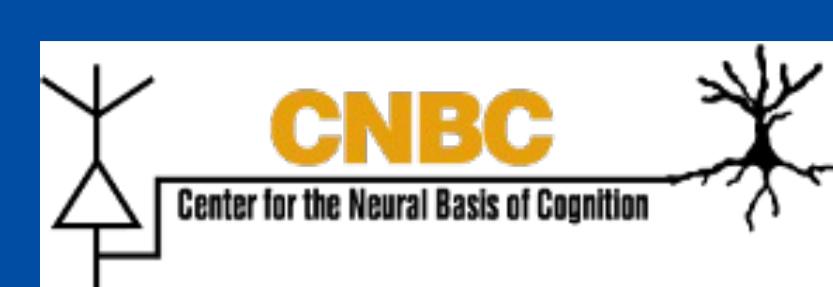


# Characterizing Fronto-Amygdala Circuitry Development During Adolescence: Implications for Internalizing Symptoms

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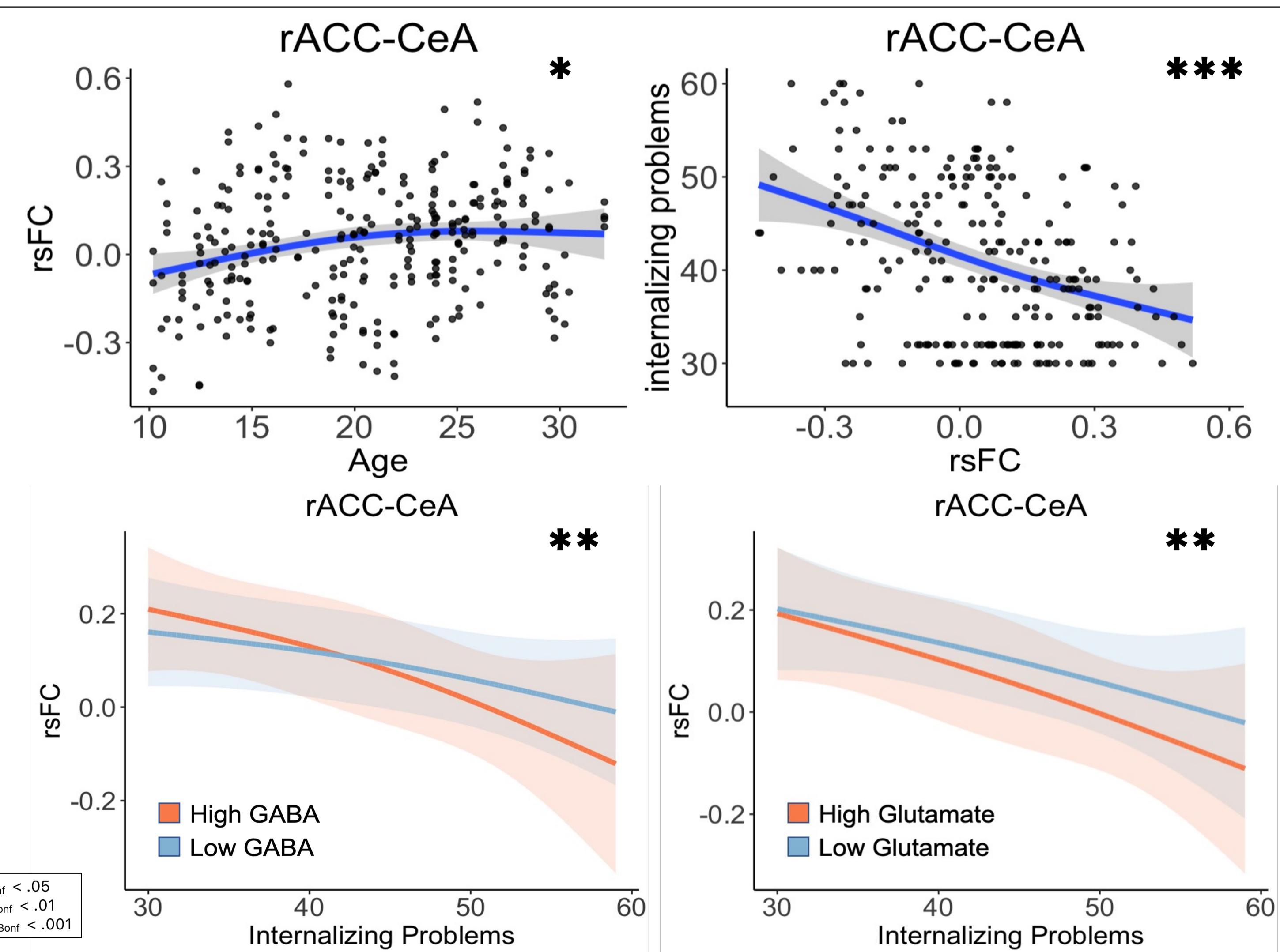
## Background & Motivation

