Machine Learning-Based Neural Biomarkers of Freezing Behaviour

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Background

SYNGAP1 haploinsufficiency is associated with neurodevelopmental disorders including intellectual disability, autism spectrum disorder, epilepsy, and fear-related phenotypes such as anxiety[1]. These arise from altered synaptic function and disrupted neural circuit development and include a prominent fear extinction deficit in *Syngap+/Δ-GAP* heterozygous rats, modelling persistent fear and anxiety-like responses[2]. Fear regulation depends on coordinated activity across cortical–limbic circuits, particularly interactions between the medial prefrontal cortex (mPFC), amygdala, olfactory bulb (OB), and periaqueductal grey (PAG) **(see fig 1)**.

In rodents, freezing behaviour provides a translational measure of fear responses and is accompanied by ~4 Hz (delta-band) directed electrophysiological oscillatory activity that synchronizes the OB → mPFC → amygdala circuit[3,4,5].

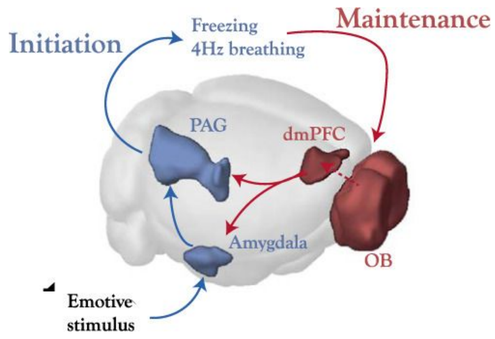


Figure 1: Fear response circuitry in rodents. Adapted from[4]

Fear-Extinction Phenotype

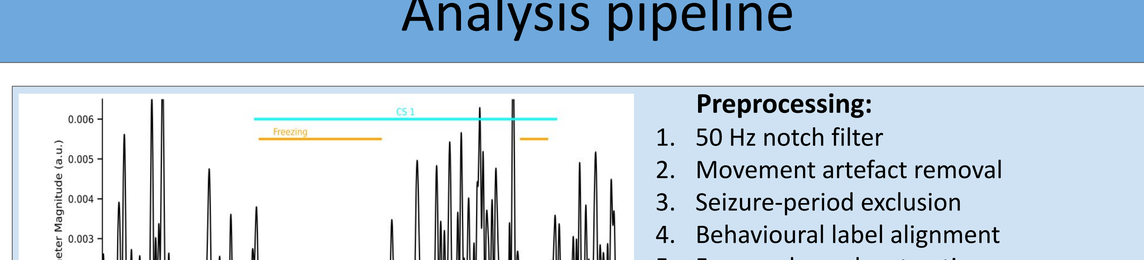


Figure 2: Locations of implanted EEG/LFP electrodes. OB: Olfactory Bulb, mPFC: Medial Prefrontal Cortex, Amyg: Amygdala

Figure 3: Schematic of visual fear conditioning paradigm. From left to right, habituation to CS box, conditioning (blue LED flash associated with a foot-shock), and then recall (10 repeats of the same LED flash).

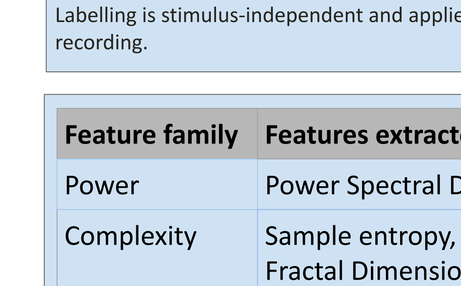


Figure 4: Freezing time percentage upon the conditioning stimulus (CS) presentation during recall of a conditioned fear response

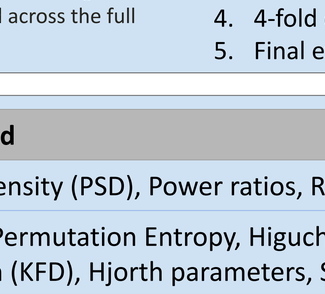


Figure 5: Extinction indices for all animals.

