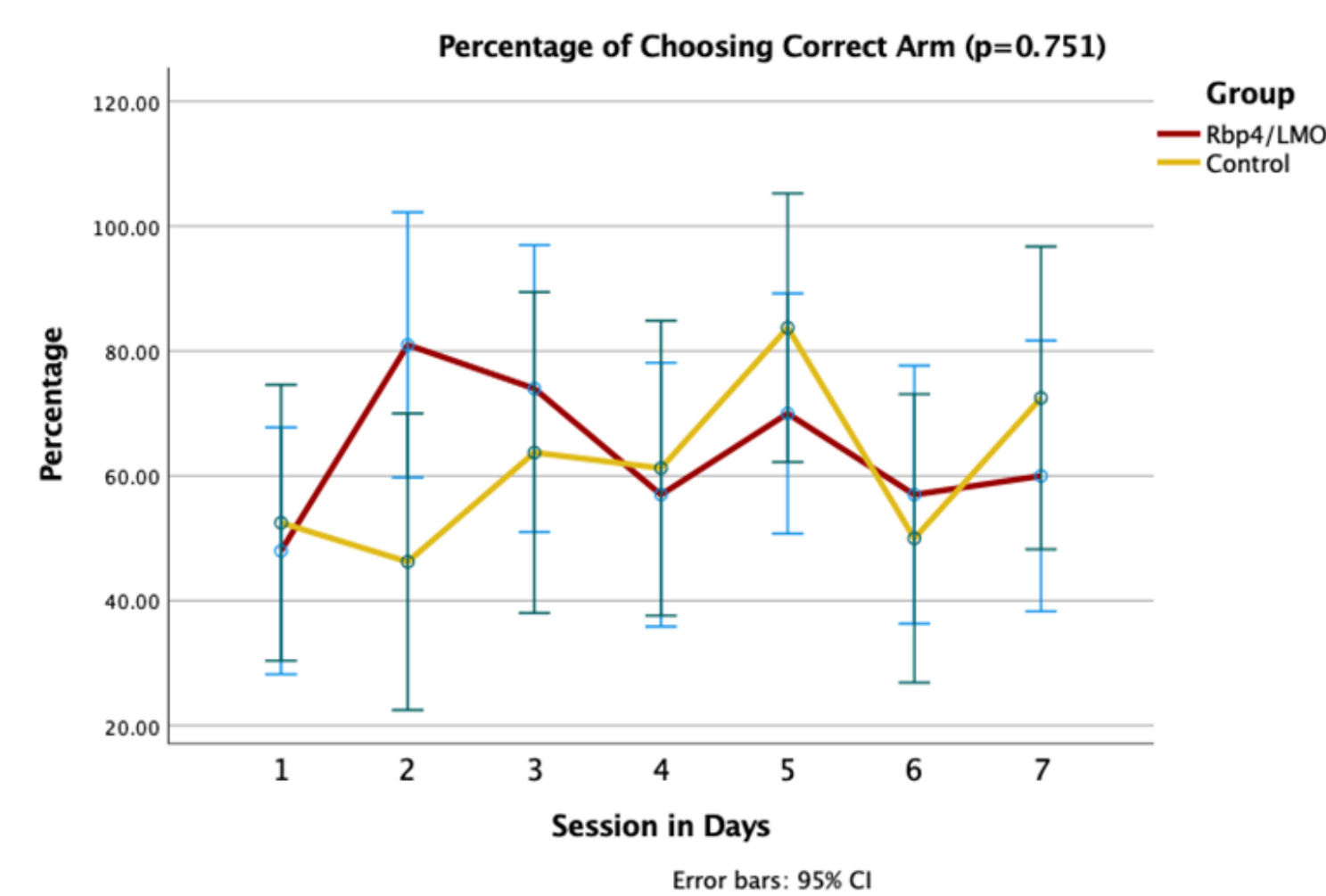


Figure 11: Percentage of choosing correct arm in water T-maze test showed no difference between Rbp4/LMO3 and the control group.

