Therapeutic Potential of Investigational Drugs for Rett Syndrome Assessed with Behavioral Profiles in Animal Models

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Aims

We used data from the SmartCube® platform and *in silico* AI predictions to identify compounds from a screening library that may be effective in the symptomatic treatment of Rett syndrome.

1. The SmartCube® Platform and Screening Schema

The Mecp2tm1.1Bird line was used as mouse model for Rett syndrome. All experiments were performed in female heterozygous mice (Rett) and wild type controls (WT), aged between 8-12 weeks.

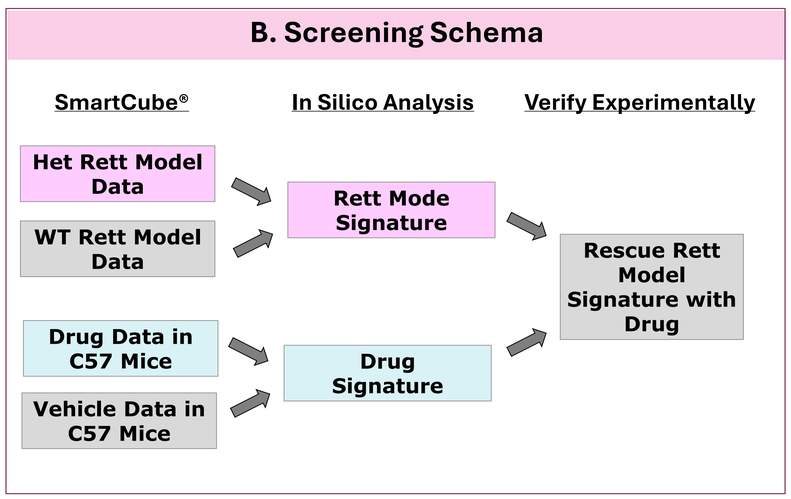
Rett and WT littermate controls were evaluated in SmartCube® (Brunner et al. 2011), an automated testing platform that presents a sequence of challenges to a mouse and collects thousands of behavioral data points from cameras and sensors (**A**).

Our *in silico* screening schema (**B**) used machine learning algorithms to analyze the Rett mouse SmartCube® signature and a database of drug signatures from SmartCube® (**B**).

A. The SmartCube® Platform

SmartCube® is an automated testing platform that presents a sequence of challenges to a mouse and collects and analyzes complex behavioral signatures.

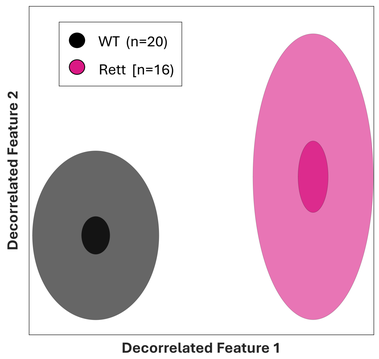
B. Screening Schema



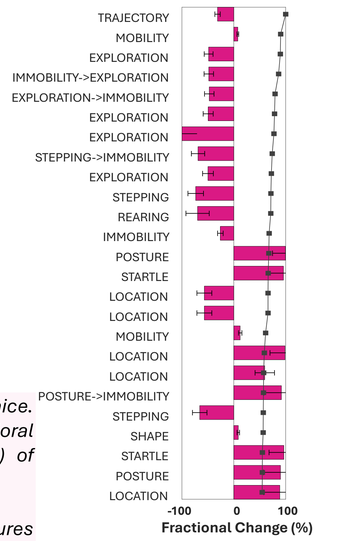
2. SmartCube® Behavioral Analysis of Rett Mice

The SmartCube® behavioral features of the Bird Rett Model and corresponding WT mice were extracted and analyzed using the Decorrelated Ranked-Feature Analysis (DRFA). The data (see cloud depiction, **C**), showed that the two genotypes could be discriminated with a >90% confidence. The top behavioral features that discriminated Rett from WT mice were identified (**D**).

C. Cloud Analysis



D. Feature Analysis



***C**. Phenotypic assessment of Rett and WT mice. DRFA identified a robust overall behavioral phenotype (Discrimination= 93.2%, p<0.0001) of the Rett mice.*

***D.** At the feature-by-feature level, some features were reduced and others increased in the Rett mice as compared against the corresponding WT mice. The strength of the features contributing to the discrimination is shown by the black line.*

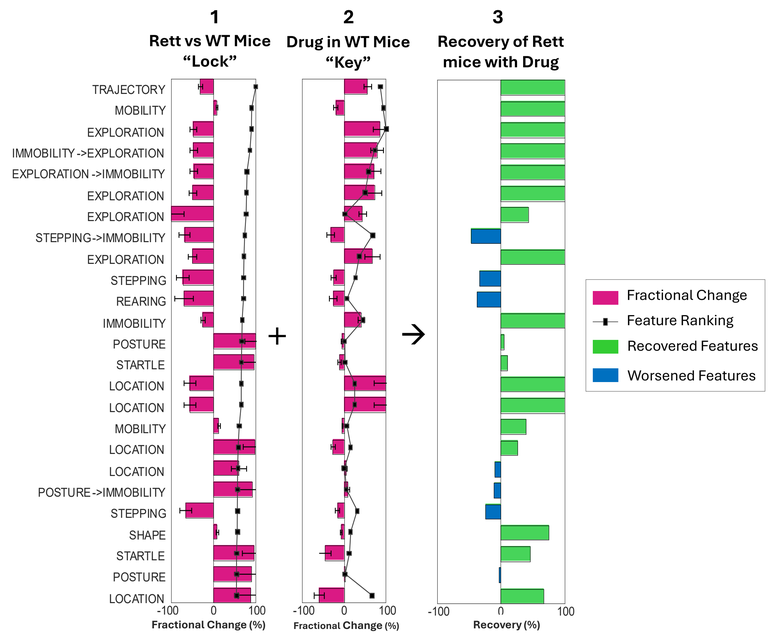
3. *In silico* Prediction of Phenotypic Reversal

Having determined the phenotype of the Rett mice, we then used the Drug-induced Behavioral Signature Analysis (DBSA) (Ambesi-Impiombato *et al.* 2023) to compare the signatures of compounds from a drug library previously tested in C57Tac WT mice.

