

# Intergenerational neural mediators of early-life anxious temperament

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**Understanding the heritability of neural systems linked to psychopathology is not sufficient to implicate them as intergenerational neural mediators. By closely examining how individual differences in neural phenotypes and psychopathology cosegregate as they fall through the family tree, we can identify the brain systems that underlie the parent-to-child transmission of psychopathology. Although research has identified genes and neural circuits that contribute to the risk of developing anxiety and depression, the specific neural systems that mediate the inborn risk for these debilitating disorders remain unknown. In a sample of 592 young rhesus monkeys that are part of an extended multigenerational pedigree, we demonstrate that metabolism within a tripartite prefrontal-limbic-midbrain circuit mediates some of the inborn risk for developing anxiety and depression. Importantly, although brain volume is highly heritable early in life, it is brain metabolism—not brain structure—that is the critical intermediary between genetics and the childhood risk to develop stress-related psychopathology.**

anxiety | primate | heritability | positron emission tomography | brain volume

Parents with anxiety and depressive disorders are considerably more likely to have children with an extremely anxious temperament (AT) (1–3). Extreme-AT children have heightened behavioral and physiological reactivity to potential threat and have a markedly increased risk to develop anxiety and depressive disorders (4, 5). These disorders emerge as inborn tendencies and environmental factors converge to disrupt the neural systems that mediate adaptive anxiety; as many as 50% of children with extreme-AT develop a psychiatric disorder (6). In addition to environmental influences that facilitate the cross-generational transfer of psychopathology (e.g., parent–child interactions), genetic variance accounts for ~35% of the likelihood that a child will develop an anxiety disorder (7, 8). The neural substrates of AT are distributed throughout the brain and range from primitive brainstem structures to primate-specific cortical subfields. Multiple brain regions causally contribute to AT, and damage to any one of these regions is sufficient to decrease, although not abolish, anxiety (9–14). Thus, the inherited risk to develop stress-related psychopathology likely manifests via its effects on multiple components of the neural circuit underlying AT. Here we use a genetic correlation approach to identify brain regions where function and structure contribute to the intergenerational transmission of AT. Genetic correlation analyses are crucial for identifying regions that are likely to mediate the genetic contributions to AT, and to distinguish them from regions that, although heritable, rely on an independent set of genetic variations.

The recent evolutionary divergence of humans and rhesus monkeys is reflected in their shared capacity for higher-order cognition, complex social behavior, and homologous neural circuits, which make the young rhesus monkey an ideal model for

understanding the neural substrates of childhood AT. Our group developed and validated a paradigm for studying the neural bases of primate AT that combines [18-F] deoxyglucose positron emission tomography (FDG-PET) imaging with behavioral and neuroendocrine responses to a potentially threatening human intruder making no eye contact (NEC) with the monkey. The NEC context is designed to elicit naturalistic adaptive defensive behaviors and captures the evolutionarily conserved tendency of high-AT individuals to inhibit behaviors that otherwise could attract the attention of potential predators. Because it measures brain metabolism over ~30 min, FDG-PET imaging is ideally suited to examine the sustained neural responses that underlie trait-like measures, such as AT. Following FDG injection, the monkey is placed in the NEC context. As FDG is taken-up into metabolically active cells, the monkey behaves freely in the NEC context, revealing its anxious disposition. The post-NEC PET scan measures the integrated brain metabolism that occurred during exposure to the ethologically relevant NEC context.

To identify the brain regions that underlie the parent-to-child transmission of psychopathology, it is critical to understand the pattern of cosegregation between the AT phenotype and its neural circuit alterations within the family tree. This approach first requires demonstrating that the phenotype is heritable, identifying

## Significance

According to the World Health Organization, anxiety and depressive disorders are a leading source of disability, affecting hundreds of millions of people. Children can inherit an extremely anxious temperament, which is a prominent risk factor for the later development of anxiety, depression, and comorbid substance abuse. This study uses high-resolution functional and structural imaging in our well-established developmental nonhuman primate model to identify the heritable neural substrate that underlies extreme childhood anxious temperament. Using a large multigenerational family pedigree, genetic correlation analyses revealed a tripartite neural circuit where metabolism likely shares a genetic substrate with early-life dispositional anxiety. Interestingly, we found that brain function—not structure—is the critical intermediary between genetics and the childhood risk to develop stress-related psychopathology.

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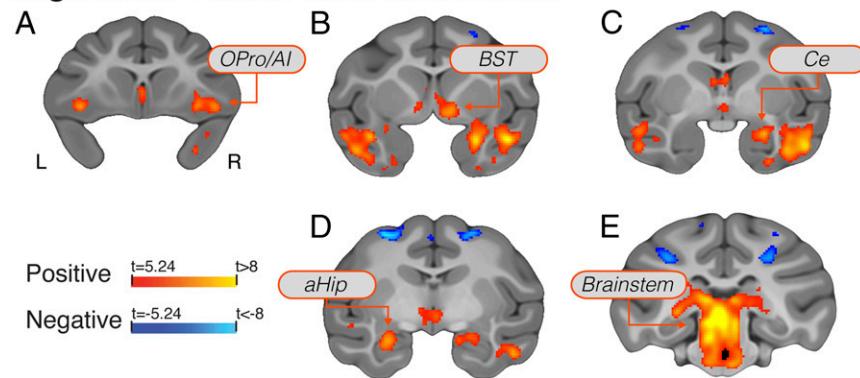
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Data deposition: The full voxelwise maps are available in [Dataset S1](#).

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## Regional AT-related brain metabolism



**Fig. 1.** Regions where brain metabolism was significantly associated with individual differences in AT ( $P < 0.05$ , Šidák-corrected for multiple comparisons across the whole-brain). Regions include the OPro/AI (A), subgenual anterior cingulate, temporal cortex, BST (B), Ce (C), aHip (D), and brainstem regions including the PAG (E).

brain regions associated with the phenotype, and determining which of these brain regions are heritable. Once this is accomplished, a genetic correlation analysis between brain function/structure and the phenotype is critical to identify the neural mediators of the heritable parent-to-child transfer of risk.

Following this strategy in a large familial sample of young rhesus monkeys, we (i) demonstrate the heritability of AT, (ii) identify neuroimaging measures that predict AT, (iii) assess the heritability of the relevant brain structural and functional measures, and finally, (iv) perform the relevant genetic correlation analyses between the neuroimaging measures and AT. To this end, NEC FDG-PET and structural brain imaging were performed in our large sample of 592 young rhesus monkeys from a large multigenerational pedigree (age: mean = 1.88 y; 327 males/265 females). Paralleling work in children, the monkey AT phenotype encompasses behavioral (freezing), communicative (decreased cooing), and physiological (increased cortisol) responses to potential threat. Specifically, our composite measure of AT was operationalized as the mean of the monkey's relative freezing levels, inhibition of coo vocalizations, and plasma cortisol concentration (15–18). In humans, the features of AT, extreme behavioral inhibition, and heightened cortisol levels are early risk factors for the later development of anxiety and depressive disorders (6, 19–21). Children who respond to strangers and novel situations with excessive apprehension or physiological arousal are likely to modify their behavior in ways that are maladaptive and over time are indicative of stress-related psychopathology. Similar to humans, monkeys with extreme AT appear to be functionally impaired across laboratory and naturalistic social settings, making the rhesus monkey model of AT ideal for understanding the pathophysiology that underlies the risk to develop anxiety and depression (21).