- Fronto-amygdala maturation in adolescence is thought to underlie improvements in emotion regulation<sup>1-3</sup> and disruptions with affective psychopathology<sup>4-7</sup>.
- Characterizing human amygdala neurodevelopment is complicated by heterogeneity in morphological, functional, and connectional characteristics of the various subnuclei<sup>8,9</sup>, possibly contributing to mixed age-related findings<sup>3,4</sup>.
- The basolateral amygdala, mainly glutamatergic<sup>10</sup>, is implicated in emotional associative learning via its integration of multimodal sensory input processing with corresponding affective physiological and behavioral responses<sup>11,12</sup>.
- The centromedial amygdala, mainly GABAergic<sup>13</sup>, is implicated in goal-directed behaviors via functional encoding and modulation of neural signals related to valence and salience<sup>14,15</sup>.
- The anterior cingulate cortex (ACC) — integrating multimodal feedback signals to monitor and improve performance — supports age-related cognitive gains<sup>16</sup>, whereas atypical connectional development relates to affective symptomatology (e.g., emotion dysregulation)<sup>4-7,13</sup>.
- Preliminary evidence suggests that ACC glutamate and GABA, which undergo maturation in adolescence<sup>17,18</sup> may relate to internalizing symptoms<sup>19-21</sup>; however, the extent to which age-related fronto-amygdala maturation relates to inhibitory and/or excitatory neurotransmission and internalizing symptoms remains unclear.
- We investigated resting-state functional connectivity (rsFC) at 7T between three major amygdala subnuclei—the basal (BnA), lateral (LnA), and central (CeA) amygdala—and the rACC and sgACC, given their involvement in affective control.

**Hypothesis:** Age-related strengthening of rACC – CeA rsFC in adolescence—differing as a function of ACC glutamate and/or GABA—will support affective development (fewer internalizing symptoms).

## Results

- We observed significant age-related rACC rsFC increases with CeA ( $F = 6.53$ ,  $p_{\text{Bonf}} = .01$ ) and LnA ( $F = 5.44$ ,  $p_{\text{Bonf}} = .03$ ), but not with the BnA or with any sgACC connections ( $ps > .05$ ).
- Stronger rACC – CeA rsFC was associated with fewer internalizing symptoms ( $F = 11.0$ ,  $p_{\text{Bonf}} < .001$ ), even after controlling for age-related effects ( $F = 8.69$ ,  $p_{\text{Bonf}} = .001$ ), such that stronger connectivity was associated with fewer internalizing symptoms. No other connection was related to internalizing symptoms ( $ps > .05$ ).
- Finally, rACC – CeA rsFC was associated with internalizing problems as a function of both ACC glutamate ( $F = 5.39$ ,  $p = .001$ ) and GABA ( $F = 4.58$ ,  $p = .004$ ). Both associations remained significant after controlling for age effects on rsFC (glutamate:  $F = 4.08$ ,  $p = .008$ ; GABA:  $F = 3.47$ ,  $p = .017$ ).

