



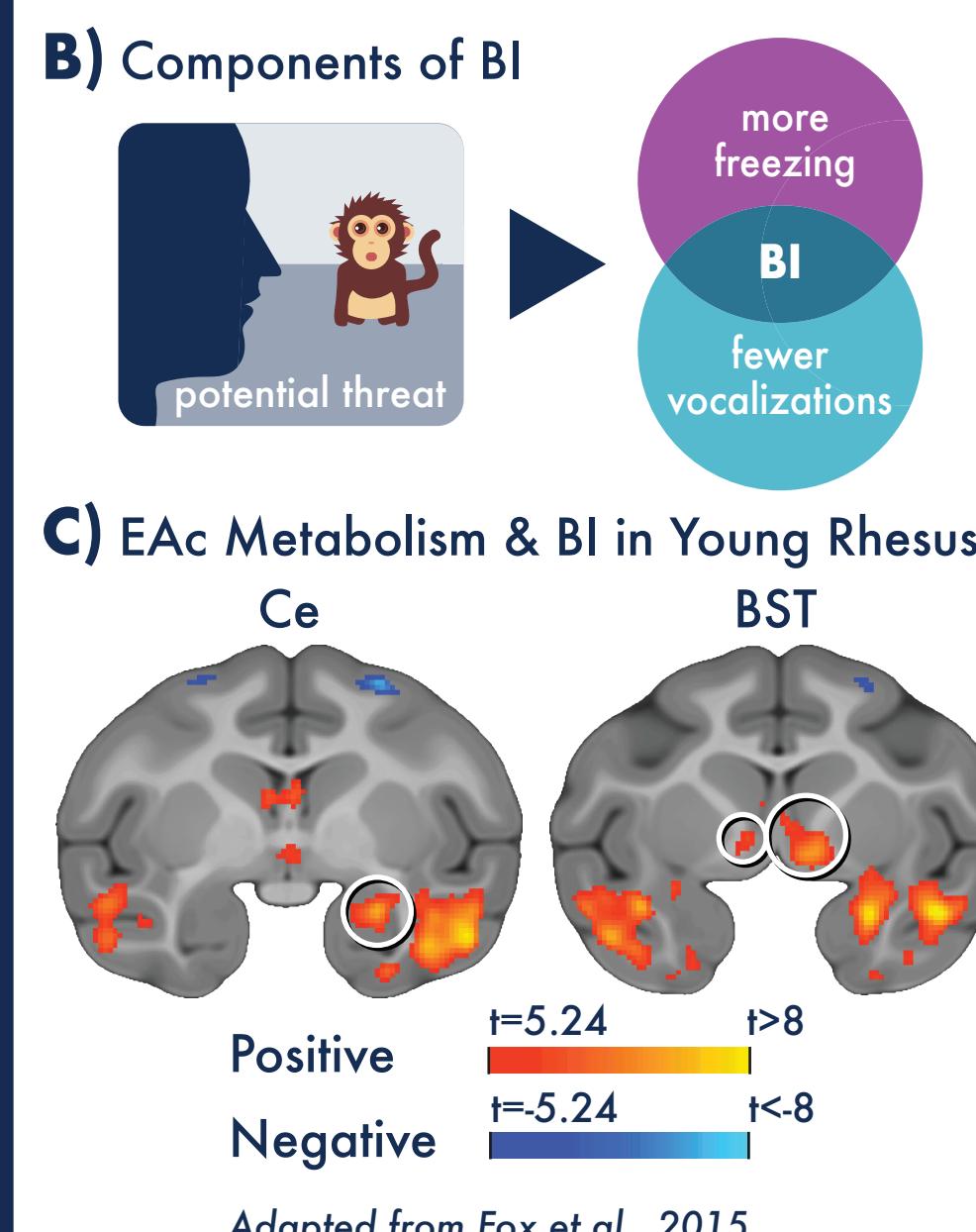
# Investigating Anxiety-Related Behavior in Young Primates: A Novel, Computer-Automated Approach