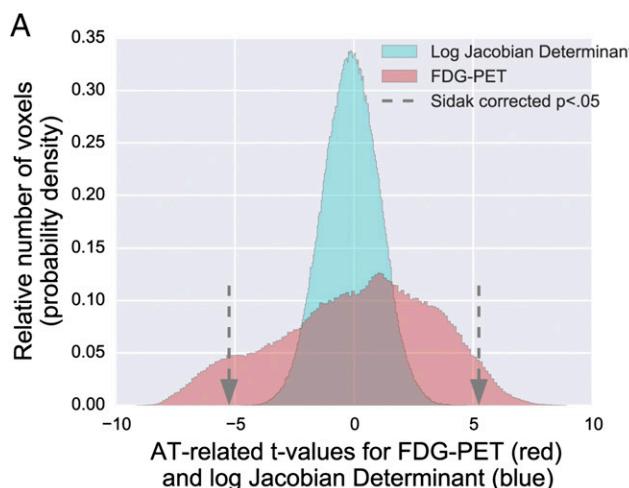
Heritability of AT was estimated as the proportion of variation in the phenotype explained by the coefficient of relatedness in the extended multigenerational pedigree (see ref. 22 and *Methods* for details). This extended pedigree approach is powerful because it accounts for the phenotypic similarity of both close and distant relationships (see *SI Appendix, SI Methods* for details). In this preadolescent sample, there was no significant effect of sex on AT ( $t = 0.830$ ,  $P = 0.407$ ), but there was a significant decrease in AT with age ( $t = -10.013$ ,  $P < 0.001$ ). Accordingly, all analyses controlled for nuisance variance in age, age<sup>2</sup>, sex, and the age  $\times$  sex interaction. Results demonstrated that the AT phenotype is heritable (AT:  $h^2 = 0.29$ ,  $P < 0.0001$ ), consistent with prior findings in young monkeys as well as the heritability estimates for human anxiety and anxiety disorders (7, 23, 24).

To identify brain regions where metabolism contributes to the cross-generational transmission of AT, it is first necessary to identify the regions where these measures predict variation in the anxious phenotype. Voxelwise robust regressions were performed between AT and FDG-PET. Results revealed significant relations between metabolism and AT ( $P < 0.05$  Šidák-corrected; also see *SI Appendix, Results and Discussion*) in regions of the orbital frontal (OFC) and anterior insular (AI) cortices [including orbital proisocortex (OPro/AI), as well as cytoarchitectonic areas 11/13/47], amygdala [including the central nucleus of the amygdala (Ce)], anterior hippocampus (aHip), bed nucleus of stria terminalis (BST), as well as midbrain regions that encompass the periaqueductal gray (PAG) (Fig. 1 and *SI Appendix, Table S1*; full voxelwise maps are available in *Dataset S1*). Importantly, these AT-related regions include areas that causally contribute to AT in mechanistic studies, including: orbital-prefrontal cortical areas involved in emotional valuation (25); the extended amygdala, an interface between emotions and their behavioral and physiological expression (26); and brainstem regions, including PAG, which are the downstream effectors required for the expression of defensive responses (27).

In parallel, to identify regions where brain volume was associated with AT, voxelwise analyses were performed to identify regions where brain volume predicted AT. Regional brain volume was measured using the log-Jacobian determinant of the nonlinear transformations to template space. Remarkably, there were no voxels in which brain volume significantly predicted AT [Šidák correction,  $P > 0.05$ , or false-discovery rate (FDR)  $q > 0.05$  corrected] (Fig. 2A; full voxelwise maps are available in *Dataset S1*). Cross-validation analyses examining the predictive utility of brain volume to predict AT or its components using elastic-net regression and supervised learning yielded the same conclusion (see *SI Appendix, Results and Discussion* and Figs. S2 and S3 for details). Thus, at least early in life, variation in the expression of AT does not involve altered regional brain volume.

The heritability of brain metabolism was identified using voxelwise heritability analyses of FDG-PET that were performed by harnessing the computational resources of the Open Science Grid's distributed high-throughput computing system (*Methods*). Results demonstrated significant heritability of glucose metabolism across the brain (Fig. 2B). We observed significantly heritable metabolic activity in nearly every AT-related region (FDR  $q < 0.05$ , corrected within regions where metabolism significantly predicted AT) (Fig. 3 and *SI Appendix, SI Results and Discussion* and *Table S1*). Within these AT-related heritable regions there was substantial variability in the magnitude of heritability estimates, with peak heritability estimates of 26%

## Distributions of AT-related t-values for brain volume and metabolism



**Fig. 2.** Histograms of voxelwise AT-related brain metabolism (red) and brain volume (blue) demonstrate significant relationships for brain metabolism but not brain volume (A). Gray arrows represent the threshold for reaching significance at a Šidák-corrected  $P < 0.05$ . Histograms of heritability estimates for brain metabolism (red) and brain volume (blue) demonstrated significant heritability for many voxels of both measures, and on average greater heritability of brain volume (see main text for details) (B). Red and blue arrows indicate the mean heritability estimates for FDG-PET and the log-Jacobian determinant, respectively.

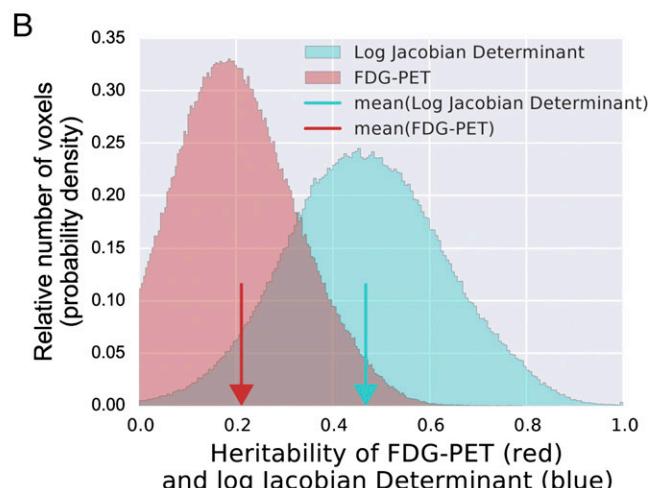
in the amygdala, 53% in the hippocampus, and 57% in the BST region. Estimates were as low as 9% in the superior temporal sulcus (full voxelwise maps are available in [Dataset S1](#)).

We assessed the heritability of regional brain volume using the same approach. Interestingly, brain volume was generally more heritable than brain metabolism ( $\chi^2 > 1,000, P < 0.001$ ), with the average  $h^2$  for brain volume  $\sim 0.5$ , whereas the average  $h^2$  for regional brain metabolism was  $\sim 0.2$ . Within the specific subset of brain regions where metabolism predicted AT, variation in volume was significantly heritable (full voxelwise maps are available in [Dataset S1](#)). For example, volume in the AT-related amygdala and OFC regions was more than 60% heritable. Nevertheless, the surprising result that brain volume did not predict that AT provides compelling evidence that these highly heritable early-life structural differences do not mediate the intergenerational transmission of the risk for anxiety and depressive disorders.

Demonstrating significant heritability of AT-related brain regions does not implicate these regions in the intergenerational transfer of AT. Because of the importance of identifying regions where brain metabolism and AT similarly fell through the family tree, we performed voxelwise genetic correlation analyses. Genetic correlation analyses are crucial for dissociating brain regions that share an overlapping genetic basis with AT from heritable regions where metabolism is driven by genes that are unrelated to the AT-phenotype. We computed bivariate genetic correlations ( $\rho_g$ ) between AT and heritable AT-related brain metabolism. Results demonstrate significant genetic correlations with AT in regions that encompass portions of the OFC/AI, extended amygdala, and brainstem (Fig. 4 and [SI Appendix](#), Fig. S4 and Table S2). To our knowledge, these data provide the first evidence for a coheritable substrate for AT and AT-related brain metabolism in a circuit, wherein the extended amygdala links prefrontal regulatory mechanisms to brainstem effector sites that initiate anxiety-related responses. Metabolism within this tripartite neural circuit is likely to share a genetic substrate with AT through which it mediates the inherited risk to develop stress-related psychopathology.