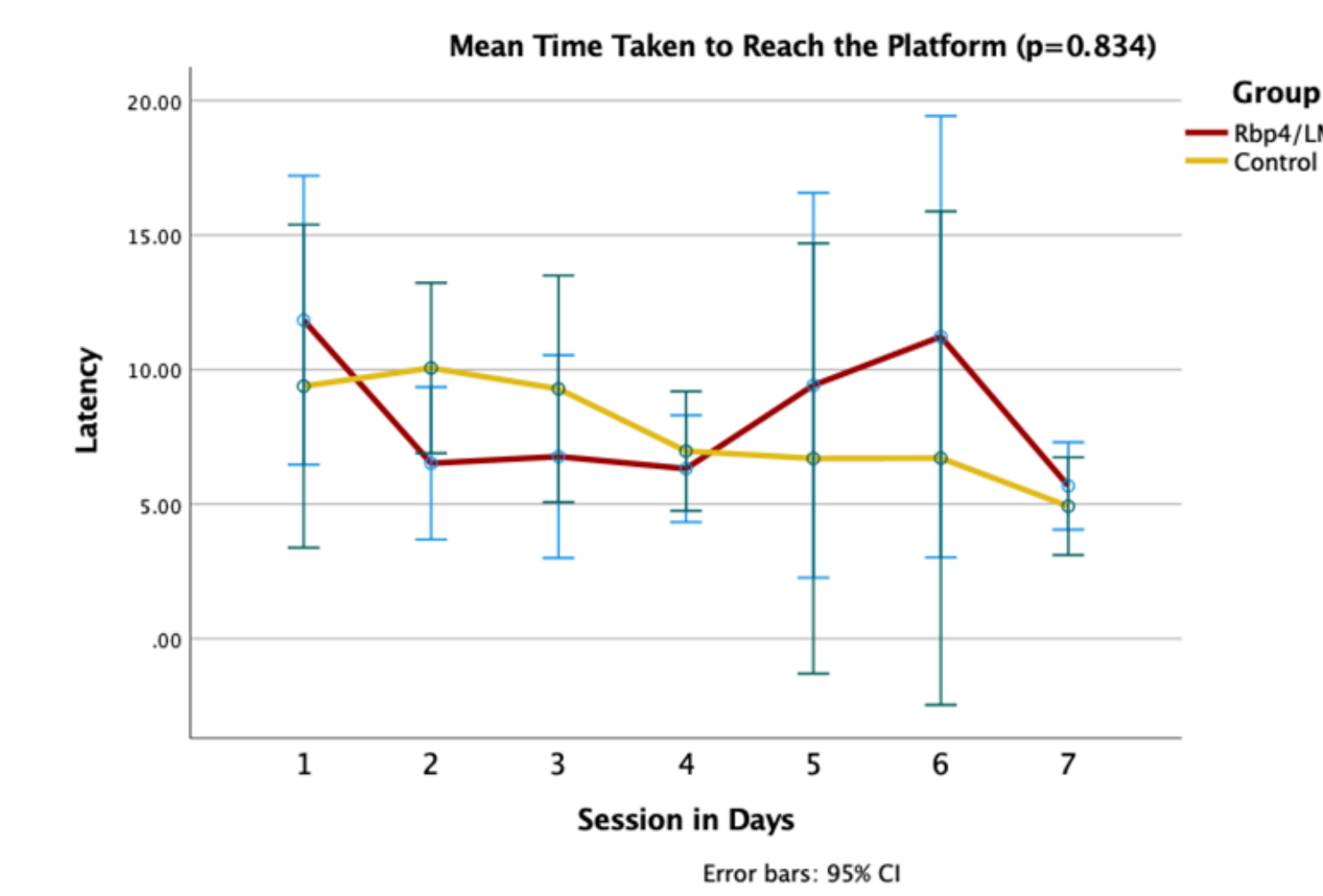


Figure 12: Mean time to reach the platform in water T-maze test showed no difference between Rbp4/LMO3 and the control group.

The data on social interaction have been collected, but still need to be analyzed.

The results of the social interaction test will allow to compare developmental hyperexcitation of pyramidal neurons of layer 5 in Rbp4/LMO3 mice to hyperexcitation of pyramidal neurons of all cortical layers in Emx1/LMO3 mice (Medendorp et al., 2021).

Availability of both sets of results will contribute to our understanding of how cortical circuits interact and whether they show differential sensitivities to developmental disturbances.

Conclusions

- This study investigated the impact of hyperexcitation of layer V neocortical pyramidal neurons during early postnatal development on adult behavior using Rbp4/LMO3 mice.
- Thus far, a limited number of behavioral tests were conducted, probing changes in motor activity, spatial memory, and exploratory behavior.
- Rbp4/LMO3 mice were found to be more explorative towards a novel object than the control group.

- No significant differences between the experimental and control mice were detected in general locomotor behavior and activity levels (Open Field), motor skills and motor learning (Rotarod), and spatial memory performance (Water T-maze).
- More behavioral tests will be conducted in Rbp4/LMO3 mice to probe the effect of developmental hyperexcitation of layer V on aspects of autism spectrum disorder (sociability, grooming), as well as aspects of other neurodevelopmental disorders (learning, anxiety, cognition).

References

Medendorp WE, Bjorefeldt A, Crespo EL, Prakash M, Pal A, Waddell ML, Moore CI, Hochgeschwender U. Selective postnatal excitation of neocortical pyramidal neurons results in distinctive behavioral and circuit deficits in adulthood. *iScience* 24:102157, 2021

Acknowledgments

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