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

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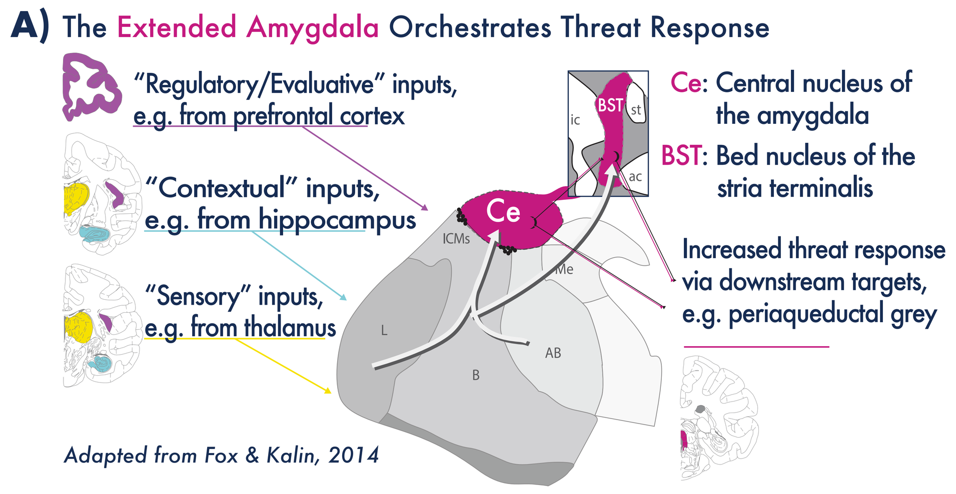
Background

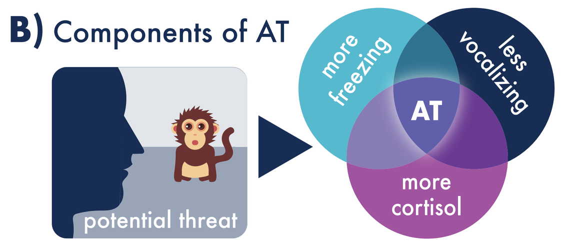
Stress and anxiety disorders adversely affect millions, degrading human health and wellbeing on a massive scale[1,2]. Understanding the neural substrates of these disorders will contribute to pharmacological and lifestyle interventions that promise to usher in a new era of human flourishing. A growing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

A) The Extended Amygdala Orchestrates Threat Response

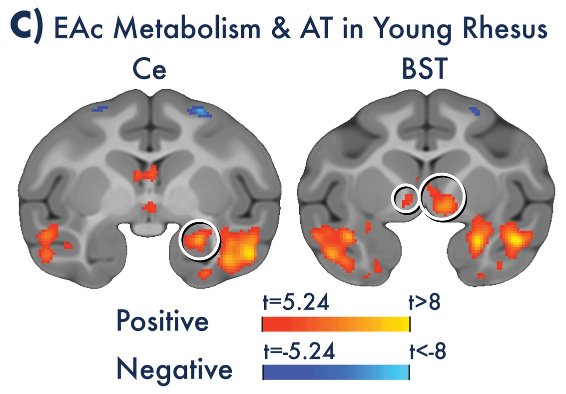


Adapted from Fox & Kalin, 2014

B) Components of AT



C) EAc Metabolism & AT in Young Rhesus



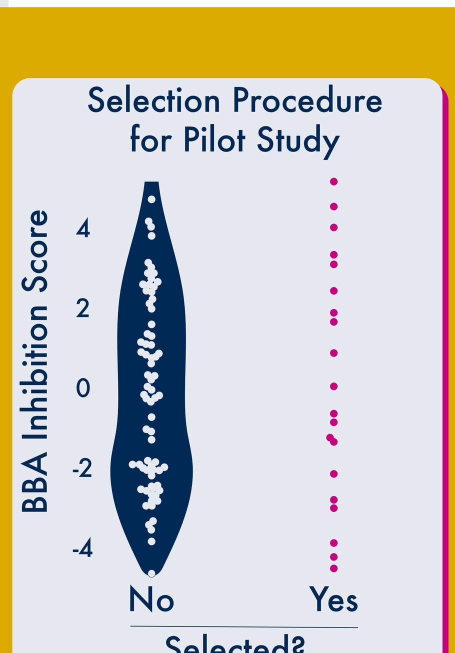
Adapted from Fox et al., 2015

Fox Lab is interested in the relationship between early-life **anxious temperament** (AT; **B**,[4]) and dysregulation of the EAc. Recently, a study of 592 rhesus monkeys (Macaca mulatta) linked early-life AT to EAc metabolism (**C**,[4]). 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.