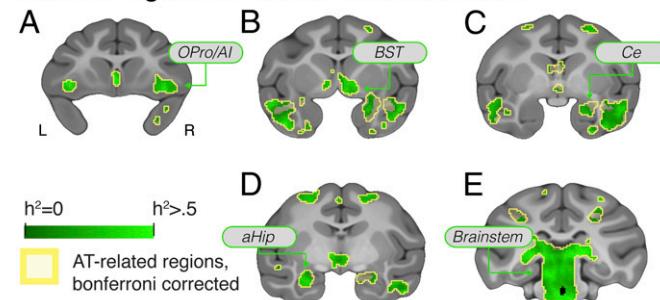
To precisely identify the locations of peak activations within the identified tripartite prefrontal-limbic-midbrain circuit, we used

## Distributions of heritability estimates for brain volume and metabolism



high-precision diffeomorphic registration and chemoarchitectonic imaging (*Methods*). High-precision diffeomorphic registration allowed us to localize significant regions on a superresolution template brain, which reflects the mean rhesus monkey brain anatomy with submillimeter precision in well-aligned areas. This integrative approach revealed that the OFC/AI cluster included regions of agranular orbital and insular cortices, with the peak  $\rho_g$  located in OPro/AI (Fig. 4A). To refine the localization of subcortical clusters, we leveraged *in vivo* chemoarchitectonic imaging of dopamine D2/D3 receptor binding ([F-18]fallypride;  $n = 33$ ) and serotonin transporter binding ([C-11]DASB;  $n = 34$ ) derived from an independent sample of young rhesus monkeys (28, 29). Using D2/D3 receptor binding to demarcate the ventral striatum, we localized the peak  $\rho_g$  within the extended amygdala to be specifically located in the BST region between the anterior commissure and the ventral striatum (Fig. 4B). In addition to the peak-region in the BST, the extended amygdala cluster encompassed portions of the subtenuclular extended amygdala immediately

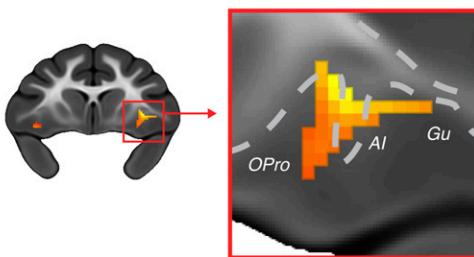
### Heritable regional AT-related brain metabolism



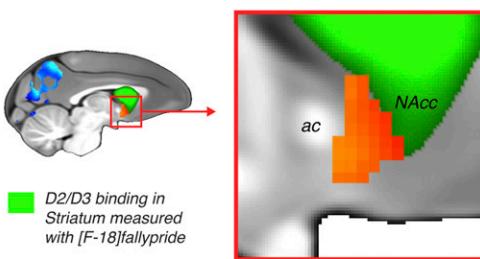
**Fig. 3.** Voxelwise heritability of brain metabolism demonstrated that nearly every region that significantly predicted AT was also significantly heritable ( $q < 0.05$ , FDR-corrected within AT-related regions). Regions include the OPro/AI (A), subgenual anterior cingulate, temporal cortex, BST (B), Ce (C), aHip (D), and brainstem regions including the PAG (E).

## AT-related regional brain metabolism sharing a genetic substrate with AT

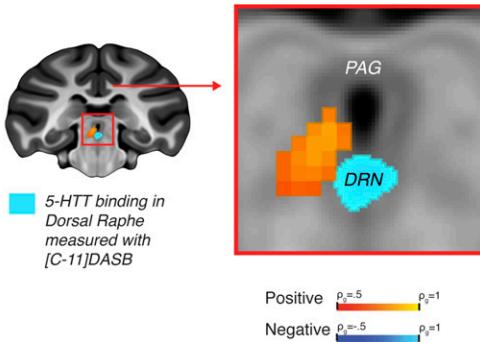
### Anatomic localization of OPro/AI



### Chemoarchitectonic Localization of BST



### Chemoarchitectonic Localization of PAG



**Fig. 4.** Regions where brain metabolism demonstrated significant genetic correlation with AT, thus sharing a genetic substrate, include: the OPro/AI (Top), BST (Middle), and the PAG (Bottom). Using high-resolution anatomical and chemoarchitectonic imaging in a separate group of monkeys, regions were precisely localized as: agranular OPro/AI (Top); the BST region lying between the anterior commissure (ac) and the [18-F] fallypride identified D2/D3-rich ventral striatum that includes the nucleus accumbens (green; NAcc) (Middle); and the vIPAG-region in the gray matter surrounding the ventricle, superior to the [11-C] DASB identified serotonin transporter-rich DRN (Bottom).

posterior to the shell of the nucleus accumbens. Using serotonin transporter binding to pinpoint the dorsal raphe nucleus (DRN), we localized the peak  $\rho_g$  within the brainstem cluster to the ventrolateral PAG (vIPAG), slightly superior and lateral to the DRN in the gray matter that encircles the ventricle (Fig. 4C).

To understand whether metabolism in the brain regions that are genetically correlated with AT are themselves genetically correlated, we performed interregion bivariate genetic correlations. We observed significant genetic correlations in metabolism between the BST and both the PAG ( $\rho_g = 0.56, P = 0.01$ ) and OPro/AI ( $\rho_g = 0.63, P = 0.006$ ), but failed to find a significant correlation between the PAG and OPro/AI ( $\rho_g = 0.40, P = 0.12$ ). These data, demonstrating significant genetic correlations in metabolism across the identified tripartite circuit, suggest that the molecular mechanisms that jointly influence the function of these regions may, in part, mediate the heritable components of

AT. These results pave the path for future molecular investigations of AT. For example, publicly available datasets can be leveraged to suggest potential molecular mediators of metabolism in these regions by identifying genes that are overexpressed in the BST-PAG or BST-OPro/AI compared with the rest of the brain (SI Appendix, SI Methods). These exploratory analyses can reveal candidate genes and molecular mechanisms that may partially mediate the cross-generational transfer AT by altering brain function within the tripartite prefrontal-limbic-midbrain circuit [e.g., somatostatin, neuropeptide Y, or the “neuropeptide hormone activity” (GO:0005184)] (Datasets S2 and S3). By identifying those brain regions that are genetically correlated with AT, informatics and molecular investigations can serve to help to prioritize future mechanistic studies aimed at altering the pathophysiology of anxiety.