The screening library was composed of reference compounds for repurposing. The outline of the analysis is represented in Figure **E**.

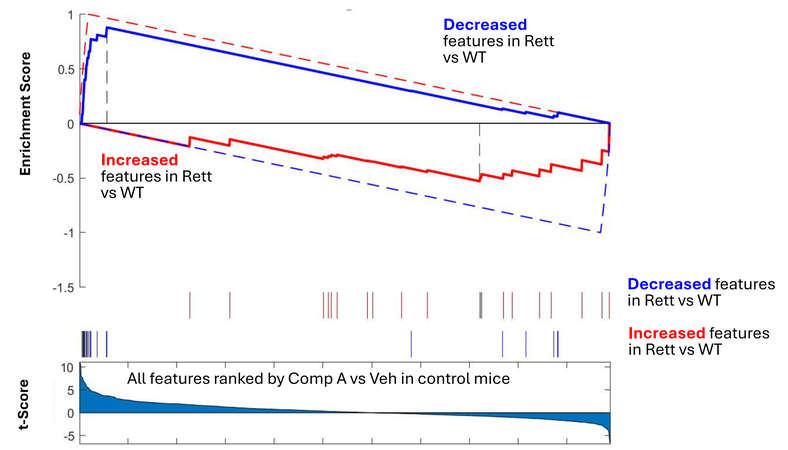
The *in-silico* screening analyses estimated the enrichment of increased and decreased features for each library compound (**F**), identifying Compound A as a potential drug for repurposing.

E. *In Silico* Screening Approach



Fractional changes of top features in Rett vs WT represent a "Lock" signature (Panel 1). Compound A treatment in control mice induces changes mostly in opposite direction, constituting a "Key" signature (Panel 2). Combining the "Key" with the "Lock" predicts recovery of the top features (Panel 3).

F. *In Silico* Screening Results

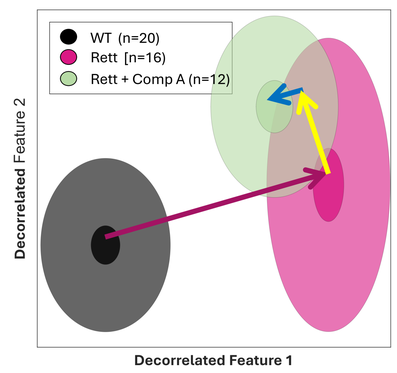


***F.** DBSA uses GSEA statistics, estimating enrichment of increased and decreased features sets combined in a single analysis. The negative Normalized Enrichment Score (NES) indicates reversal of Rett phenotype by **Compound A** at 5 mg/kg. Normalized Enrichment Score (NES)= -2.9, Odds Ratio of Leading-Edge features (OR) = 21.3, P<0.001*

4. *In vivo* Validation Results

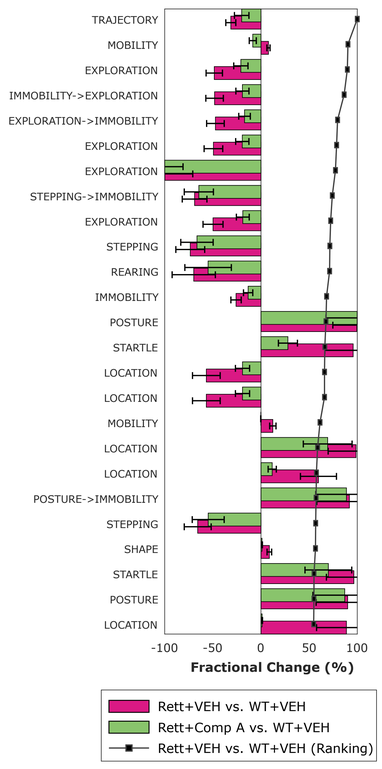
Compound A was tested at several doses in Rett mice in SmartCube®. The results indicated that this compound partially rescued the Rett phenotype (**G**), partially normalizing many of the features (**H**) that were altered in Rett mice.

G. Cloud Analysis



Discrimination: 93.2% (P<0.0001)
Recovery: 23.9% (P=0.0362)

H. Feature Analysis



***G.** Treatment of Rett mice with Compound A at 5 mg/kg showed partial recovery of the phenotype (23.9%, p=0.0362).*

***H.** At the feature-by-feature level, compound A corrected many of the top ranked features that are changed in Rett mice when compared to WT.*

Conclusions

Our AI *in silico* screening method successfully identified Compound A as a potential symptomatic treatment for Rett Syndrome, validating this approach as a viable screening method for drug repurposing for Rett Syndrome and other neurodevelopmental disorders. Our repurposing platform uses behavioral and/or EEG data and can also be used for drug discovery and development.

References

Brunner, D., et al. (2011). Method For Predicting Treatment Classes Using Behavior Informatics. US 7882135 B2. (Priority date 2001.)

Ambesi-Impiombato, A., K. Cox, S. Ramboz, D. Brunner, M. Bansal and E. Leahy (2023). "Enrichment analysis of phenotypic data for drug repurposing in rare diseases." Front. Pharmacol. 14: 1128562.