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## Background

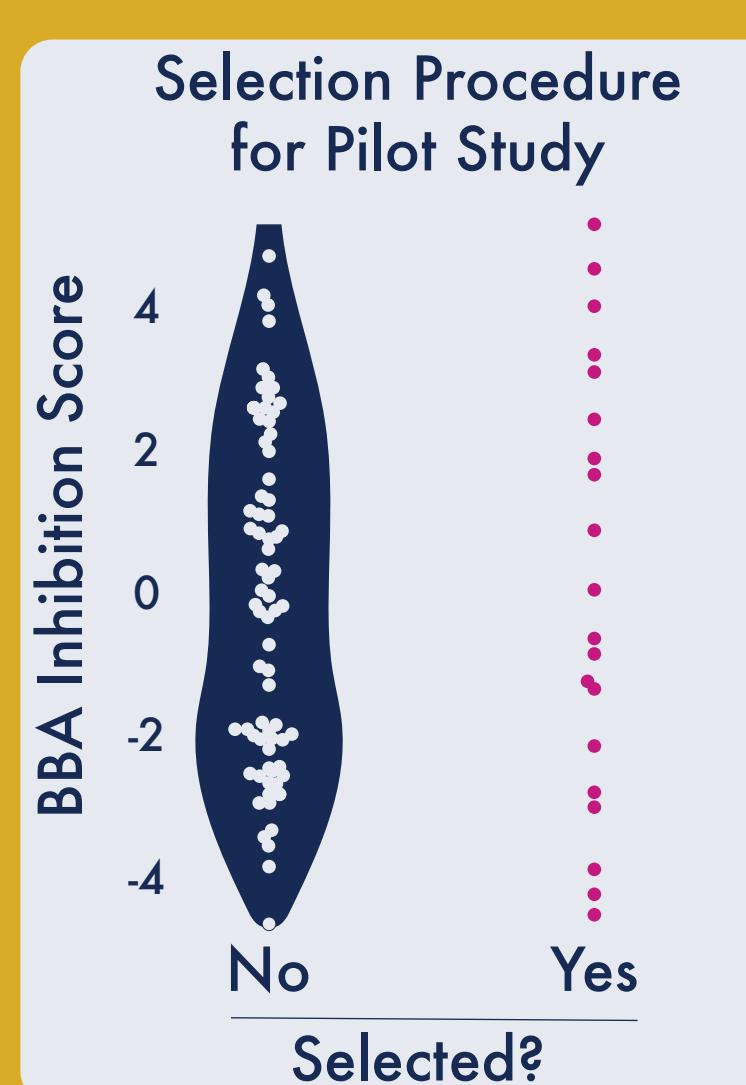
Stress and anxiety disorders adversely affect millions, degrading human health and wellbeing on a massive scale<sup>1,2</sup>. Understanding the neural substrates of these disorders will contribute to pharmacological and lifestyle interventions that promise to usher in a new era of human flourishing. A growing body of research implicates individual variation in the **extended amygdala** (EAc; **A**) in aberrant threat-processing<sup>3,4</sup>. In light of the emerging view that maladaptive threat response underlies stress-related psychopathology<sup>5</sup>, the EAc forms a clinically intriguing circuit.



Fox Lab is interested in the relationship between early-life **behavioral inhibition** (BI; **B**,<sup>4,5</sup>) and dysregulation of the EAc. Recently, a study of 592 rhesus monkeys (*Macaca mulatta*) linked early-life BI to EAc metabolism (**C**,<sup>4</sup>). However, questions about individual EAc variation warrant additional large-scale studies, ideally with animals raised in naturalistic conditions.

Hand-scoring hundreds of hours of subject footage in such studies is a major bottleneck. To overcome this obstacle, we have developed a machine-learning toolkit that automatically analyzes behavior. Here we detail a pilot study in which our methods were validated, showing great promise.

## Human-Intruder Pilot Study



The California National Primate Research Center's (CNPRC) Bio-Behavioral Assessment (BBA) program has categorized over 4,000 animals' early-life biobehavioral organization since 2001<sup>7</sup>. The BBA program has been instrumental in improving our understanding of how the biobehavioral organization of nonhuman primates raised in large, naturalistic colonies can change across the lifespan. Using BBA data to ensure our subjects represented the full spectrum of behavioral-inhibition scores, we selected a stratified sample of 20 females (of 98 total) aged 2-3 years [mean age = 2.75 years; SD = 0.47] for our pilot study (**A**).