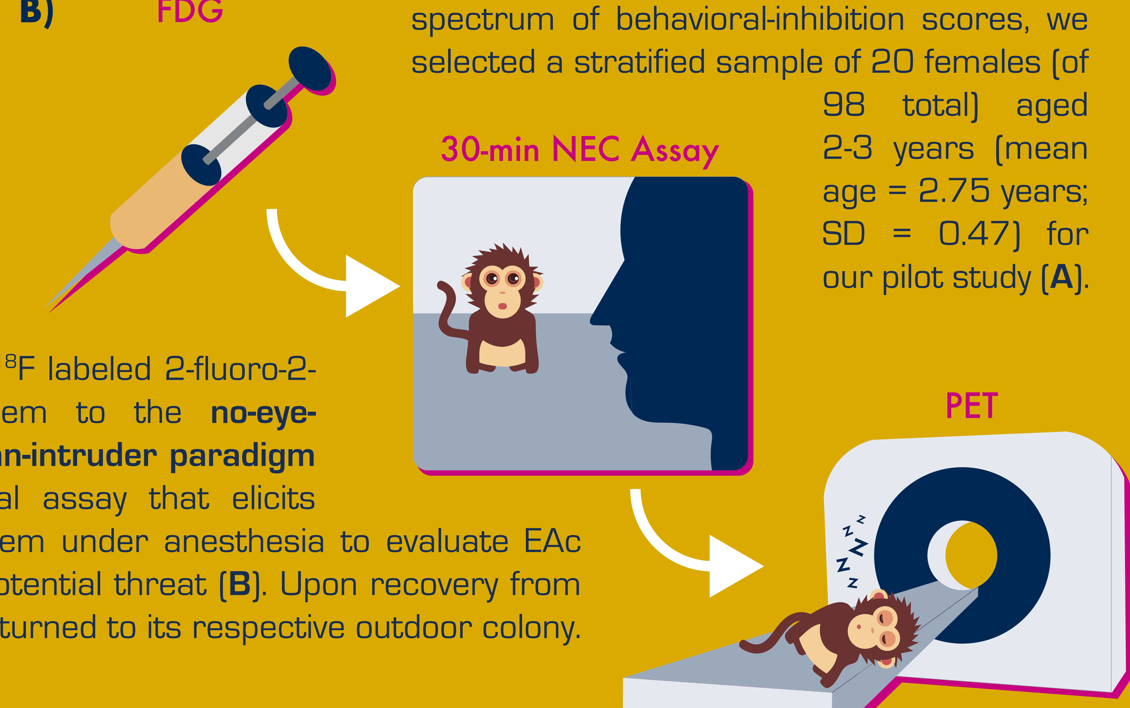
Human-Intruder Pilot Study

A)



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[6]. 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)



We injected each animal with ^{18}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[7]), 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

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[2]Rice, D. P., & Miller, L. S. (1995). The economic burden of affective disorders. *British Journal of Psychiatry* [Supplement], April(27), 34-42.

[3]Fox, A. S., & Kalin, N. H. (2014). A translational neuroscience approach to understanding the development of social anxiety disorder and its pathophysiology. *American Journal of Psychiatry 171*, 1162-1173.

[4]Fox, A. S., et al. (2015). Intergenerational neural mediators of early-life anxious temperament. *Proc. Natl. Acad. Sci. U. S. A. 112*, 9118-9122.

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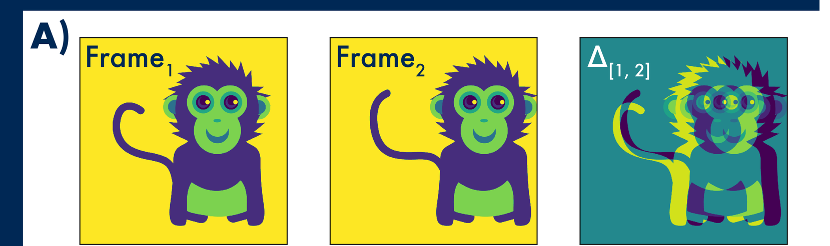
[7]Kalin, N. H., & Shelton, S. E. (1998). Ontogeny and stability of separation and threat-induced defensive behaviors in rhesus monkeys during the first year of life. *American Journal of Primatology, 44*(2), 125-13