Through a tripartite prefrontal-limbic-midbrain circuit, the OPro/AI, BST, and PAG regions work in concert to integrate and evaluate potentially threatening information to initiate and enact anxiety-related responses. Interestingly, this circuit, genetically correlated with individual differences in early-life anxiety, incorporates survival-related regions that span evolutionary history, where selective pressures shaped each stage of central nervous system development. The OPro/AI, BST, and PAG working together may also underlie the pathophysiology of anxiety and affective disorders (21, 30, 31). The primate OPro and AI are thought to be involved in predicting and maintaining affective representations of current and future events, which can be communicated to other AT-related brain regions to guide emotional responding (25, 32, 33). Increased OPro/AI activity may give rise to the excessive anticipatory anxiety associated with anxiety disorders (30, 34, 35). Furthermore, lesions to the monkey OFC that include the OPro/AI are sufficient to decrease anxiety-related behavior, as well as metabolism within the BST region (9, 10). The BST is required for the prolonged threat preparedness (16, 36–40) and is well-suited to integrate cortical affective inputs to coordinate emotional responses, via direct projections to the brain-stem regions required to mount defensive behaviors (31, 41). Although understudied in relation to human psychopathology, dysregulation of the BST likely underlies the hypervigilance characteristic of patients with extreme anxiety and anxiety disorders (31, 42). The evolutionarily old PAG is organized into functionally distinct columns that initiate specific behavioral and physiological responses, including those characteristic of anxiety, such as fleeing, inhibition of motoric activity, and increased passive coping (27, 43–45). It is likely that dysregulated PAG activity, in part, underlies the extreme behavioral inhibition, freezing behavior, and increased autonomic reactivity that is associated with panic symptoms that are common to anxiety disorders. Although temperamental anxiety is instantiated in distributed circuits throughout the brain, these new data specifically implicate the OPro/AI, BST, and PAG as key components that likely work together to mediate some of the inherited predisposition for extreme early-life temperamental anxiety.

Here, we identified previously unknown relationships between AT and metabolism in brain systems that regulate, initiate, and enact anxiety-related behavior that, when passed down from parent to child, likely result in early-life anxiety. We found that the genetic alterations underlying the inborn risk for anxiety and depressive disorders, which are only beginning to be identified, are likely instantiated within the molecular systems that alter metabolism within the OPro/AI, BST, and PAG. Furthermore, we demonstrated the utility of combining empirical data from this study with large-scale publicly available gene-expression databases to gain insight into the molecular systems underlying the heritable components of AT. Regions that do not share a genetic substrate with AT, including the amygdala and aHip, likely play a role in mediating environmental influences, such as caregiver-style, trauma, and other critical socio-emotional environmental

factors on AT. Surprisingly, although early-life regional brain volume was highly heritable, we did not find any evidence linking it to early life anxiety. By identifying the neural systems that share a genetic substrate with AT and likely mediate a part of the genetic risk to develop stress-related psychopathology, these data provide a novel framework for understanding the relationship between genetic variation and early-life anxiety. Elucidating how inherited molecular alterations affect brain function in this tripartite anxiety circuit will help guide the development of novel interventions aimed at helping families with debilitating anxiety enhance the mental health of their at-risk children.

## Methods

For this study, 592 young rhesus monkeys that were part of a large multi-generational pedigree were phenotyped with well-established behavioral, physiological, and brain-imaging methods. Each animal was injected with FDG and exposed to the potentially threatening NEC context in which a human intruder presents their profile to the monkey for 30 min before receiving a PET scan. This paradigm allows us to obtain simultaneous measurements of our composite AT measure and integrated regional brain metabolism during exposure to the NEC context. On a separate day, a structural T1-weighted MRI scan was acquired on each animal. Based on the T1-weighted MRI, the log-Jacobian determinant of the transformation to standard space was computed as to measure relative brain volume. Brain imaging measures were regressed against AT to identify AT-related brain regions. Heritability of local brain volume and brain metabolism was estimated at each voxel based on each pair of animals' degree of relatedness. Bivariate heritability estimates were similarly computed to examine the

degree to which AT and regional brain metabolism share a genetic substrate. All experiments were performed according to the federal guidelines of animal use and care and with the approval of the University of Wisconsin-Madison Institutional Animal Care and Use Committee. More information can be found in *SI Appendix, SI Methods*.

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# Supplementary Information: Intergenerational neural mediators of early-life anxious temperament

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## Including:

- Supplementary Results/Discussion (with Figures) (pg. 2-6)
- Supplementary Methods (pg. 6-12)
- Supplementary References (pg. 12-13)
- Supplementary Legends (pg. 14)
- Supplementary Tables (pg. 15-17)

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## Supplemental Results & Discussion

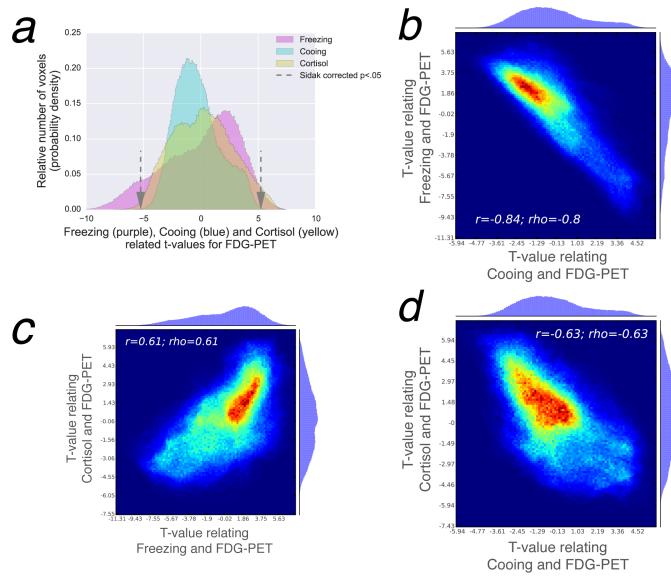
### **Brain metabolism and the components of AT**

To ensure that the relationship between the composite measure of AT and brain metabolism was not primarily determined by an individual component of the composite AT phenotype, we performed three separate voxelwise regressions between NEC-related FDG-uptake and freezing, cooing, and cortisol. Results demonstrated significant relationships between brain metabolism and each AT-component, i.e. freezing, cooing and cortisol (Fig S1a, all Šidák corrected p's<.05). We then sought to determine if the three components of AT were likely to share a neural substrate by examining the spatial correlation of AT-relatedness across voxels. Although the components of AT are not highly related (see (1)), spatial correlations across voxels demonstrate a similar pattern of brain-phenotype relationships between components of AT (Fig S1b,  $r^2_{[Freezing,Cooing]}=.71$ ; c,  $r^2_{[Freezing,Cortisol]}=.37$ ; d,  $r^2_{[Cooing,Cortisol]}=.40$ ; all p's<.0001). While the scatter plots reveal some phenotype specific voxels, most of the regions that we highlight commonly relate to each of our three anxiety-related measures. Consistent with our previous research, these data suggest that the components of AT are, in part, associated with a shared neural substrate for anxiety-related responding (1, 2). For these reasons, we have focused on AT as a whole. It will be critical for future research to further differentiate general AT-related regions, from those that specifically relate to a particular phenotypic expression of anxiety.

### **Cross-validation analysis of imaging-AT relationships**

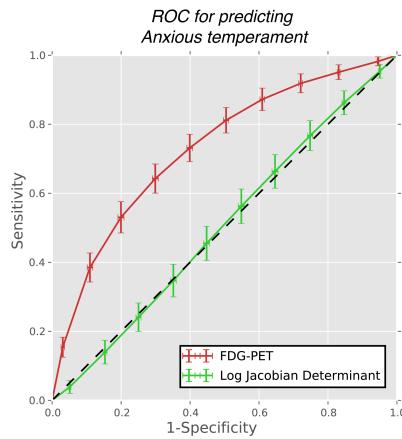
To obtain reliable estimate of the utility of brain metabolism and local brain volume in predicting extreme early-life AT, we performed supervised learning analyses with repeated cross-validation. These analyses use every brain voxel as a predictor and AT as the outcome variable. Analyses were performed separately for brain metabolism (i.e. FDG-PET) and local brain volume (i.e. log jacobian determinant). Prior to statistical analysis, each voxel was residualized for the potentially confounding effects of age, sex, site, MRI scanner, prior exposure to NEC, scan order, and affine registration parameters. These residuals were used as predictors in regression analyses.