**B)** FDG

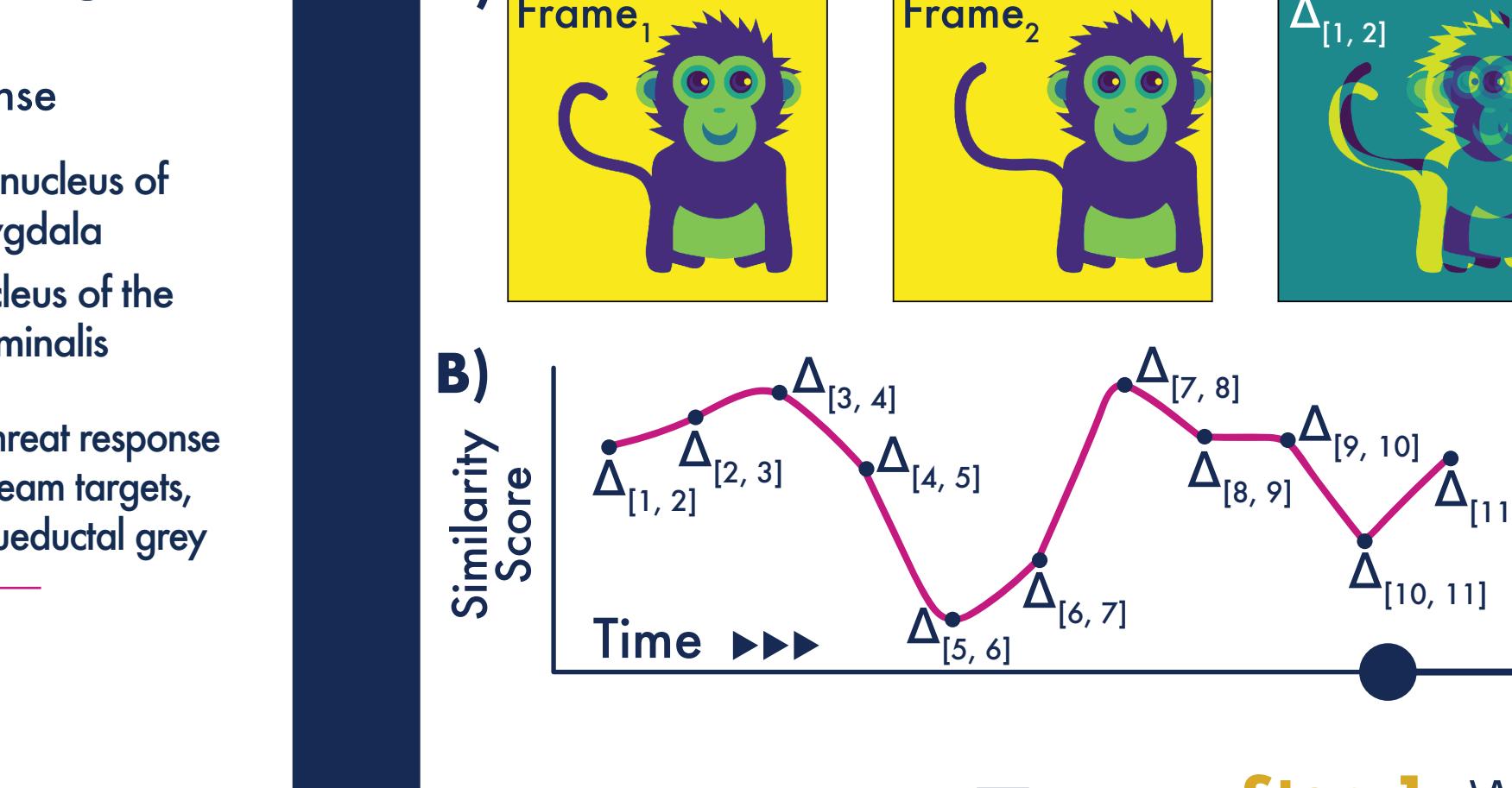
We injected each animal with <sup>18</sup>F labeled 2-fluoro-2-deoxy-D-glucose, subjected them to the no-eye-contact condition of the human-intruder paradigm (NEC; an anxiogenic behavioral assay that elicits freezing<sup>8</sup>), and PET-scanned them under anesthesia to evaluate EAc metabolism in response to a potential threat (**B**). Upon recovery from anesthesia, each animal was returned to its respective outdoor colony.