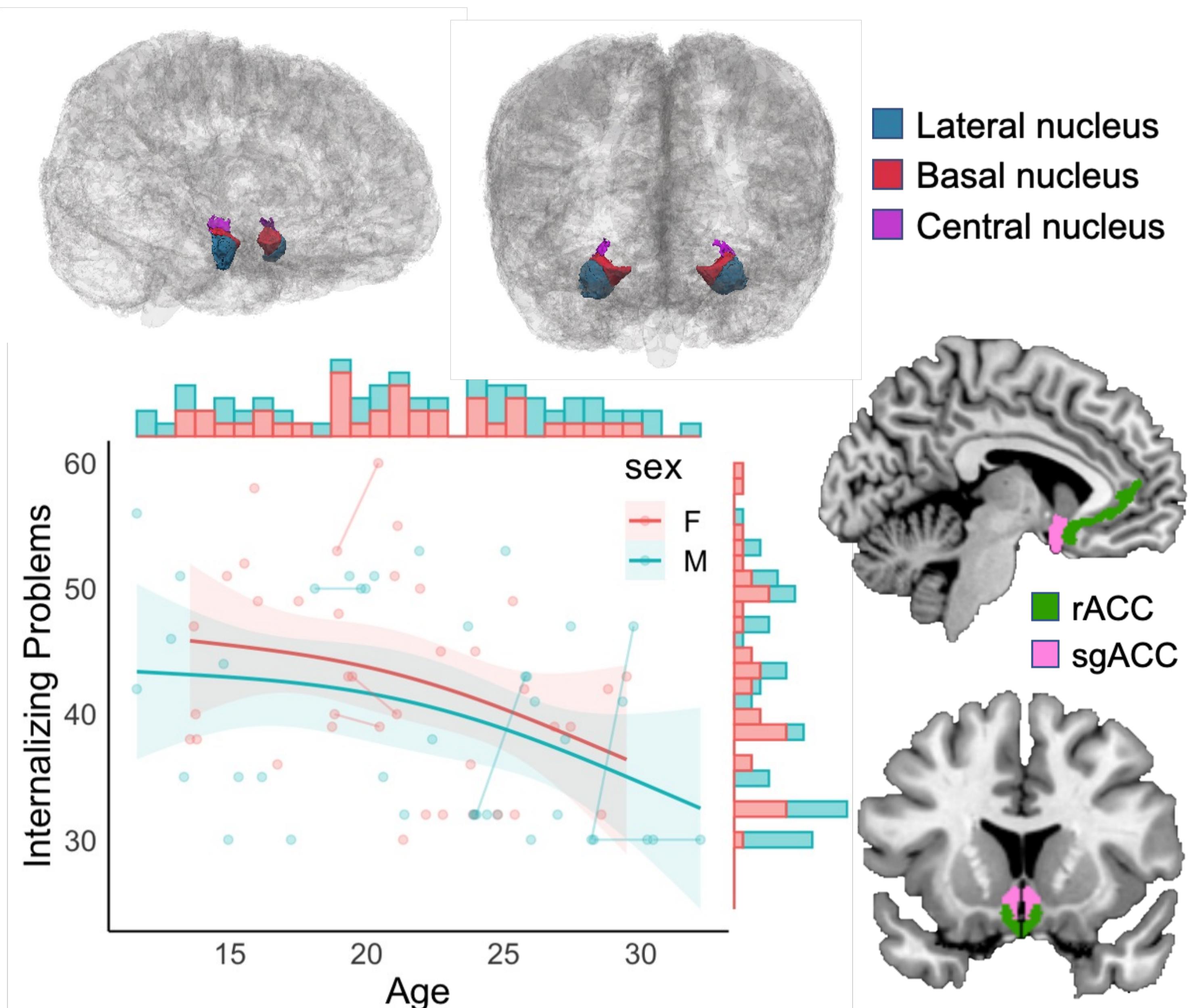


## References

- Silvers, J.A., Insel, C., Powers, A., et al. (2016). *Cerebral Cortex*, 27(7), 3502-3514.
- Bostochitsch, L.G., Heath, T.C., Maitson, W.L., et al. (2019). *Human Image*, 191, 278-291.
- Gee, D.G., Humphreys, K.L., Flannery, J., et al. (2013). *Journal of Neuroscience*, 33(10), 4584-4593.
- Jalbrzikowski, M., Larsen, B., Hallquist, M.N., et al. (2017). *Biological Psychiatry*, 82(7), 511-521.
- Jin, J., Delaporte, L., Chen, H.W. (2021). *Biological Psychiatry: CNT*, 7(3), 249-255.
- Vijayakumar, N., Allen, N.B., Denison, M. (2017). *NeuroImage*, 156, 403-411.
- Roy, A.K., Fudge, J.L., Kelly, C., et al. (2013). *Journal of the American Academy of Child and Adolescent Psychiatry*, 52(5), 290-299.
- Bzdok, D., Laird, A.R., Zilles, K., et al. (2012). *Human Brain Mapping*, 34(12), 3247-3266.
- Mishra, A., Rogers, B.P., Chem, L.M., et al. (2014). *Human Brain Mapping*, 35(4), 1247-1260.
- Sharp, B.P. (2017) *Translational Psychiatry*, 7(8), e1194.
- Gore, F., Schwartz, E.C., Brangiers, B.C., et al. (2019). *Cell*, 163(1), 134-145.
- Rosen, J.M., McEwen, D.J., et al. (2019). *Journal of Neuroscience*, 39(11), 4501-4509.
- McDonald, A.J. (1999). *Progress in Neurobiology*, 33(1), 257-332.
- Warlow, S.M. & Berridge, K.C. (2021). *Behavioral Brain Research*, 411, 113376.
- Warlow, S.M., Naffziger, E.E., Berridge, K.C. (2020). *Nature Communications*, 11(1), 2716.
- Bush, G., Luu, P., Posner, M.I., et al. (2000). *Trends in Cognitive Science*, 4(6), 215-222.
- Shimizu, M., Suzuki, Y., Yamada, K., et al. (2017). *Pediatric Research*, 82(5), 749-752.
- Perica, M.I., Calabro, F., Foran, W., et al. (preprint). *bioRxiv*.
- Ho, T.C., Teresi, G.I., Segara, J.R., et al. (2020). *Frontiers in Psychiatry*, 12, 642976.
- Moriguchi, S., Takamia, A., Noda, Y., et al. (2018). *Molecular Psychiatry*, 24(7), 952-964.
- Duman, R.S., Saucor, G., & Krystal, J.H. (2019). *Neuron*, 102(1), 75-90.
- Fischl, B. (2012). *NeuroImage*, 62(2), 774-781.
- Siagian, Z., Saito, D., & Saito, T. (2017). *NeuroImage*, 155, 370-382.
- Markov, S., & Petrides, M. (2014). *European Journal of Neuroscience*, 40(6), 2777-2796.
- Ebeschutani, C., Bernstein, A., Martinez, J.I., et al. (2011). *Journal of Clinical Child and Adolescent Psychology*, 40(2), 338-346.
- Achenbach, T.M., Ivanova, M.Y., & Rescorla, L.A. (2017). *Comparative Psychiatry*, 59, 4-18.

## Methods

- We collected high-resolution (7 Tesla) rsfMRI data (2x2x2 mm, 8min3s), in 68 healthy participants (ages 11.6 – 32.2), with up to 2 visits, for a total of 78 scans. Most ( $n = 62$ ) additionally had ACC MRSI data.