## AT-component related brain volume and metabolism



**Figure S1:** Histograms displaying the distribution of t-values reflecting the relationship between brain metabolism and each component of AT, i.e. freezing, cooing, and cortisol can be seen in (a). Grey arrows represent the threshold for reaching significance at a Šidák corrected  $p<.05$ . Although the components of AT are not highly related (see (1)), spatial correlations across voxels demonstrate a similar pattern of brain-phenotype relationships between components of AT (b,  $r^2_{[Freezing,Cooing]}=.71$ ; c,  $r^2_{[Freezing,Cortisol]}=.37$ ; d,  $r^2_{[Cooing,Cortisol]}=.40$ ; all p's<.0001).

**Receiver operating characteristic curve for brain metabolism and brain volume in predicting extreme AT**



**Figure S2:** ROC curves using FDG-PET (red) and log jacobian determinant (green) to predict AT using elastic net regularized regressions. The dashed black line indicates chance predictions, and curves that near the upper left corner represent better predictors of AT.

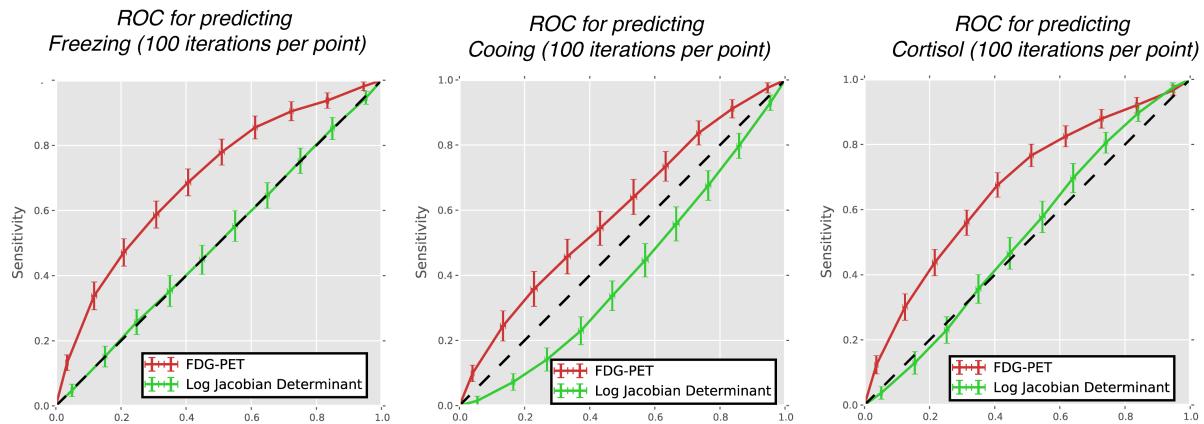
the correlation coefficient between the predicted and actual AT values in the test sample. To estimate the amount of variance that could be predicted, this procedure was repeated 1000 times.

To determine the utility of brain metabolism and brain volume in predicting extreme AT, we performed cross-validation analyses to determine the sensitivity and specificity of our AT-prediction. Receiver operating characteristic (ROC) curves were computed for classification of individuals into a high-AT groups defined at various percentiles (i.e. 0, 5, 15, 25, 35, 45, 55, 65, 75, 85, 95, 100). Sensitivity was computed as the number of correctly predicted AT-individuals divided by the total number of high-AT individuals in the test-set, while specificity was measured as the number of correctly predicted non high-AT individuals divided by the total number of non high-AT individuals in the test-set. Predictive measures should ideally result in high sensitivity and specificity. Complementing our univariate analyses in the full sample, cross-validation of extreme-AT prediction demonstrated significant predictive validity for FDG-PET, but not for the log jacobian determinant (Fig S2). Similar analyses were performed to examine the relationship between neuroimaging measures and the components of AT (i.e. Freezing, Cooing, and Cortisol). Like to AT, examination of the relationship between neuroimaging measures and each of AT's components revealed significant predictive validity for FDG-PET, but not for the log jacobian determinant (Fig S3).

These results indicate that there is valuable AT-related information in the FDG-PET data, but fail to support the hypothesis that early-life AT is associated with altered regional brain volume.

Cross-validation techniques were used to compute voxelwise parameter estimates in a training dataset and use those parameter estimates to examine a test dataset. The training (four-fifths: n= 473) and test (one-fifth: n=119) samples were randomly drawn from the set of 592 subject scans. Supervised learning was performed using an elastic net regularized regression to estimate the best-fitting set of voxels and parameter estimates to predict AT in the training sample, using AT as the dependent measure and all voxels as predictors (3). These voxels and parameter estimates were then used to compute the estimated levels of AT in each subject in the test sample. When the number of predictors is greater than the number of samples, standard regression techniques will fail, as the set of linear equations becomes over-parameterized and rank-deficient. Regularized regression techniques provide a reasoned method for selecting which voxels best predict the AT phenotype and how to optimally weight them. Elastic net regularization combines the LASSO and ridge-regression approaches to prevent over-fitting of the data and punish overly-sparse solutions. Elastic net regularization was used to 'lightly' regularize the regression (i.e. lasso & ridge parameters  $\lambda_1=.001$ ,  $\lambda_2=.009$ , respectively). The amount of variance explained was defined as the square of

## Receiver operating characteristic curve for brain metabolism and brain volume in predicting the components of AT

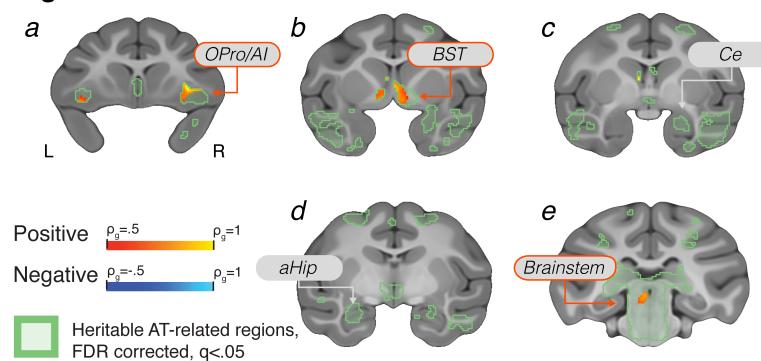


**Figure S3:** ROC curves using FDG-PET (red) and log jacobian determinant (green) to predict Freezing (left), Cooling (middle) and Cortisol (right) using elastic net regularized regressions. The dashed black line indicates chance predictions, and curves that near the upper left corner represent better predictors of AT.

### Heritability of brain metabolism

The FDG-PET findings in this manuscript replicate and extend our previously published work, which were performed in the initial subjects from this sample ( $n=238/592$ ; (4)). In  $n=238$  we found heritability differed across AT's neural substrates. Of particular interest, we found that anterior hippocampal metabolism was heritable, whereas Ce metabolism was not. This finding replicates in the non-overlapping portion of the present sample ( $n=354/592$ ; FDR  $q < .05$ ). Power analyses revealed that we had a  $\sim 35\%$  chance of detecting a voxel that was 25% heritable as different from zero in the initial sample, whereas in the combined  $n=592$  sample we obtained  $\sim 95\%$  power for this same test. In  $n=592$ , although some Ce voxels failed to reach significance, we also identified heritable voxels within the Ce-region (i.e. peak  $h^2$  in Ce:  $p=.0003$ ). Consistent with our prior work, the peak hippocampal metabolism was observed to be nearly twice as heritable (e.g. peak  $h^2$  in aHip=.50). The differential heritability of ATs neural substrates provides important information that can help us to understand the biology of anxiety. In particular, these data suggest that different genetic pathways are likely to influence the function of the amygdala and anterior hippocampus. Moreover, by extending these results to include genetic correlation analyses, we can begin to identify those regions that are most likely to mediate the heritable risk to develop anxiety and depressive disorders (See main text as well as Figure S4).