## References

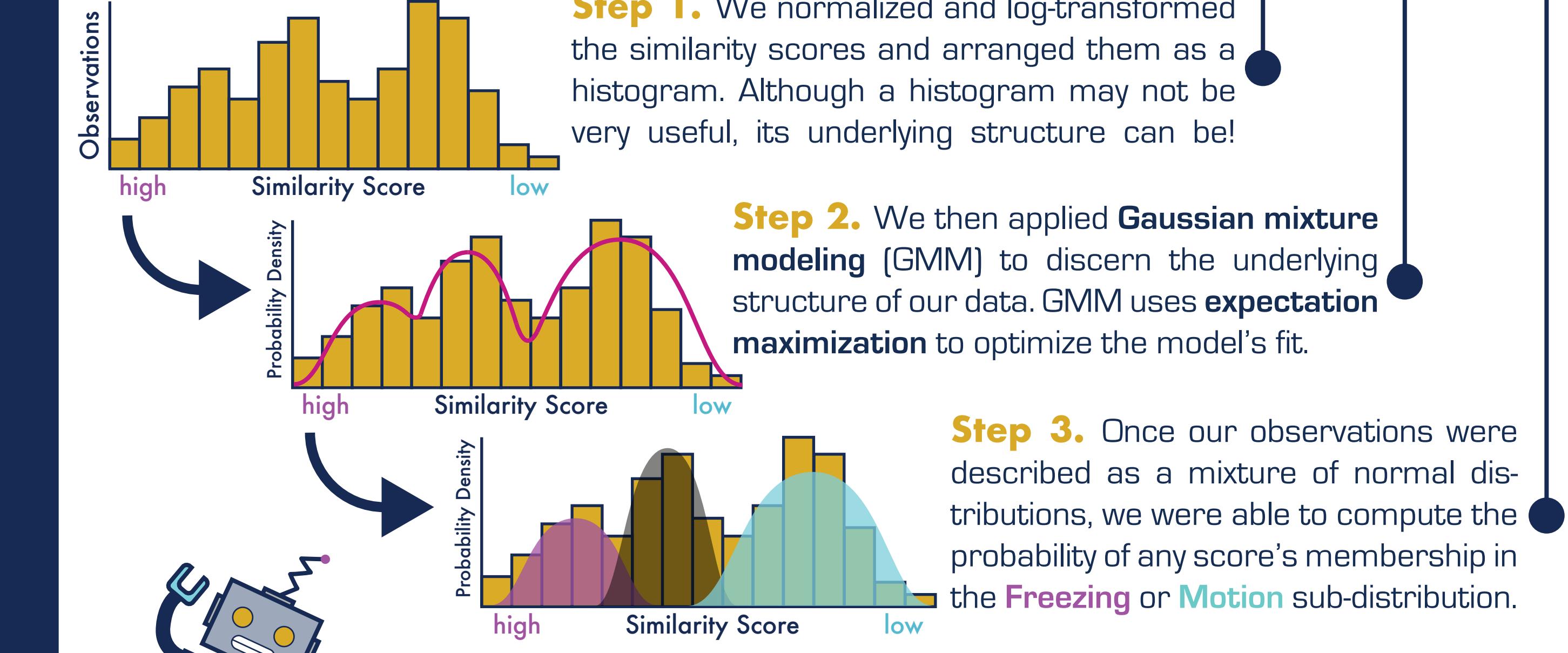
- <sup>1</sup>Bandelow, B., & Michaelis, S. (2015). Epidemiology of anxiety disorders in the 21st century. *Dialogues in Clinical Neuroscience*, 17(3), 327-335.
- <sup>2</sup>Rice, P., & Miller, L. S. (1992). The economic burden of anxiety disorders. *British Journal of Psychiatry [Supplement]*, April(27), 34-42.
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- <sup>4</sup>Fox, A. S., et al. (2015). Intergenerational neural mediators of early-life anxious temperament. *Proc. Natl. Acad. Sc. U. S. A.* 112, 9118-9122.
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- <sup>8</sup>Kalin, N. H., & Shelton, S. E. (1998). Ontogeny and stability of separation and threat-induced defensive behaviors in rhesus monkeys during the first year of life. *American Journal of Primatology*, 44(2), 125-135.