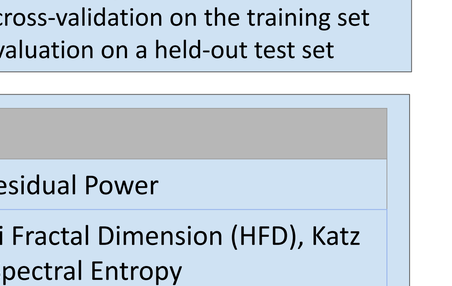


Figure 6: Cumulative probability distribution of freezing events time lengths.

Analysis pipeline

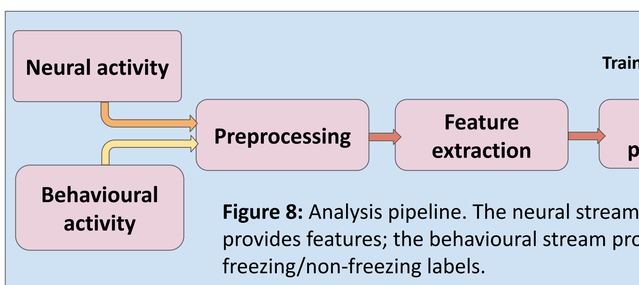


Figure 7: Head-fixed accelerometer-based freezing labelling aligned to CS 1 onset. Accelerometer magnitude relative to CS onset. The cyan bar marks the CS 1 stimulus window; yellow bars mark epochs automatically labelled as freezing. Epochs continuously below the threshold (0.001 a.u.) were labelled as freezing; all other periods were labelled as non-freezing. Labelling is stimulus-independent and applied across the full recording.

Preprocessing:

1. 50 Hz notch filter
2. Movement artefact removal
3. Seizure-period exclusion
4. Behavioural label alignment
5. 5-second epoch extraction
6. Band-pass decomposition into 5 frequency bands: δ (1–4 Hz), θ (4–8 Hz), α (8–12 Hz), β (12–30 Hz), γ (30–48 Hz)

Classification:

1. Elastic Net logistic regression classifier
2. Optuna-based hyperparameter optimisation
3. Balanced accuracy-based model selection
4. 4-fold cross-validation on the training set
5. Final evaluation on a held-out test set

Feature family	Features extracted
Power	Power Spectral Density (PSD), Power ratios, Residual Power
Complexity	Sample entropy, Permutation Entropy, Higuchi Fractal Dimension (HFD), Katz Fractal Dimension (KFD), Hjorth parameters, Spectral Entropy
Connectivity	Imaginary Coherence (iCoh), Directed Phase Lag Index (dPLI), Transfer Entropy, Cross-Correlation

Figure 8: Analysis pipeline. The neural stream provides features; the behavioural stream provides freezing/non-freezing labels.

Classification Results & Feature Importance

	Inner CV	Holdout
Accuracy	0.953	0.962
ROC AUC	0.985	0.993
F1	0.955	0.963
Recall	0.977	0.978
Specificity	0.930	0.946

Elastic Net Classifier Performance: *Syngap+/Δ-GAP*

	Inner CV	Holdout
Accuracy	0.922	0.939
ROC AUC	0.968	0.977
F1	0.924	0.941
Recall	0.948	0.963
Specificity	0.896	0.916

Elastic Net Classifier Performance: Wild-type

What is SHAP?

- SHAP (SHapley Additive exPlanations) explains individual predictions by assigning each feature a contribution score
- Higher mean |SHAP| = greater overall importance to the classifier

