Characterization of Instinctive Defensive Responses to Looming Stimuli in Nlgn3$^{-/y}$ Rats

Tianhui Ding, Supervisor: Vijay Kapgal
Kind lab, The University of Edinburgh, Institute for Neuroscience and Cardiovascular Research

Institute for Neuroscience and Cardiovascular Research

Simons Initiative for the Developing Brain

The Patrick Wild Centre

1. Background

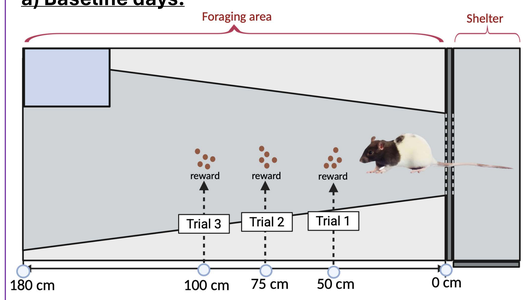
Emerging evidence demonstrates a strong connection between **neuroligin-3** (*1NLGN3) dysfunction and **autism spectrum disorder.** Previous study employed conditioned threat paradigm have revealed significant distinct patterns of freeze and flight responses between wild-type and *Nlgn3* knockout rats (Anstey *et al.*, 2022). The loss of NLGN3 appears to bias neural circuits toward flight rather than freeze, driven by increased excitability of neurons specifically in the *2 **dPAG,** indicating synaptic alterations resulting from loss of *Nlgn3* may disrupt neural circuits underlying these instinctive behaviours**.** However, it remains unclear whether these behavioural differences are present in these rats in response to innate fear under naturalistic, ecologically relevant conditions. In this study, we hypothesize that *Nlgn3*$^{-/y}$ rats will exhibit enhanced flight responses and elevated active avoidance behaviour in response to looming stimuli. Building on this, our study introduces predator-mimicking aerial stimuli which **aiming** to examine and characterize the **innate fear behaviour** of these rats.

*1 NLGN3: an adhesion protein expressed on the postsynaptic membrane that binds to neurexins to mediate synaptic formation and function.
*2 dPAG: the abbreviation for dorsal periaqueductal gray, which is a critical midbrain output hub for generating active defensive behaviours (i.e. flight).

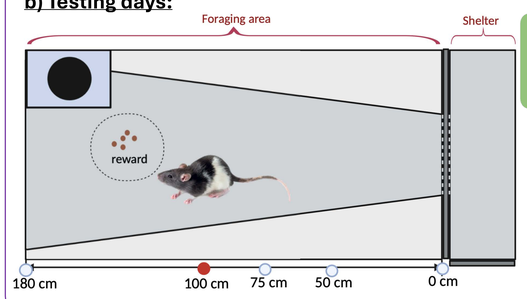
2. Methods

2.1 Looming avoidance tasks

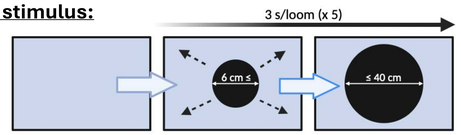
a) Baseline days:



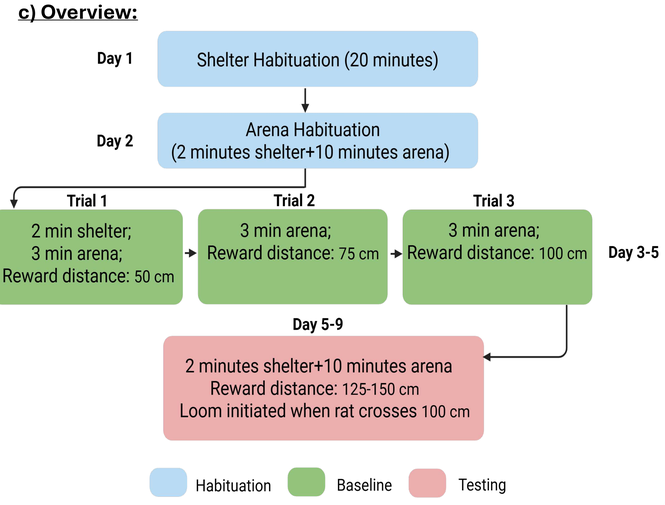
b) Testing days:



Looming stimulus:



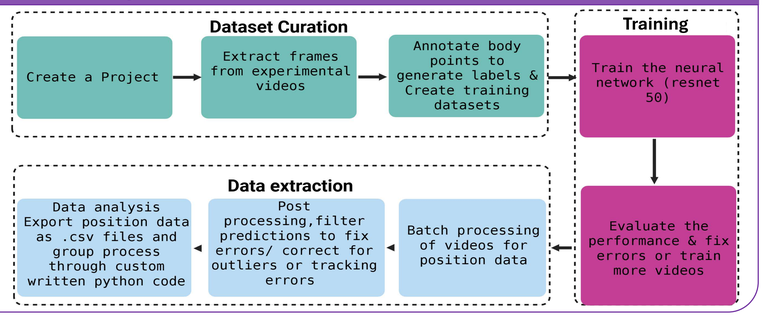
c) Overview:



2.2 Data Analysis & Visualization

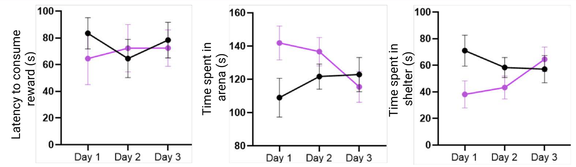
Data analysis and visualization

- Data were analyzed using GraphPad Prism (statistical test applied: repeated-measure 2-way ANOVA)
- Advanced analyses such as trajectory track were obtained using DeepLabCut.



