Freezing is Associated with Higher Amygdala Metabolism in Female Adolescent Monkeys

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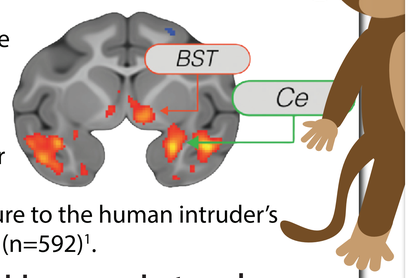
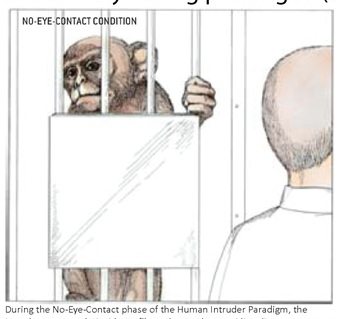
The Role of the Extended Amygdala

The extended amygdala has long been implicated as playing a critical role in the experience of fear and anxiety. Previous studies using FDGPET found that metabolism in the Ce and BST was associated with heightened signs of fear and anxiety during prolonged (30 min) exposure to the human intruder's profile (n=592)[1].





During the No-Eye-Contact phase of the Human Intruder Paradigm, the intruder presents their side profile to the monkey, avoiding direct eye contact.
Image: Kalin, 1997

Human Intruder Paradigm

The Human Intruder Paradigm was developed in macaques by Kalin and Shelton[2] to model earlier studies done on behaviorally inhibited children. Here, we used the No-Eye-Contact (NEC) phase of the Human Intruder Paradigm.

The California National Primate Research Center

With the majority of animals living in large, half-acre outdoor field corrals, the California National Primate Research Center (CNPRC) offers a unique opportunity to study animals who have been reared in natural social groups, similar to those found in the wild.

Methods

Animal Selection: Twenty 2-3 year old, female, rhesus macaque monkeys were selected using a stratified sampling approach from a list of 98 potential animals.

FDG-PET: Each animal was injected with [18F]fludeoxyglucose (FDG) prior to behavioral testing. Immediately after the injection, animals were exposed to a NEC-Human Intruder Paradigm for 30 minutes. After behavioral testing, animals underwent a positron emission tomography scan (PET) using a piPET scanner.

Behavioral data: Similarity of video frames were vectorized and a one dimensional gaussian mixture model was used to probabilistically categorize each frame into different freezing/motion categories[3].

FDG-PET Data Collection Timeline

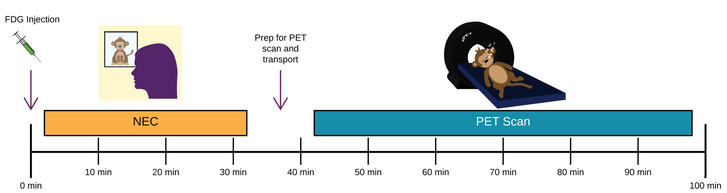
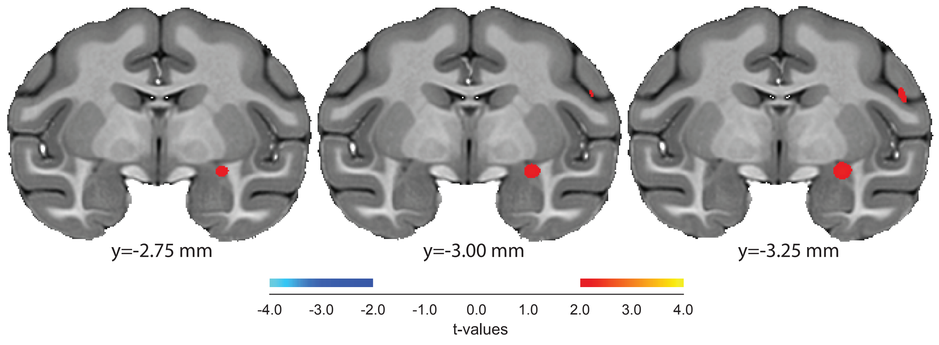


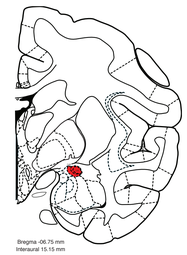
Image Processing: All T1-weighted images were manually masked to exclude non-brain tissue. Linear registration was performed using Advanced Normalization Tools (ANTS) to align each animal's PET scans to their respective T1 image. T1 weighted images were aligned to the National Institute of Mental Health Macaque Template (NMT) using ANTS. Finally, a non-linear registration was performed to align PET scans to template space.

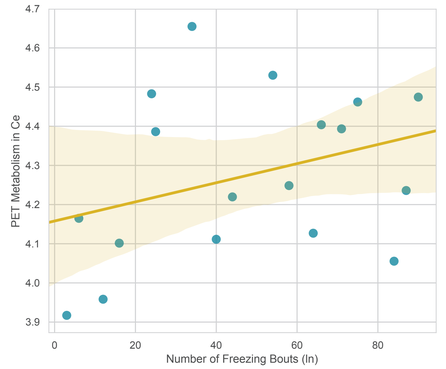
Results

Whole brain voxelwise analyses using FSL's randomize demonstrated a significant correlation between amount of time freezing to the human intruder and metabolism in a cluster that included the dorsal amygdala region, including portions of the central nucleus of the amygdala (Ce) (p<.05, two-tailed uncorrected).