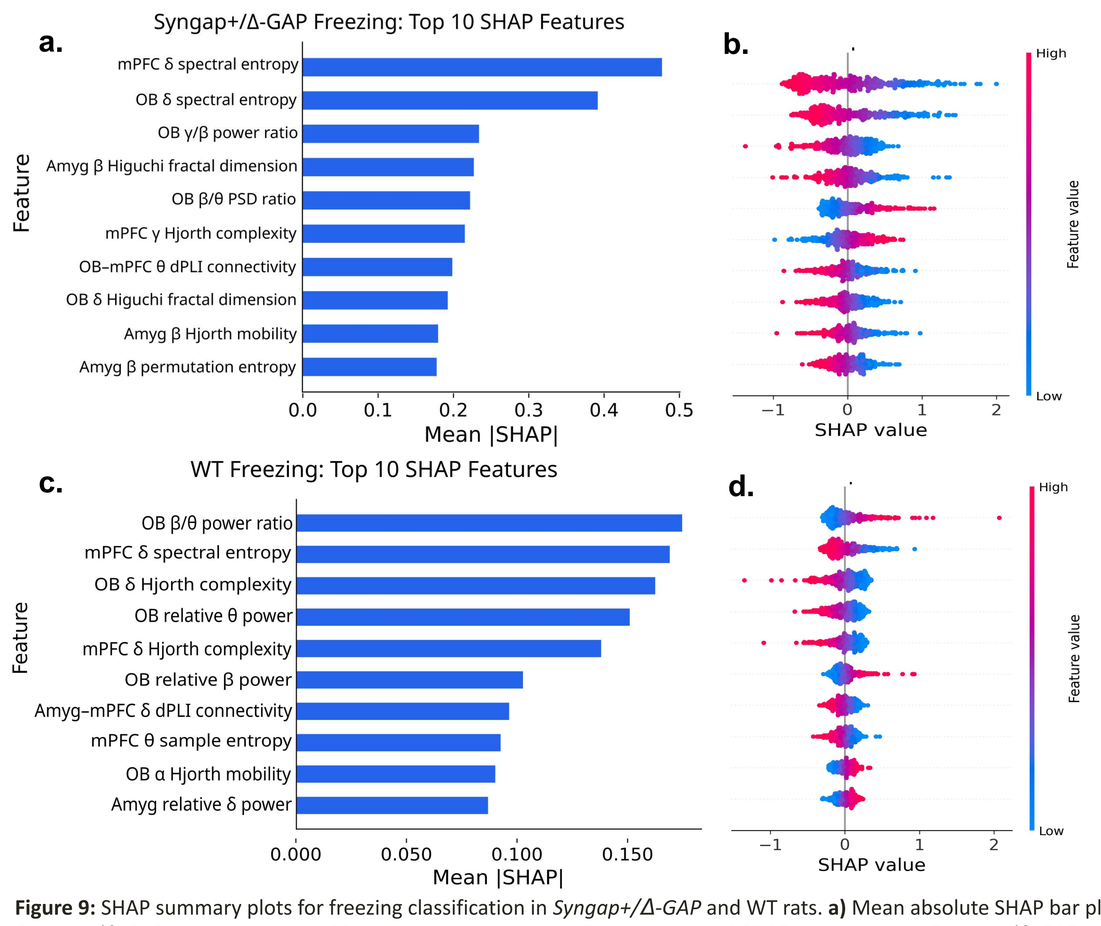
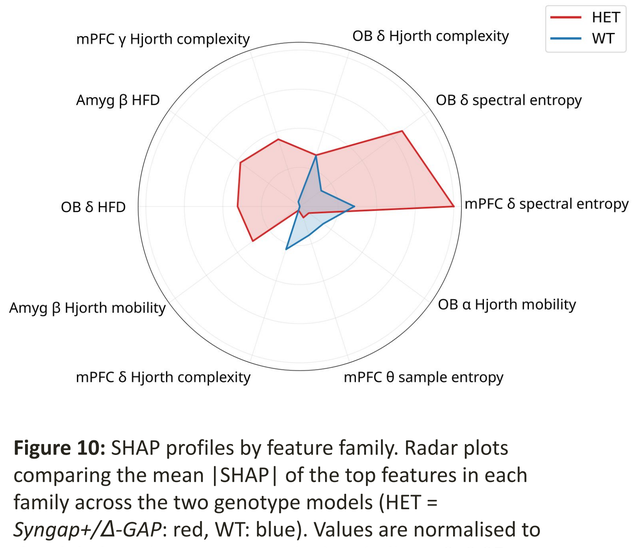


Figure 9: SHAP summary plots for freezing classification in *Syngap+/Δ-GAP* and WT rats. **a)** Mean absolute SHAP bar plot for *Syngap+/Δ-GAP*, showing the top 10 features ranked by overall importance; **b)** SHAP beeswarm for *Syngap+/Δ-GAP*, showing the distribution and direction of feature effects across samples; **c)** mean absolute SHAP bar plot for WT; and **d)** SHAP beeswarm for WT. coloured by feature value (blue = low, red = high).



