

# Early-life Inhibited Temperament is Associated with White-Matter Integrity in Emotion-Related Pathways of the Primate Brain in Adolescent Females

Lillian J. Campos<sup>1,3</sup>, Do P.M. Tromp<sup>2</sup>, John P. Capitanio<sup>3</sup>, Andrew S. Fox<sup>1,3</sup>

Department of Psychology, University of California, Davis<sup>1</sup>, University of Wisconsin-Madison<sup>2</sup>, California National Primate Research Center, University of California, Davis<sup>3</sup>

**The Biobehavioral Assessment (BBA)** is a program at the California National Primate Research Center started in 2001 with the goal of characterizing the behavioral and psychological responses of the majority of the animals at the center<sup>1</sup>. As part of the BBA, animals undergo about 26 hours of testing that includes: human intruder data, blood sampling data, visual memory testing, novel object testing, and many others. Since its inception, the BBA program has a database of more than 4000 animals.

## Defining Inhibited Temperament

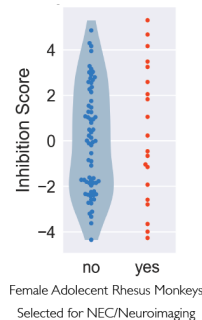
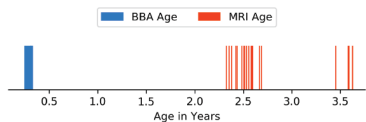
Our measure of **inhibited temperament** is based on behavioral data collected by the Biobehavioral Assessment (BBA) program at the California National Primate Research Center (CNPRC). Animals that are labeled as having inhibited temperament show: (a) low activity (b) low displacement (c) low emotionality and (d) low aggression on Day 1 and Day 2 of BBA testing<sup>2</sup>. We z-scored and averaged all variables to create our measure of inhibited temperament.

## Methods



### Animal Selection

Twenty 2-3 year old female rhesus monkeys (macaca mulatta), previously assessed for inhibited temperament at ~3-4 months were selected using a stratified sampling approach from 98 young rhesus monkeys that were a part of the BioBehavioral Assessment.



### DTI

Diffusion Tensor Imaging (DTI) analyzes the movement of water diffusion in white matter in the brain. In white matter, water diffuses along the same direction as white matter fibers and myelin because water is less likely to permeate through fatty substances. Because of this, water diffusion models in white matter look anisotropic and their grey matter counterparts, look isotropic. Tensors are then used to compute the direction of water flow and the amount of diffusion in each voxel. In this study we measured the fractional anisotropy (FA) of each tensor.

For DTI processing, two 60-direction diffusion weighted scans per animals were collected using a Siemens Skyra 3T MRI Scanner and a dedicated rhesus 8-channel surface coil. Images were preprocessed, standardized, and normalized using neuroimaging software like FSL, Camino, and DTI-TK. Fiber tractography analyses were performed using TrackVis.

### How to DTI?

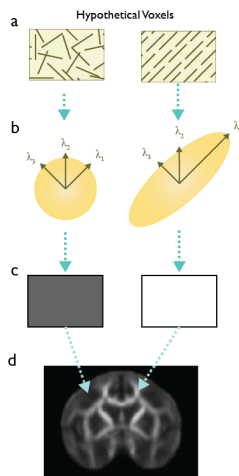
1. Preprocessing: Eddy distortions and EPI distortions were corrected using FSL's eddy\_correct command and TOPUP; respectively. FSL's bet command was used to extract the brain. A scheme file with b-values and b-vectors was created using the command fslchrm. Camino's modelfit command was used to run the tensor model estimation from the images collected. A sanity check was done in order to see if the scheme file was applied well and if the tensors were fitted properly.

2. Standardization was conducted using DTI-TK: tensors were adjusted to the same diffusivity units, extreme outliers were removed, and tensors were made symmetric and positive-definite.

3. Normalization was conducted using DTI-TK: an initial mean was made, and rigid, affine, and diffeomorphic registrations were performed.