### AT-related regional brain metabolism shares a genetic substrate with AT



**Figure S4:** Regions where brain metabolism showed a significant genetic correlation with AT include include orbital proisocortex/anterior insula (OPro/AI; shown in [a]), bed nucleus of the stria terminalis (BST; shown in [b]), and periaqueductal gray (PAG; shown in [e]), with no significant results in central nucleus of the amygdala (Ce; shown in [c]), anterior hippocampus (aHip; shown in [d]) or other brainstem regions (shown in [e]).

*Identifying candidate genes using publically available databases*

To begin the process of identifying molecules that might mediate the heritable component of the risk to develop anxiety and depressive disorders, we examined regional variation in the levels of gene expression from publically available human brain data. Specifically, we compared gene expression in regions genetically correlated with both AT and each other, to gene expression in the rest of the brain. By virtue of their preferentially high expression in brain regions that mediate the intergenerational transfer of AT, these genes are candidates for future mechanistic studies aimed at identifying the molecular underpinnings of the heritable risk to develop anxiety and depressive disorders. All analyses were performed based on microarray-measured gene expression in the human brain using the differential expression tool at <http://human.brain-map.com>, provided by the Allen Institute for Brain Science (AIBS)(5). These analyses are not intended to implicate genetic variation within these genes in AT. Rather, these analyses based on our non-human primate brain imaging data aim to provide researchers with a list of potential molecular systems within the tripartite OPro/AT-BST-PAG circuit that may contribute to the intergenerational transmission of anxiety. Because our between-region genetic correlation analyses revealed significant genetic correlations in the metabolism between BST and both PAG and OPro/AI, we targeted differential gene expression searches to those genes preferentially expressed in regions most homologous to our BST-PAG and BST-OPro/AI clusters. Specifically, independent differential gene expression searches were performed in the corresponding BST-PAG (i.e. central gray of the midbrain similar to PAG, and left bed nucleus of stria terminalis ["CGMB BST-L"] versus all gray matter ["GM"]) and BST-OPro/AI regions (i.e. posterior orbital gyrus similar to OPro, short insular gyrus similar to AI, and left bed nucleus of stria terminalis ["POrG SIG BST-L"] versus all gray matter ["GM"]) of the human brain. These tests were used to identify the top 200 genes with the greatest fold-change increase in expression between POrG, SIG, & BST-L compared to the rest of the brain (Dataset S2a), and a similar list of the top 200 genes with the greatest fold-change increase in expression in PAG & BST-L compared to the rest of the brain (Dataset S2b). We restricted these exploratory analyses to genes that showed greater expression in target areas compared to the rest of the brain, as interpreting relatively decreased expression as unrelated to the heritability of AT would require: 1) accepting that these are true null findings, and 2) assuming that lack of expression in human brains during adulthood implies that these molecules do not play a role in the cross-generational transfer of AT. Analyses revealed several well-known molecules implicated in stress-related psychopathology (e.g. serotonin transporter [SLC6A4], corticotropin-releasing hormone [CRH]), as well as several promising targets for stress-related intervention (e.g. neuropeptide Y [NPY], somatostatin [SST], and serotonin receptor 2C [HTR2C]), and several molecules that represent novel candidates for examination in relation to AT (Dataset S2). Based on our genetic correlation analyses, the genes in these lists are reasonable candidates for contributing to the function of the tripartite OPro/AI-BST-PAG circuit and the heritable components of AT.

In order to assess the relevance of these gene lists to anxiety, and to identify broader anxiety-relevant molecular processes, we performed gene ontology analyses using Enrichr (6). Specifically, using gene set enrichment analyses, we examined the relative number of genes identified in Dataset S2 that belong to curated gene-sets with known functions (7). These analyses revealed an over-representation of genes in the Biological Processes (n=75; Dataset S3a), Cellular Component (n=15; Dataset S3b), and Molecular Function ontologies (n=25; Dataset S3c). Specific over-represented ontologies include, "neuropeptide hormone activity" (GO:0005184), "synapse part" (GO:0044456), and "behavioral fear response" (GO:0001662). These informatics-based proposals for molecular processes contributing to the function of the tripartite prefrontal-limbic-midbrain circuit, provide a "proof-of-concept" for combining our large-scale brain imaging studies in non-human primates with publically-available neurogenetic datasets to gain translational insight. A deeper understanding of the mechanisms and regulators of regional gene expression will

continue to refine these candidates for the molecules responsible for the heritable risk to develop anxiety and depressive disorders.

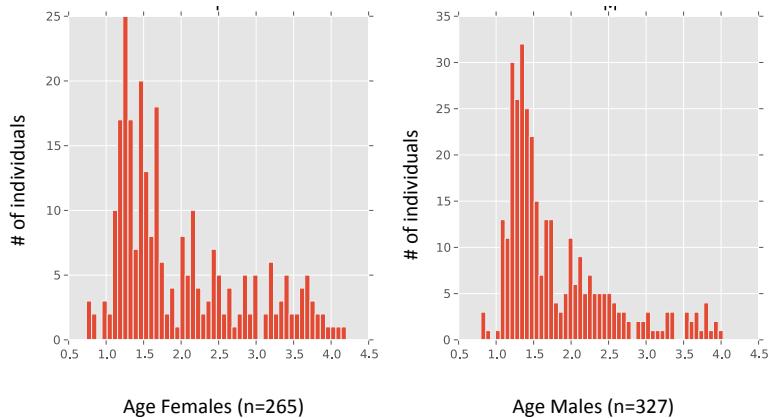
## Supplementary Methods

### *Method overview*

Each animal was injected with FDG and exposed to the NEC-context in which a human intruder presents their profile to the monkey for 30-minutes prior to receiving a PET scan. This paradigm allows us to obtain a measurement of integrated regional brain metabolism during exposure to the NEC-context. Brain metabolism was regressed against AT to identify AT-related brain regions. Heritability of local brain volume and brain metabolism was estimated at each voxel based on each pair of animals' degree of relatedness. Bivariate heritability estimates were similarly computed to examine the degree to which AT and regional brain metabolism share a genetic substrate. All experiments were performed according to the federal guidelines of animal use and care and with the approval of the University of Wisconsin Madison Institutional Animal Care and Use Committee.

### *Subjects*

Five hundred and ninety-four young rhesus monkeys that were part of a large multi-generational pedigree were phenotyped for brain metabolism and stress-related behaviors. Paternity tests were performed when paternity was in question, which resulted in 2 animals being excluded from all analyses, resulting in five hundred and ninety-two animals included in all analyses (Age:  $\mu=1.88$ ,  $sd=0.78$ ; 327M/265F; see histogram below). All animals were mother-reared, and pair-housed in a vivarium on a 12 hour light/dark cycle with a 6 am light onset at the Harlow Primate Laboratory and the Wisconsin National Primate Research Center. All studies were performed during the light cycle. We attempted to test every young rhesus monkey at the University of Wisconsin-Madison's Harlow Lab and Primate Center that was available for study. The availability of animals during the period data collection determined the final sample size. Animals that underwent prior drug administration or surgery were excluded. Although, a small number of animals underwent prior behavioral testing at some point in their lives as a part of experiments in other laboratories, these effects were considered to be random with respect to the effects of interest, part of the environmental influences, and were, therefore, not specifically examined. The typical life span of a rhesus macaque is approximately 25, and since most animals are weaned between 6 months and 1 year and begin puberty when they are between 3 and 4 years old, the majority of animals are considered to be roughly equivalently aged to pre-pubescent children between 3-12 years old.