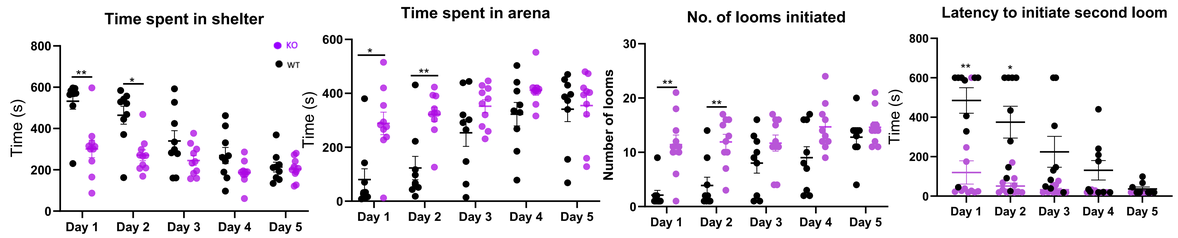
3. Results

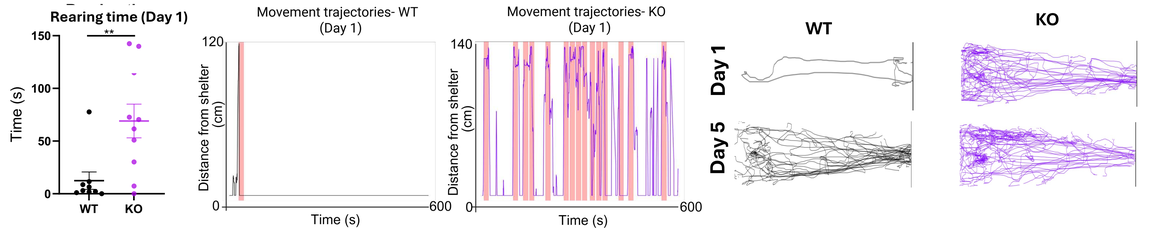
Habituation is observed in both WT and *Nlgn3*$^{-/y}$ rats across the baseline days.



1. ***Nlgn3*$^{-/y}$ rats show earlier habituation, whereas WT rats display a slower habituation profile during the testing period.**
2. ***Nlgn3*$^{-/y}$ rats display altered avoidance responses to looming stimuli.**
   Nlgn3$^{-/y}$ rats demonstrate reduced avoidance responses toward looming stimuli.
   - Exhibit attenuated fear responses (i.e. reduction in flight behaviour.)
   - Relative actively exposed in the open field.
   - Increased exploratory activity such as rearing.
   - Significantly higher frequency of looming initiation on the first testing day.

KO
WT





Repeated measure two-way ANOVA: Genotype: p=0.0001*; Interaction: p=0.0001*; multiple comparison test: p=0.0001*; p=0.01*.

4. Future Direction

Future studies could investigate whether diminished fear responses stem from dysfunction in a specific brain region or from disrupted coordination among multiple regions involved in the freeze-flight circuits, whether the variability results from distinct types of sensory input, and re-express *Nlgn3* across different brain regions to assess whether this intervention can rescue the observed behavioural phenotype.

5. Conclusion

1. Wild-type control group shows typical avoidance responses and displays slower habituation in looming avoidance tasks.
2. *Nlgn3* $^{-/y}$ rats exhibit altered innate fear behaviour, characterized by diminished avoidance responses to looming stimuli, and show faster habituation in comparison to WT rats.
3. The findings suggest that the defensive neural pathways underlying innate fear may function differently in *Nlgn3*$^{-/y}$ rats.

6. Acknowledgement & Reference

I would like to thank my supervisor, Dr. Vijay Kapgal, for supporting me throughout this incredible journey to learn, grow and gain invaluable experiences, as well as Prof. Peter Kind for giving me the opportunity to work in his lab, and the Summer Vacation Scholarship Program in Biomedical Sciences for providing me financial support. In addition, special thanks to Dr. Domagoj Anticic for helping with the DeepLabCut coding and analysis.

Reference:

- Anstey, N.J. et al. (2022) 'Imbalance of flight–freeze responses and their cellular correlates in the NLGN3−/Y rat model of autism', Molecular Autism, 13(1). doi:10.1186/s13229-022-00511-8.