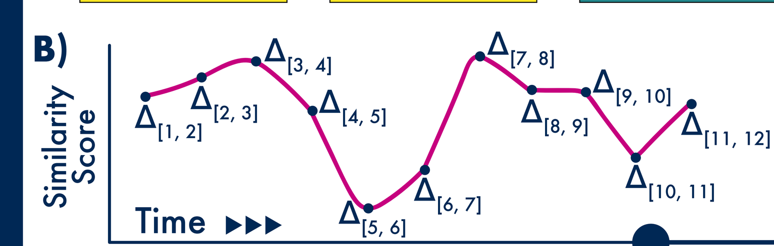
Machine-Learning Approach

Converting Full-Motion Video to Vectorized Data

A)



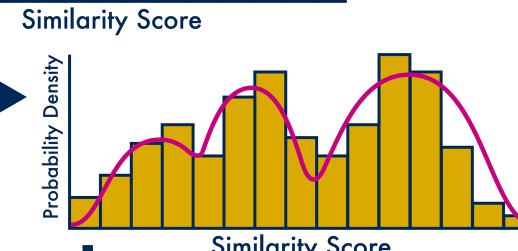
B)



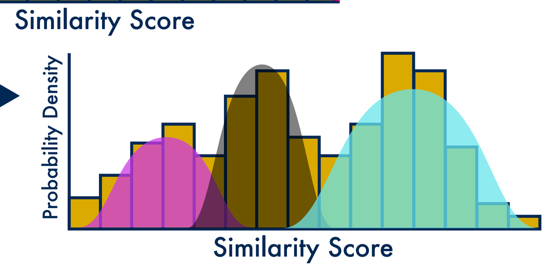
We recorded each subject's response to the NEC at 30 fps. Using Python, we broke the video into frames, converted the frames to greyscale, and vectorized them before computing similarity scores (r^2) between frames (**A**). Next, we fit these scores to the video's time course (**B**). To test whether patterns in these data mapped to anxiety-like behaviors, **we passed them into a generative clustering algorithm.**

One-Dimensional Gaussian Mixture Modeling

Step 1. We normalized and log-transformed the similarity scores and arranged them as a histogram. Although a histogram may not be very useful, its underlying structure can be!



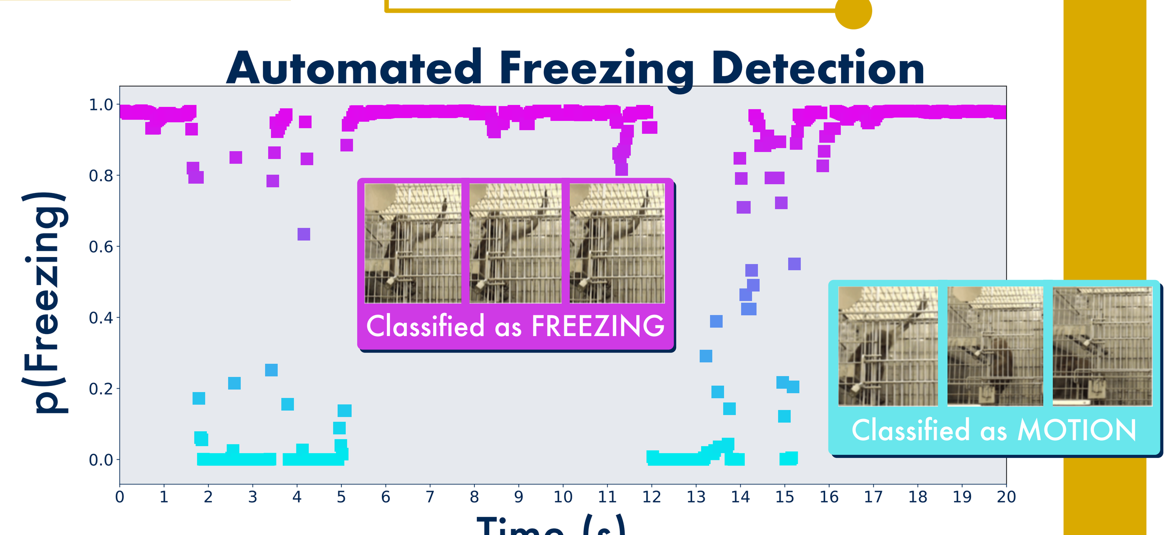
Step 2. We then applied **Gaussian mixture modeling** (GMM) to discern the underlying structure of our data. GMM uses **expectation maximization** to optimize the model's fit.



