Elucidating Relationships within Neurological Screening Batteries via Random Forest-Based Hypothesis Testing

Sambit Panda[1], Leslie R. Wilson[2], Jariatu Stallone[2], Dalisa Kendricks[2], Jesse D. Cushman[2]

[1]Johns Hopkins Medical Institute, [2]Neurobehavioral Core, NIEHS

Email: spanda3@jhu.edu
Website: sampan.me

SHIRPA

Standardized set of experimental procedures used as a rapid neurological assessment of mice, with up to **40 tests**. These include:

View Jar | Grip Strength | Open Field

And so much more…

- Body position
- Motor activity
- Acoustic startle
- Piloerection
- Negative geotaxis
- Etc.

Mouse	Group	Locomotor Activity	Grip Strength
10	WT	24	0.1
7.2	Mutant	18	0.12
34	Mutant	17	0.22
20	WT	33	0.05
8.8	Mutant	15	0.3

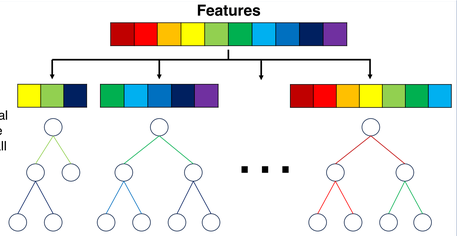
Mutant

WT

Goal:
Jointly analyze SHIRPA screens and rank the screens by importance.

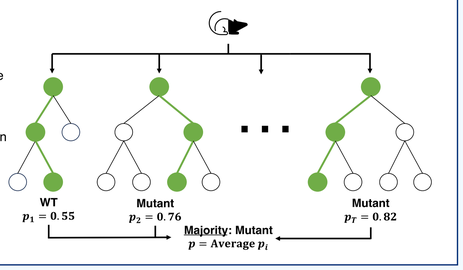
Random Forest (RF)

Training:



Feature Importance can be computed by looking at the total decrease in node impurity across all trees.

Testing:

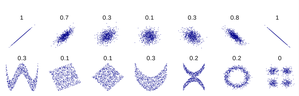


Similarity can be computed by looking at how often two observations lie in the same leaf node across all trees.

KMERF Test

Compute similarities via RF

Exact Equivalence



Estimate Pairwise Distance Matrices

Linear Algebra

Calculate Distance Correlation Statistic

RF Feature Importance

Permutation Test

Calculate Feature Importance

Calculate P-value

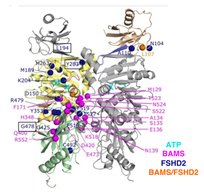
Bosma Arhinia Microphthalmia Syndrome (BAMS) Study

Background:

Bosma Arhinia Microphthalmia Syndrome (BAMS) is defined by a triad:
- Arhinia (absent nose)
- Anterior eye defects – Microphthalmia, Cataracts, Coloboma (iris)
- Hypogonadotropic Hypogonadism – GnRH Deficiency

90% of cases involve a SMCHD1 mutation

Pediatric Neuroendocrinology Group



Coker 2014, Chen 2015, Brideau 2015, Wilke 2017, Pedersen 2019

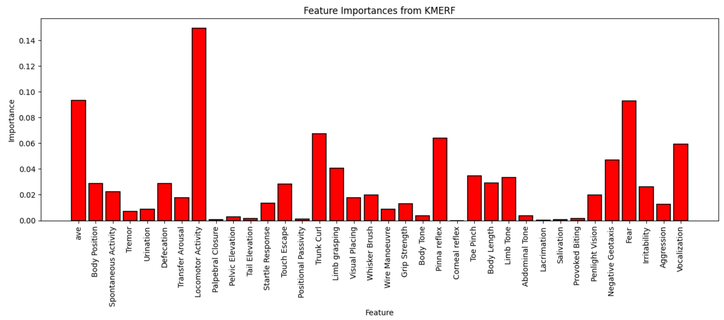
Dataset:

L141F*Smchd1 (Arhinia) Mouse line was created by the NIEHS KO Mouse Core.