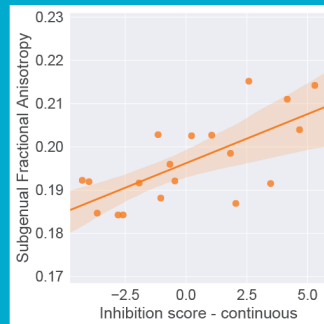
### What is FA?

$$FA = \frac{\sqrt{\frac{3}{2}} \sqrt{\frac{\lambda_1 - \lambda_2}{\lambda_1 + \lambda_2 + \lambda_3}}}{\sqrt{\frac{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}}$$

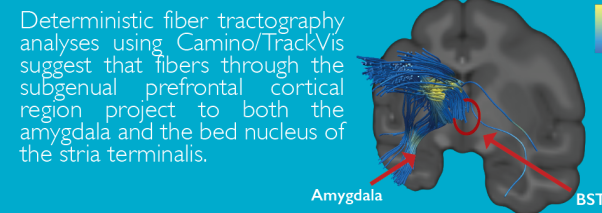


## Results

### Inhibited Temperament is significantly correlated with FA in subgenual prefrontal cortical white matter

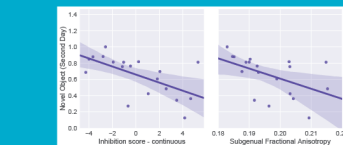


Whole-brain voxelwise analyses between inhibited temperament and fractional anisotropy (FA) were conducted using FSL's randomise. Results demonstrated a significant positive correlation between inhibited temperament and FA in the subgenual prefrontal cortical white matter ( $p < 0.005$ , two-tailed uncorrected).



### But there's more ...

Exploratory analyses into other inhibited temperament-related BBA measures revealed subgenual FA to be associated with less activity on day 1 and 2 ( $t = -2.39$ ,  $p = 0.029$ ;  $t = -1.86$ ,  $p = 0.08$ ), less emotionality on day 1 and day 2 ( $t = -3.07$ ,  $p = 0.007$ ,  $t = -1.75$ ,  $p = 0.098$ ), decreased ratings of how vigilant ( $t = -2.93$ ,  $p = 0.009$ ), gentle ( $t = -1.92$ ,  $p = 0.072$ ), and confident ( $t = -4.35$ ,  $p < 0.001$ ) the animal was, as well as decreased time interacting with a novel object placed in their enclosure during the second half of day 1 as well as the morning of day 2 ( $t = -1.77$ ,  $p = 0.094$ ;  $t = -2.41$ ,  $p = 0.028$ ).



Non-Independent exploratory analysis revealed that animals with inhibited temperament had reduced contact with a novel object on the second day of BBA testing. It also revealed that animals that were less likely to have contact with a novel object had higher FA in subgenual prefrontal cortical white matter.



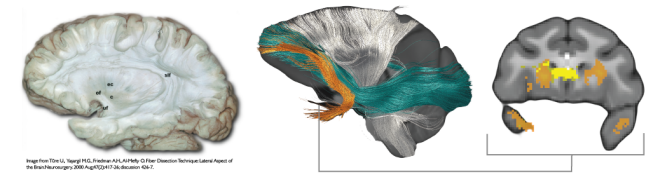
We're in the process of analyzing behavioral and other concurrent neuroimaging data from these adolescent female monkeys, including freezing, coping, cortisol, as well as FDG-PET in the NEC context. We hypothesize that those animals with an extremely inhibited temperament, will freeze more during adolescence, and that this freezing will be associated with increased FA in the subgenual region and increased metabolism in the BST.

### Acknowledgements

We thank the California National Primate Research Center and its staff, UC Davis Imaging Research Center, and especially Sarah Grisso and Jennifer Kendrick. This study was funded via the CNPRC and the University of California, Davis.

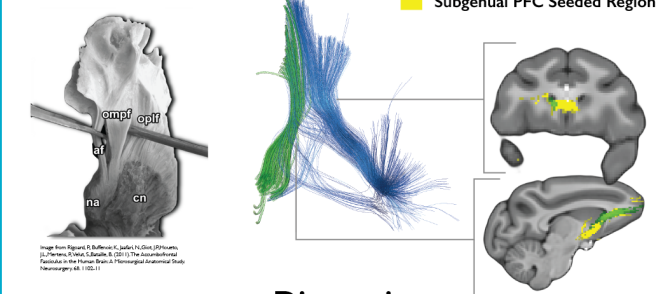
## Tractography

Extraction of the Uncinate Fasciculus revealed modest overlap between the subgenual seed and the uncinate fasciculus.