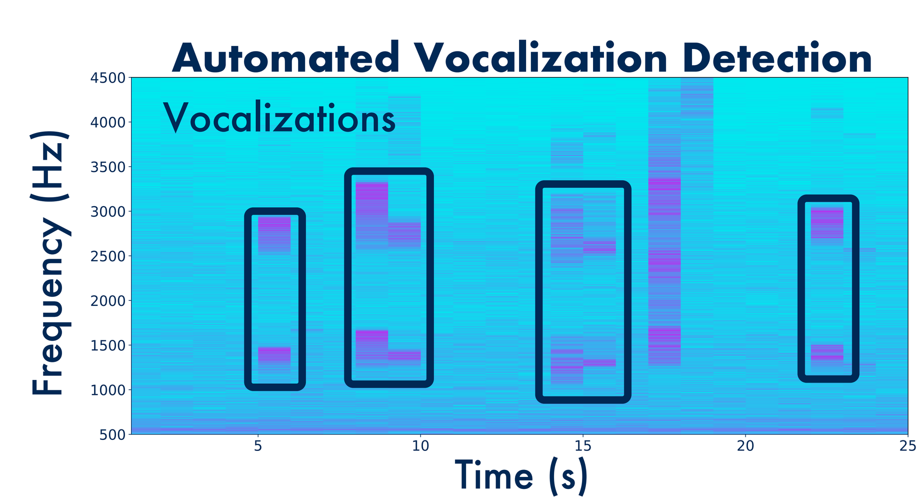
Step 3. Once our observations were described as a mixture of normal distributions, we were able to compute the probability of any score's membership in the Freezing or Motion sub-distribution.

Machine Learning Can Accurately Classify Behavior

We reconstructed the time course of the NEC by plotting the posterior probability of each similarity score's membership within our GMM's Freezing sub-distribution. This allowed us to assess the probability of subjects' freezing behavior at any time, and to precisely compare behavioral and multimodal data.



Automated Spectral Analysis of Vocalizations



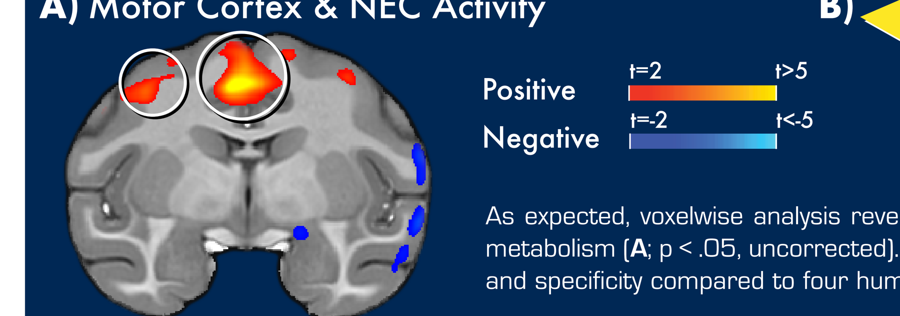
Since reduced vocalizing is a component of anxious temperament[4], Fox Lab has developed a spectral analysis tool that automatically:

1. Isolates subjects' audio signals
2. Fourier-transforms those signals
3. Finds patterns in the Fourier data, and
4. Outputs vocalization metrics.

Results

Validation

A) Motor Cortex & NEC Activity



B)

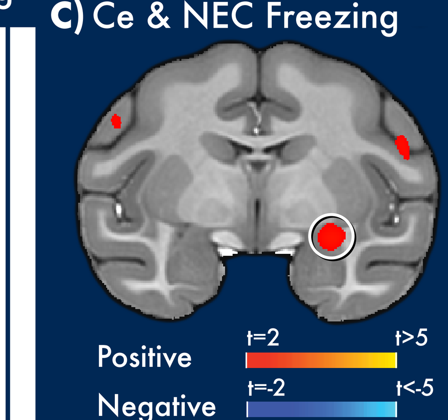
Validated Against Human Raters!