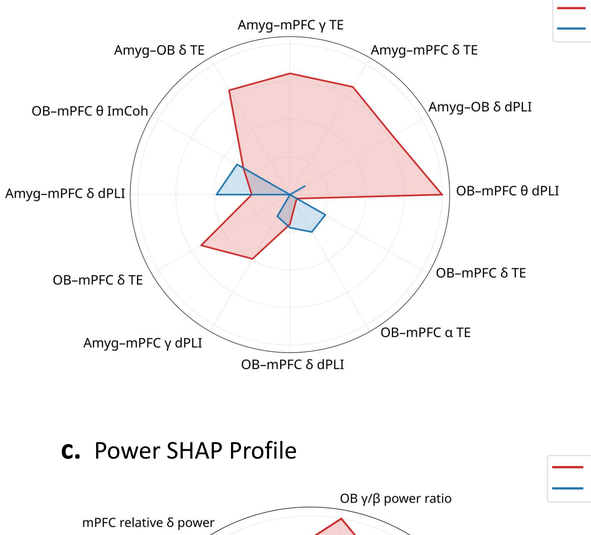
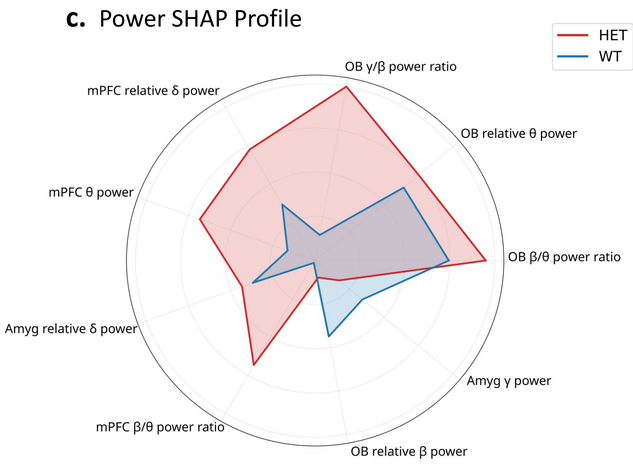


Figure 10: SHAP profiles by feature family. Radar plots comparing the mean |SHAP| of the top features in each family across the two genotype models (HET = *Syngap+/Δ-GAP*: red, WT: blue). Values are normalised to the global maximum; concentric rings indicate 0, 0.25, 0.50, 0.75 and 1.0. **a)** Complexity. **b)** Power. **c)** Connectivity.



Conclusion

1. Elastic Net classifiers decoded freezing from mPFC, amygdala and OB signals with 0.96 accuracy in *Syngap+/Δ-GAP* and 0.94 in WT rats.
2. SHAP analysis identified candidate neural biomarkers of freezing in both genotypes: WT biomarkers included OB β/θ power ratio, mPFC δ spectral entropy, OB δ Hjorth complexity, OB relative θ power, and mPFC δ Hjorth complexity, while *Syngap+/Δ-GAP* biomarkers were mPFC δ spectral entropy and OB δ spectral entropy only.
3. The two biomarker sets partially overlap but are differentially weighted: *Syngap+/Δ-GAP* freezing leans on complexity features, WT on a broader mix of power and complexity.
4. This suggests a weakening of the canonical oscillatory organisation of freezing.
5. Genotype classification was also explored and suggested genotype-specific neural differences, although these results are not shown on this poster.

Future Directions

- Compare power-only and complexity-only feature spaces to clarify their relative explanatory value for freezing classification within each genotype.
- Develop a time-resolved model to predict freezing transitions and identify distinct neural signatures of freezing onset, maintenance, and offset.
- Evaluate the predictive value of habituation-day neural signatures for later recall-day freezing and extinction-related outcomes.

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SFARI SIMONS FOUNDATION AUTISM RESEARCH INITIATIVE

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