### *Multi-generational family pedigree*

Subjects were part of a large multi-generational pedigree of 1928 animals across 8 generations. Scanned subjects from this pedigree consisted of 592 animals, with 350,464 possible relationships. The structure of this multi-generational pedigree consists of: 2 parent-offspring pairs, 28 full-sibling pairs, 44 other pairs of 1<sup>st</sup> degree relatives, 11 avuncular pairs, 1340 half sibling pairs, 1388 other pairs of 2<sup>nd</sup> degree relatives, 3293 pairs of 3<sup>rd</sup> degree relatives, 6991 pairs of 4<sup>th</sup> degree relatives, 73138 pairs of animals related less than 4<sup>th</sup> degree, and 83419 unrelated pairs.

#### *No-Eye-Contact (NEC) context*

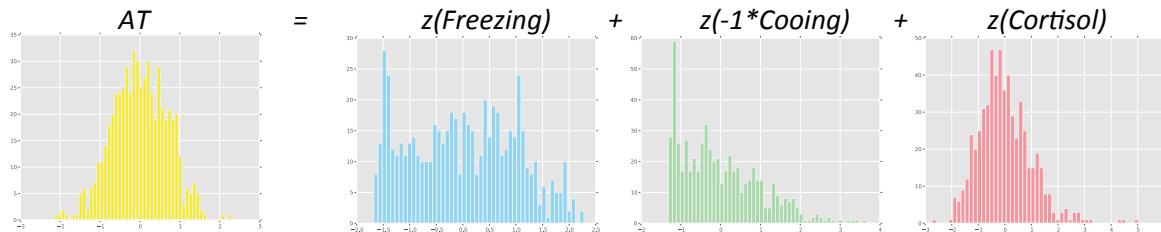
In the NEC-context of the human intruder paradigm a potentially threatening human intruder stands ~2.5 meters away and presents their profile to the monkey while making no-eye-contact (NEC) for 30 minutes (4, 8). In contrast to being alone or exposed to a human intruder staring at the monkey, the NEC context reliably elicits freezing behavior.

#### *Measuring glucose metabolism using [18-F] deoxyglucose PET*

Subjects received an intravenous injection of FDG immediately prior to the 30-minute NEC-context. Following 30-minute exposure to the NEC-context, whole blood was collected for quantifying cortisol and subjects were anesthetized with a 15mg/kg intramuscular injection of ketamine, intubated, and placed in the PET scanner. Anesthesia was maintained using 1-2% isoflurane gas. FDG and attenuation scans were acquired using a Siemens/Concorde microPET P4 scanner (9). Images were reconstructed using standard filtered-backprojection techniques with attenuation- and scatter-correction. This technique results in FDG-PET scans that represent the integrated brain metabolism throughout NEC-exposure. FDG-PET images were transformed to standard space as described below, and intensity-normalized so that the mean brain value was equivalent across individuals. A 2mm FWHM smoothing kernel was applied to account for variation in brain anatomy and registration.

#### *Anxious Temperament (AT) measurements during NEC*

AT estimates were computed based on behavior and physiology during NEC exposure as in other published work (1, 2, 4, 10). Behavior was measured during NEC exposure by a trained rater blind to pedigree information. Freezing was defined as a lack of movement for greater than 3-seconds, and was scored in seconds per 5-min of NEC exposure (30-min total). Mean freezing scores were log-scaled, and standardized after removing the linear effects of age and sex. Cooing was measured as the frequency of coo-vocalizations during each 5-min period of NEC-exposure. Mean cooing frequencies were square-root transformed, and standardized after removing the linear effects of age and sex. Plasma cortisol ( $\mu\text{g}/\text{dL}$ ) was quantified based on samples taken immediately after NEC-exposure. Cortisol was quantified in duplicate using the DPC Coat-a-count radioimmunoassay (Siemens, Los Angeles, CA). Cortisol values were standardized after removing the linear effects of age, sex, and the time-of-day in which samples were taken. A composite measure of AT was computed as the combination of standardized freezing, reductions in cooing and cortisol measures. More specifically, freezing minus cooing plus cortisol all divided by three, as can be seen in the histograms below.



### *Measuring local brain volume using Magnetic Resonance Imaging (MRI)*

Magnetic resonance images (MRI) were collected within 2 months of NEC-exposure. MRI was collected under anesthesia (see above) using a General Electric Discovery 3T scanner (GE Inc., Fairfield, CT) and standard quadrature extremity coil. Anatomical scans were obtained with a 3D T1-weighted, inversion-recovery, fast gradient echo prescription (TI/TR/TE/Flip/NEX/FOV/Matrix/Bandwidth: 600ms/8.65ms/1.89ms/10°/2/140mm/256×224/61.1 kHz) with whole brain coverage (128 slice encodes over 128 mm) reconstructed to 0.27×0.27×0.5 mm on the scanner). Each MRI scan was manually segmented into brain and non-brain tissue. T1-brain images were then transformed to standard space (methods described below), and transformation parameters were saved. The transformations to standard space were decomposed into linear (affine) and non-linear (warp) maps. The proportion of volumetric change between each animal's original scan and template space was quantified as the absolute value of the jacobian determinant of the non-linear transformation. This procedure is akin to computing the number of original-space voxels that became a single voxel in template space. To put volumetric expansions and reductions on the same scale, data were log transformed. Importantly, this procedure accounts for individual differences in total brain volume. This procedure produced a single map for each subject representing the relative volume at each voxel in the brain. These log jacobian determinant maps were smoothed by 2mm in standard space, and used for statistical computations.

### *Study-specific template creation and individual-subject registration*

We created a study-specific template because this unique dataset of 592 young rhesus monkey T1-anatomical scans together constitutes our best estimate of the macro-structure of the young rhesus monkey brain anatomy. Study-specific T1-anatomical template creation was performed using an iterative procedure using Advanced Normalization Tools (ANTS; [http://sourceforge.net/projects/advants/](http://sourceforge.net/projects/advants;); (11, 12)). Each subject's T1-anatomical image was first aligned to a predefined template-space using a non-linear symmetric diffeomorphic image registration in ANTS. Nonlinear registration was performed using a symmetric diffeomorphic image registration and a .25 gradient step-size; a pure cross correlation cost-function with window radius 2 and weight 1; the similarity matrix was smoothed with sigma=2; and this process was repeated at 4 increasingly fine levels of resolution with 30, 20, 20, and 5 iterations at each level respectively. The average of all 592 individual-subject T1's in 'template-space' was computed and taken to be the study-mean. Similarly, the non-linear deformation-field was also averaged and taken to be the deformation-mean. The deformation-mean was inverted and 15% of this deformation was applied to the study-mean, to obtain the first iteration of the study-specific template. To maintain comparability to other studies, and to printed brain atlases, the affine transformation was *not* inverted and applied to the study-mean. The same procedure was performed by aligning each subject's T1-weighted anatomical image to the initial study-specific template. After averaging the images and deformations, a new study-specific template was created by applying 15% of the newest mean-deformation to the newest study-mean. This process was repeated 4 times, to obtain a final study-specific template. Each subject's original T1-anatomical images were then aligned to this study-specific template using the ANTS non-linear registration, as previously described. Each animal's FDG-PET image was aligned to its T1-anatomical image using a rigid body mutual information warp, and the transformation from T1 to template-space was then applied to the FDG-PET image.