Chronbach's α:	ICC:	Sensitivity:	Specificity:
.979	.961	92%	100%

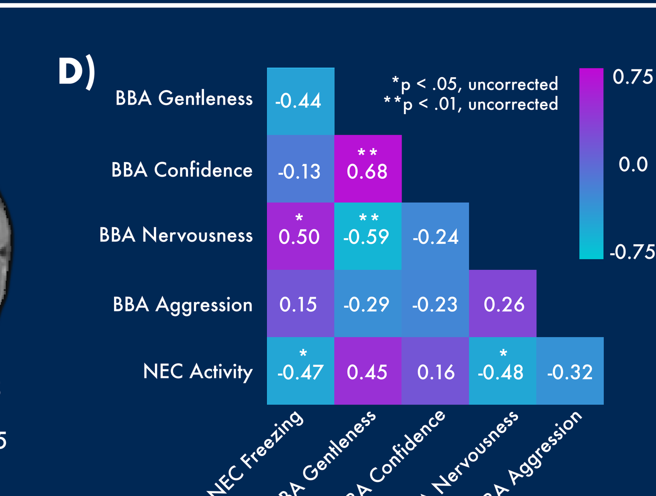
As expected, voxelwise analysis revealed a significant association between subjects' NEC activity and motor-cortex metabolism (**A**; p < .05, uncorrected). Our machine-learning approach achieved high measures of reliability, sensitivity, and specificity compared to four human raters in a trial classifying 100 randomly sampled NEC video segments (**B**).

Findings

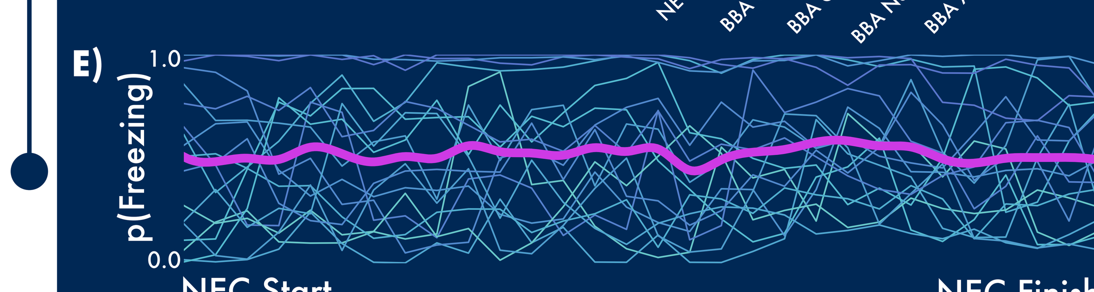
C) Ce & NEC Freezing



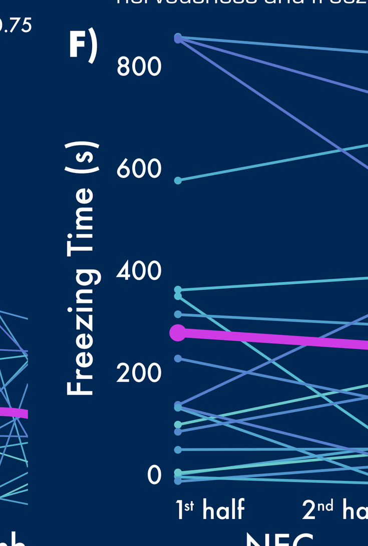
D)



E)



F)

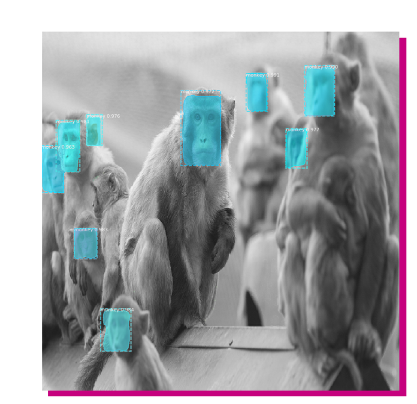


Voxelwise analysis revealed a significant association between subjects' total NEC freezing episodes and Ce metabolism (**C**; p < .05, uncorrected). Regression analysis revealed a significant association between early-life nervousness and freezing 2-3 years later. Intriguingly, the general trend in these data support the view that a tendency to express negative affect early in life can predict aberrant threat-processing (i.e., anxious temperament) many years later (**D**, [3,4]). We predicted that our subjects would adjust to the presence of the NEC's human intruder. Curiously, however, neither subjects' probability of freezing per minute (**E**) nor their total freezing time across the two halves of the NEC (**F**) 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 the neural substrates and behavioral nuances of anxious temperament. 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

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