## Machine-Learning Approach

### Converting Full-Motion Video to Vectorized Data

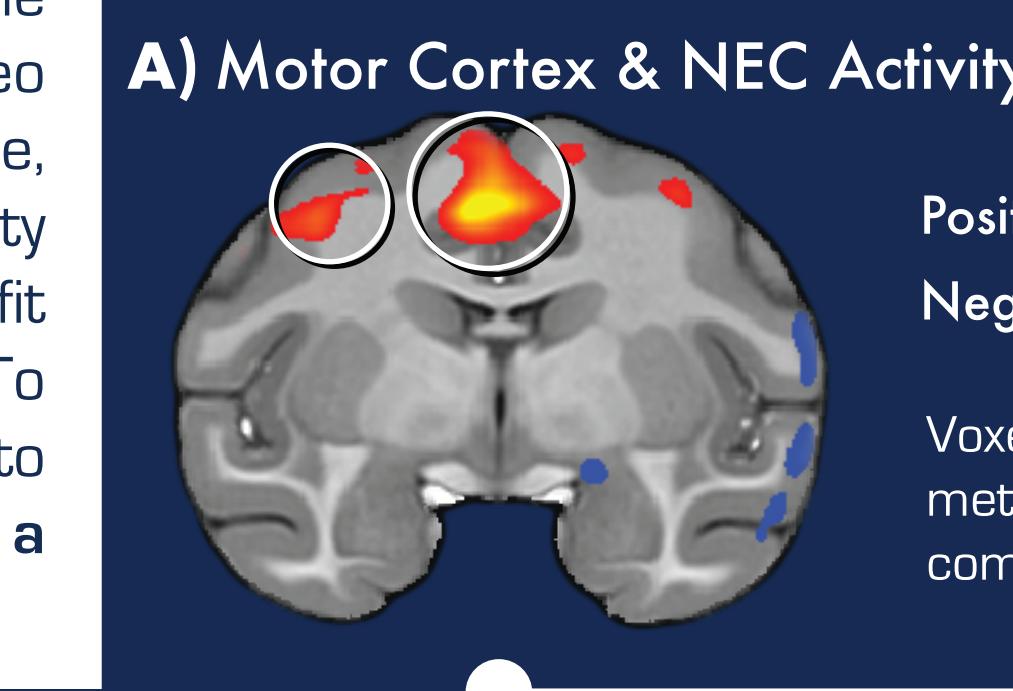


### One-Dimensional Gaussian Mixture Modeling



## Results

### Validation



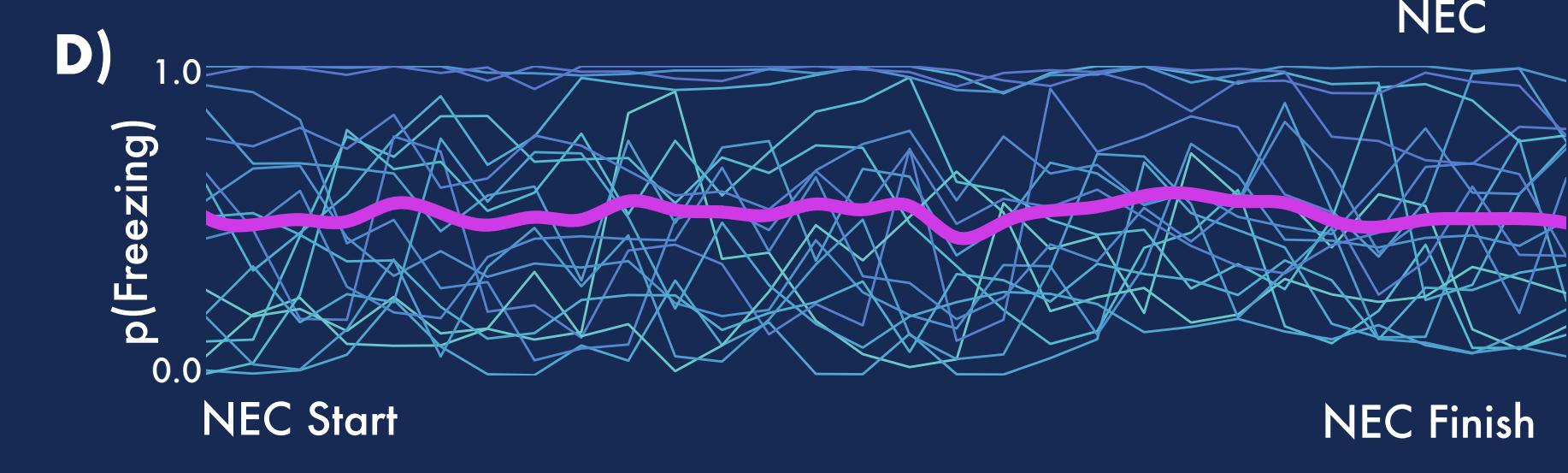
**B)** Validated Against Human Raters!