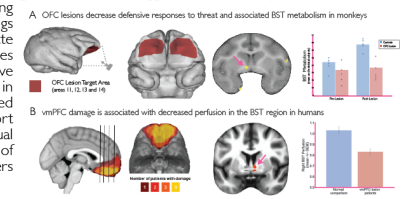
Extraction of the Accumbens fasciculus revealed substantial overlap with tracts coursing through the significant subgenual seed region.

- Uncinate Fasciculus
- Inferior Longitudinal Fasciculus
- Accumbens Fasciculus
- Subgenual PFC Seeded Region



## Discussion

Even though some emotion-related circuits are evolutionarily conserved across species, primates have increasingly incorporated these pathways into larger and more complex networks that include recently evolved cortical regions. Here, we have provided initial evidence that naturally occurring variation in white matter pathways that link prefrontal regions that project to both the amygdala and the BST, modulate inhibited temperament in downstream primate brains. Previous studies demonstrated that targeted lesions of the macaque orbitofrontal cortex (OFC) are sufficient to decrease freezing and anxiety-related metabolism in downstream targets, including the BST (Fox, Shelton, Oakes, et al., 2010). Moreover, surgical resection of the ventromedial prefrontal cortex in humans demonstrated a similar effect, on downstream function in the BST (Motzkin et al., 2015). Recently, Kenwood and colleagues (Under Review) found that "strip-lesions," which disconnect the prefrontal cortex from downstream targets, decrease a composite measure of anxious temperament, along with BST metabolism, and disrupt BST-PFC functional connectivity. Together, these data suggest prefrontal projections to the BST play an important role in increasing anxiety and inhibition. These findings support the idea that the primate prefrontal cortex, which provides humans with enhanced cognitive capacities, also plays an important role in generating an anxious or inhibited disposition. Ultimately, these data support the proposed role of subgenual white-matter in the development of anxiety and depressive disorders (Riva-Posse, et al., 2018).



Fox AS, Shaqfeh AH (2018). The central extended amygdala in fear and anxiety: Closing the gap between mechanistic and neuroimaging research. *Neuroscience Letters*. In Press.

### References

1. Capitanio JP, Capitanio JP. Latent variables affecting behavioral response to the human intruder test in rhesus monkeys (Macaca mulatta). *American Journal of Primatology*. 2013;75:314-323.
2. Capitanio JP. BBA database.
3. Tromp D. PhD (2009, May 1). DTI-based 1. From Scanner to Tensor. Retrieved from <http://www.difusion-mag.org>. DOI: 10.15200/ucdavis.145633.1599
4. Fox AS, Shelton SE, Oakes TR, Davidson RJ, Kahn NH (2010). Orbitofrontal Cortex Lesions Alter Anxiety-Related Activity in the Primate Bed Nucleus of Stria Terminalis. *Journal of Neuroscience*. 30(20):7023-7027.
5. Kenwood MH, Chen J-A, Fox AS, Tromp D, P.H., Zhou X, Rees H, K., Hayes S.H., Babbitt M.S., Buvaykova I.C., Nummy S.A., Kahn N.H. (Under Review) Fibers coursing through the OFC are critical for the modulation of primate dispositional anxiety.
6. Motzkin J, C. Philipp, C. L. Chen, J. A. Kahn, N. H. Babbitt, M. K. Rees, H. Kenwood, M. H. (2015) Ventromedial prefrontal cortex damage alters resting blood flow to the bed nucleus of stria terminalis. *Cortex: a Journal Devoted to the Study of the Nervous System and Behavior*. 64:281-288. <https://doi.org/10.1016/j.cortex.2014.11.013>
7. Riva-Posse F, Choi S, K. Haldamer, P.E. Crowley, A. L. Garlow, S. J. Rapoport, J. K., ... Haggberg S. S. (2018). A connectomic approach for subcallosal cingulate deep brain stimulation surgery: prospective targeting in treatment-resistant depression. *Molecular Psychiatry*. 23(4), 843-849. <https://doi.org/10.1038/s41380-017-759>