Number of Freezing Bouts and PET Metabolism

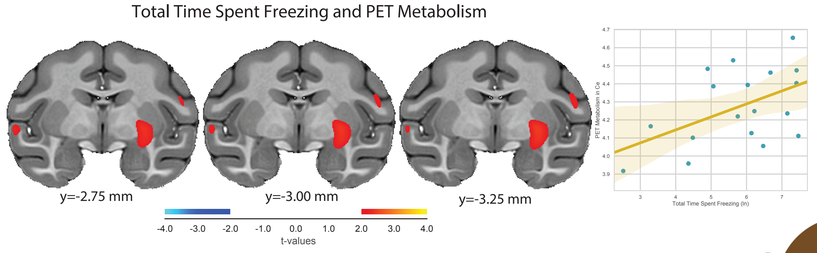






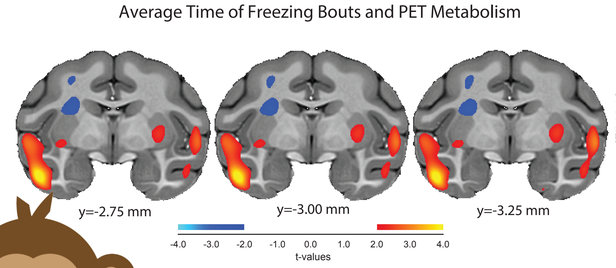
The total amount of time an animal froze to the human intruder, was also correlated to Ce metabolism

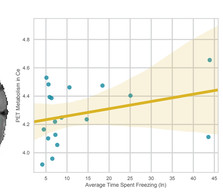
Total Time Spent Freezing and PET Metabolism



Interestingly, Ce was not found to be correlated with the average time an animal spent freezing to the human intruder.

Average Time of Freezing Bouts and PET Metabolism





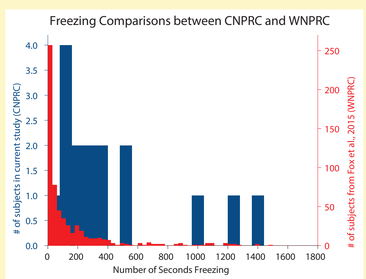
Discussion

Previous work in NHPs found Ce & BST metabolism to be strongly associated with freezing during NEC exposure1. Here, we replicated the Ce metabolism finding in animals that have been reared in large naturalistic social groups. Intriguingly, we did not find that elevated BST metabolism, which has been previously associated with heritable effects, to be associated with NEC-induced freezing in this sample. However, this study was underpowered to detect such an effect, so we interpret this with caution. Of note, previous studies have suggested that the amygdala likely plays a role in mediating environmental influences4, which are likely to be larger in this naturalistic upbringing. Future research will have to disassociate between heritable and environmental influences on brain function.

Overlapping activation in the dorsal amygdala region between our CNPRC sample and WNPRC sample from Fox et al., 2015.

CNPRC animals appear to freeze more than the animals in Fox et al., 2015.

Freezing Comparisons between CNPRC and WNPRC



Acknowledgements

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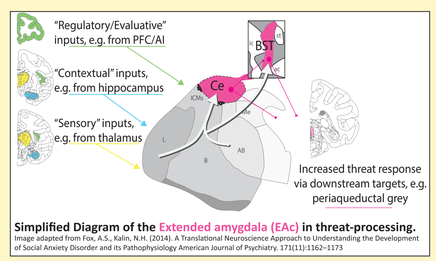
Future Directions

Understanding the neural circuitry that is involved in the experience of fear and anxiety is crucial for the development of optimal behavioral and/or pharmacological interventions.

Extend to a larger population.

Understand sex differences in freezing-related brain function. Because women are two times more likely to develop stress related psychopathology[5], we plan to continue using non human primate models to understand this well established risk.



Simplified Diagram of the Extended amygdala (EAc) in threat-processing.
Image adapted from 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(11):1162–1173

Broaden these studies to animals across the lifespan into young-adults and aged animals.

Elucidate the role of environmental influences. Because animals at the CNPRC are housed in large, half acre corrals, we plan on combining in-lab measures with naturalistic observations while animals are in their home corrals.

References

[1]Fox AS, Oler JA, Shackman AJ, Shelton SE, Raveendran M, McKay DR, Converse AK, Alexander AL, Davidson RJ, Blangero J, Rogers J, & Kalin NH (2015). Intergenerational neural mediators of early-life anxious temperament. Proceedings of the National Academy of Sciences. 112(29):9118-9122
[2]Kalin, N & Shelton, S (1989). Defensive behaviors in infant rhesus monkeys: environmental cues and neurochemical regulation. Science 243, 1718 .
[3]Holley D, Campos LJ, Zhang YZ, Capitanio, JP, Fox AS, "Investigating Anxiety-Related Behavior in Young Primates: A Novel, Computer-Automated Approach", Society of Biological Psychiatry, May 18, 2019.
[4]Oler JA, Fox AS, Shackman AJ, Kalin NH (2016). The Central Nucleus of the Amygdala is a Critical Substrate for Individual Differences in Anxiety. Living without an amygdala (Amaral DG, Bauman M, & Adolphs R, Eds.). Guilford Press. 2016
[5]Kessler, R. C. et al. (2005).Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch. Gen. Psychiatry. 62, 593–602