Chronbach's $\alpha$ : .979	ICC: .961	Sensitivity: 92%	Specificity: 100%
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Voxelwise analysis revealed a significant association between subjects' NEC activity and motor-cortex metabolism (**A**;  $p < .05$ , uncorrected). Our machine-learning approach achieved high validation measures compared to four human raters in a trial classifying 100 randomly sampled NEC video segments (**B**).

### Findings

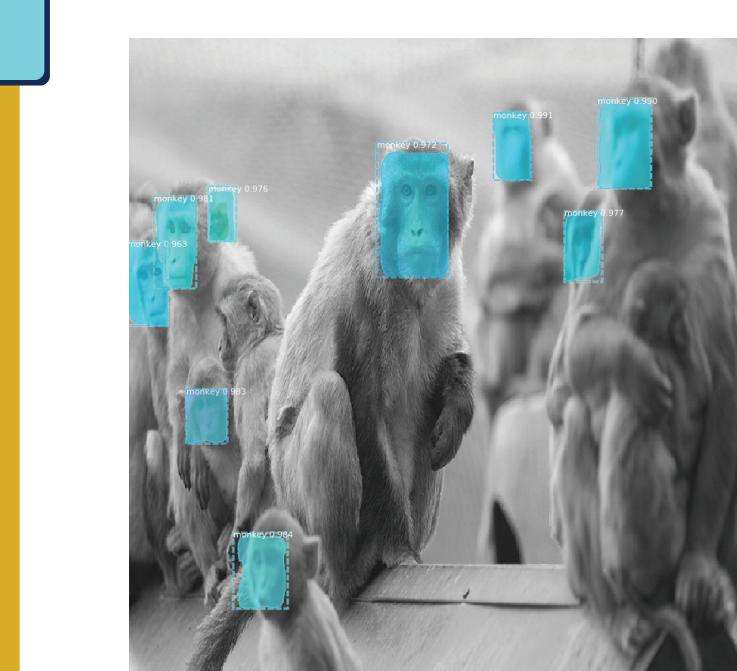
Regression analysis revealed a significant association between early-life nervousness and freezing 2-3 years later (**C**). Intriguingly, the general trend in these data supports the view that a tendency to express negative affect early in life can predict behavioral inhibition many years later<sup>3,5</sup>. We predicted that our subjects would adjust to the presence of the NEC's human intruder. However, neither subjects' probability of freezing per minute (**D**) nor their total freezing time across the two halves of the NEC (**E**) supported this hypothesis.



## Outlook

The rise of machine learning presents exciting opportunities for behavioral neuroscientists. The analyses described here would be immensely time consuming using less-sophisticated methods, yet they are rendered trivial by the right machine-learning toolkit.

Fox Lab will continue to push the boundaries of computer automation. For instance, we plan to pioneer deep learning facial-recognition to track specific colony animals as they freely engage in complex social behaviors.



By developing novel, computer-based approaches to relate multimodal in-lab measures (e.g., NEC freezing and vocalizations, neuroimaging, hematology, etc.) to naturalistic behaviors (e.g., tendency to approach conspecifics, solitary hunching, etc.), we hope to shine new light on behavioral inhibition, from neural substrates to complex social behaviors. In conclusion, machine-learning approaches like ours promise to reveal a host of intriguing findings that might otherwise remain inaccessible, hidden behind the veil of human perceptual and computational limitations.

## Acknowledgments and Contact

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Please contact Dan Holley ([dholley@ucdavis.edu](mailto:dholley@ucdavis.edu)) with questions or feedback.

Thank you for your interest in our work!