- Subject-specific amygdala subregional ROIs were parcellated from structural MRI images using FreeSurfer<sup>22,23</sup> and cortical segmentations were created using an anatomical atlas<sup>24</sup>.
- We computed rsFC by extracting rsfMRI time series data, taking the first principal component across all voxels within each ROI, computed Pearson correlation coefficients between ROI seeds, and normalized connectivity values using Fisher's Z transformation.
- Internalizing symptomatology was assessed using the YSR/ASR<sup>25,26</sup>.
- We used generalized additive mixed models (GAMMs) to characterize linear and non-linear relationships. Sex, laterality, and cortical hemisphere were modeled as covariates. All  $p$ -values are Bonferroni corrected for multiple comparisons.



## Discussion

- Consistent with our hypothesis, age-related strengthening of rACC – CeA rsFC may reflect more mature fronto-amygdala circuitry across males and females and support effective emotion regulation (e.g., fewer internalizing symptoms).
- The CeA — consisting of GABAergic neurons — has been shown to amplify or narrow incentive salience<sup>14</sup> (e.g., approach/avoidance behaviors). Our results suggest that maturation of connections with the rACC in adolescence may downregulate exaggerated affective responses to environmental stimuli.
- We also found preliminary evidence suggesting that this effect might be most pronounced in individuals with higher concentrations of ACC glutamate or GABA.
- Our findings elucidate trajectories of fronto-amygdala development, previously unresolved at 3T, possibly due to important differences in sample age distributions, lower TSNR, and/or lower spatial and temporal resolution at 3T.
- Taken together, age-related strengthening of rACC – CeA connectivity during adolescence represents one novel target for internalizing symptoms and may be modulated via glutamatergic and/or GABAergic processes.

## Future Directions

- Future research leveraging larger, longitudinal samples with increased MRSI specificity (e.g., rACC) is needed to further replicate and extend our findings.
- Additionally, future work should consider testing our model in affective psychopathologies (e.g., depression, anxiety) as well as in externalizing symptomatology to determine the generalizability and/or specificity of our results.
- Finally, investigators should investigate the functional neurophysiology of fronto-amygdala circuitry (versus rsfMRI) to determine whether age-related rACC – CeA engagement during task-fMRI supports emotion regulation in adolescents.

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