WT 16 Males | **Het Mutant** 15 Males | **Homo Mutant** 11 Males

WT vs Homo Mutant:

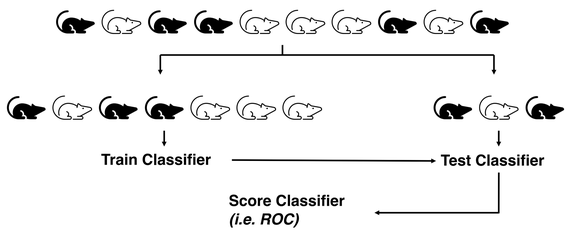
1. Run the KMERF on the dataset

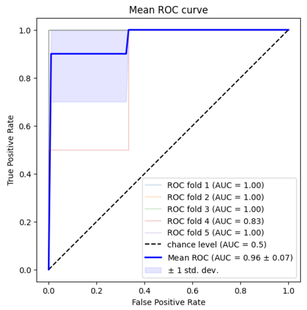


Test Statistic: 0.85

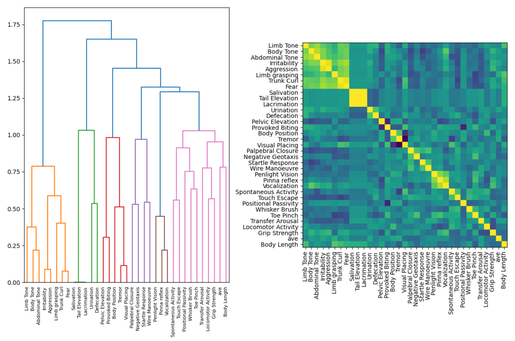
P-value: 0.002

2. Evaluation of Performance for Random Forest (or any classifier)





3. Hierarchical Clustering of Correlations of Features

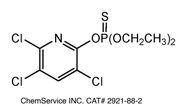


Note:
- *Other pairwise comparisons are not significant for this dataset, so feature importances for them are not as meaningful. Accuracy was hovering around chance for those.*
- *Only males were included since homo females are embryonically lethal. Further analysis will need to be done to explore this phenomenon.*

Chlorpyrifos (CPF) Study

Background:

- **Chlorpyrifos** is an organophosphate Insecticide and Acetylcholinesterase (AChE) inhibitor
- It is banned from use by the EPA in 2021 (EPA-HQ-OPP-2021-0523-001)



ChemService INC. CAT# 2921-88-2

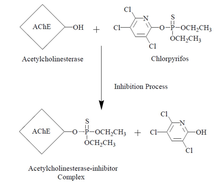


Fig.1. **Inhibition of acetylcholinesterase by the pesticide chlorpyrifos.**
Račáková 2006

Goal: Test Efficacy of SHIRPA to detect neurobehavioral deficits and determine subsequent threshold for studies using CPF

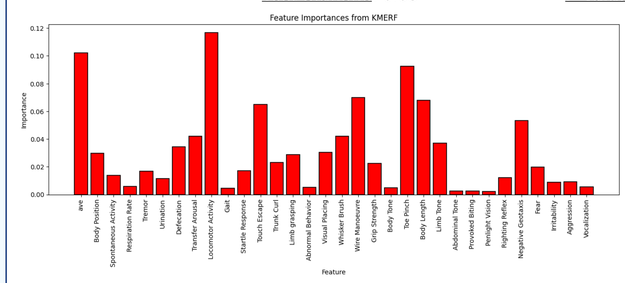
Dataset:

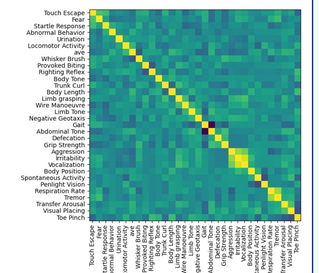
Mice were dosed with **CPF (5 mg/kg)**

WT 11 Females, 8 Males | **Exposed** 11 Females, 9 Males

SHIRPA Battery:

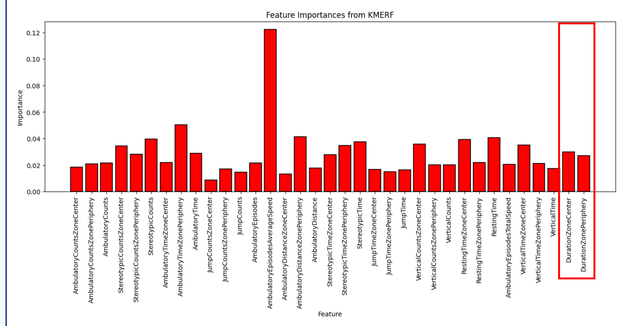
Test Statistic: 0.68 **P-value:** 0.06

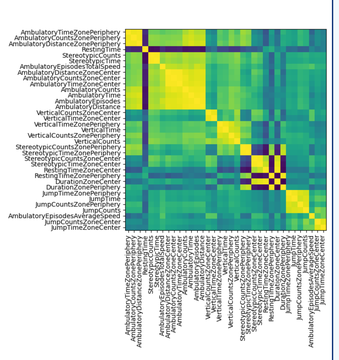




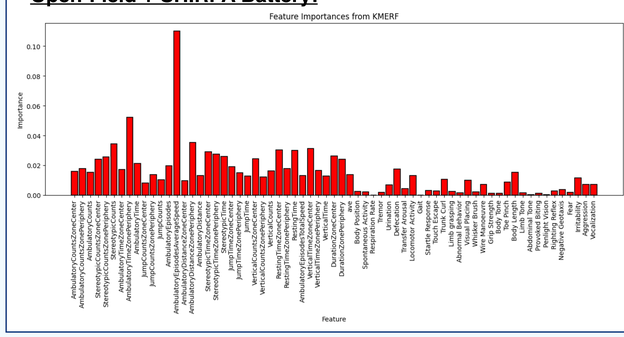
Open-Field Data:

Test Statistic: 0.67 **P-value:** 0.005





Open-Field + SHIRPA Battery:



Test Statistic: 0.73 **P-value:** 0.003

Summary:
- *Addition of open-field measures creates a significant difference between the exposed and unexposed mice.*
- *KMERF found measures that were previously ignored as being being important.*
- *Caveat: Feature importances for continuous features are upweighted.*

Conclusion

- SHIRPA screens alone seem to be a good metric to determine differences between groups, and significant differences exist in all groups studied.
- KMERF discovered novel behavioral features in the open field results that had previously been ignored.
- We show the utility of machine-learning approaches like KMERF to find underlying dependencies that conventional approaches cannot, and how we can apply these methods to improve current neurological screening batteries.

References & Acknowledgements

- Hatcher, J. P., Jones, D. N. C., Rogers, D. C., Hatcher, P. D., Reavill, C., Hagan, J. J., & Hunter, A. J. (2001). Development of SHIRPA to characterise the phenotype of gene-targeted mice. Behavioural brain research.
- Breiman, L. (2001). Random forests. Machine learning, 45, 5-32.
- Székely, G. J., Rizzo, M. L., & Bakirov, N. K. (2007). Measuring and testing dependence by correlation of distances.
- Shen, C., Panda, S., & Vogelstein, J. T. (2018). Learning Interpretable Characteristic Kernels via Decision Forests. arXiv preprint arXiv:1812.00029.
- Shaw, N. D., Brand, H., Kupchinsky, Z. A., Bengani, H., Plummer, L., Jones, T. I., ... & Talkowski, M. E. (2017). SMCHD1 mutations associated with a rare muscular dystrophy can also cause isolated arhinia and Bosma arhinia microphthalmia syndrome. *Nature genetics*, *49*(2), 238-248.
- Lan, A., Stein, D., Portillo, M., Toiber, D., & Kofman, O. (2019). Impaired innate and conditioned social behavior in adult C57Bl6/J mice prenatally exposed to chlorpyrifos. Behavioral and Brain Functions, 15(1), 1-9.
- Shen, C., & Vogelstein, J. T. (2021). The exact equivalence of distance and kernel methods in hypothesis testing. AStA Advances in Statistical Analysis, 105, 385-403.

Acknowledgements: NeuroData Lab (Cencheng Shen, Joshua Vogelstein), **Neurobehavioral Core (**Korey Stevanovic, Jared Glorius, DaNashia Thomas, Sydney Fry), **Pediatric Neuroendocrinology Group** (Mert Icyuz Natalie Shaw), **Jensen Lab (**Rebecca Arechevala, Joseph Valdez, Patricia Jensen), **Comparative Medicine Branch**