### *Measuring serotonin transporter binding using [11-C] DASB*

Serotonin transporter binding was measured using the radioligand [*11-C*]DASB in a sample of 34 animals. Data from these [*C-11*]DASB-PET scans have been previously published (13, 14). Methods for acquisition were fully described in (13). Reconstructed DASB binding estimates were transformed to the n=592 study-specific template using ANTS. Methods are identical to those described above, and transformations were applied to the DASB images.

#### *Measuring dopamine D2/D3 receptor binding using [*18-F*]Fallypride*

Dopamine D2/D3 receptor binding was measured using the radioligand [*18-F*]Fallypride, in a set of 33 animals. Data from these [*18-F*]Fallypride-PET scans and full methods have been previously published (15). Reconstructed Fallypride binding estimates were re-transformed to the n=592 study-specific template using ANTS. Methods are identical to those described above, and transformations were applied to the Fallypride images.

#### *Statistical analysis: Voxelwise correlations*

Voxelwise robust regressions between AT and brain metabolism were performed using fMRIStat (<http://www.math.mcgill.ca/keith/fmrifstat/>) and robustfit in MATLAB (<http://www.mathworks.com/>) (16). To account for potential confounds, all regressions entered potentially the confounding variables age, sex, MRI scanner, prior exposure to NEC, and order of acquisition. We implemented Šidák correction for multiple comparisons to examine AT to brain correlations (17).

#### *Statistical Analysis: Spatial Correlations*

To assess the similarity of the spatial relationships between voxelwise maps, we performed spatial correlations. Each image was converted to a vector, masked for voxels within the brain, and the two vectors were correlated using Pearson's and Spearman's correlations.

#### *Statistical analysis: Heritability analyses*

Heritability analyses were performed using SOLAR (<http://solar.txbiomedgenetics.org/>) based on pedigree information (18). Heritability analyses always controlled for age, age<sup>2</sup>, sex and the age x sex interaction. Heritability analyses were performed at each voxel in the brain for both FDG-PET and log jacobian determinant with the help of the center for high-throughput computing at the University of Wisconsin-Madison and the Open Science Grid (OSG). Heritability analyses were computed as described below, and we corrected for multiple comparisons using the False Detection Rate (FDR) technique (19).

To estimate the heritability of a single trait (i.e. AT or brain metabolism at a single voxel), we first computed the trait's covariance matrix, which we will call  $\Omega$ , where location  $i,j$  in the matrix is filled with the covariance in X between subject  $i$  and subject  $j$ .

$$\Omega_X = Cov[X, X] = \begin{bmatrix} cov(X_1, X_1) & cov(X_1, X_2) & \dots & cov(X_1, X_n) \\ cov(X_2, X_1) & cov(X_2, X_2) & \dots & cov(X_2, X_n) \\ \vdots & \vdots & \ddots & \vdots \\ cov(X_1, X_n) & cov(X_2, X_n) & \dots & cov(X_n, X_n) \end{bmatrix}$$

Where covariance is defined as:

$$cov[X_i, X_j] = E[(X_i - E[X_i])(X_j - E[X_j])]$$

and, at least in this case, the expectation can be defined as:

$$E[X] = \mu(X) = \frac{1}{N} \sum_{i=1:N} X_i$$

The relatedness matrix ( $\Phi$ ) can be computed based on the pedigree, as:

$$\Phi = \frac{1}{2} R$$

where  $R$  is the matrix of each pair of animals relationship to each other, with the  $r$  for a parent and a child  $.5 = (2^{-1})$ ,  $r$  for siblings  $.5 = (2^{-2} + 2^{-2})$ , and so on according to the table below, and beyond.

*Table for relatedness matrix:*

r	relationship	degree of relationship
<b>100%</b>	identical twins; clones	0
<b>50%</b>	parent-offspring	1
<b>50%</b>	full siblings	2
<b>37.5%</b>	3/4 siblings or sibling cousins	2
<b>25%</b>	grandparent-grandchild	2
<b>25%</b>	half siblings	2
<b>25%</b>	aunt/uncle-nephew/niece	3
<b>25%</b>	double first cousins	4
<b>12.5%</b>	great grandparent-great grandchild	3
<b>12.5%</b>	first cousins	4
<b>12.5%</b>	quadruple second cousins	6
<b>9.38%</b>	triple second cousins	6
<b>6.25%</b>	half-first cousins	4
<b>6.25%</b>	first cousins once removed	5
<b>6.25%</b>	double second cousins	6
<b>3.13%</b>	second cousins	6
<b>0.78%</b>	third cousins	8
<b>0.20%</b>	fourth cousins	10

Using the covariance and relatedness matrices, one can estimate the putatively genetic and environmental variance of a quantitative phenotypic trait in the form:

$$\Omega \approx 2\Phi\sigma_g^2 + I_n\sigma_e^2$$

where:

$\Omega$  is the covariance matrix of the phenotype

$\Phi$  is the  $n \times n$  kinship matrix for the pedigree

$\sigma_g^2$  is the variance in the trait due to additive genetic ( $g$ ) effects

$I_n$  is the  $n \times n$  identity matrix

$\sigma_e^2$  is the variance due to unmeasured random effects, i.e. environmental ( $e$ )

It is worth noting that the variance attributed to the environment in this mode, is considered to be random for each subject, and not shared between subjects.

The variance parameters  $\sigma$  can be estimated by maximizing the likelihood function:

$$\mathcal{L}(\sigma_g^2, \sigma_e^2 | y) = -\frac{n}{2} \ln(2\eta) - \frac{1}{2} \ln(\Omega) - \frac{1}{2} (x - \mu_x)' \Omega^{-1} (X - \mu_X)$$

After estimating this model, the heritability ( $h^2$ ) can be estimated based on the variance in genetic and environmental effects, by calculating:

$$h^2 = \frac{\sigma_g^2}{(\sigma_g^2 + \sigma_e^2)}$$

Computing the probability of this heritability and is computed by comparing the log likelihood of the model above and the difference between this model and another where  $\sigma_g^2$  is constrained to equal 0, i.e.:

$$\chi_1^2[\sigma_g^2] = -2\mathcal{L}_{\sigma_g^2=0} + 2\mathcal{L}$$

#### *Statistical analysis: Bivariate heritability analyses*

Bivariate heritability analyses were performed using SOLAR (<http://solar.txbiomedgenetics.org/>) based on pedigree information (18, 20, 21). Bivariate heritability analyses always controlled for age, age<sup>2</sup>, sex and the age by sex interaction. Bivariate heritability analyses examining the shared heritability of AT and FDG-PET were performed at each voxel in the brain as described below, and were corrected for multiple comparisons using the False Detection Rate (FDR) (19). Because there were no significant correlations between AT and the log-jacobian determinant, bivariate heritability was not performed on this dataset. Analyses were performed at the center for high-throughput computing at the University of Wisconsin-Madison and the Open Science Grid (OSG).

Bivariate heritability analyses are performed using methods similar to the heritability analyses detailed above, with a covariance matrix that represents both traits and their interaction. More specifically,

$$\Omega_B = \begin{bmatrix} \Omega_X & \Omega_{YX} \\ \Omega_{XY} & \Omega_Y \end{bmatrix}$$

Where  $\Omega_X$  and  $\Omega_Y$  are as  $\Omega$  above, and the bivariate portions are:

$$\Omega_{XY} \cong 2\Phi\sigma_{g_{XY}}^2 + I_n\sigma_{e_{XY}}^2$$

with  $\phi$  defined as before, and the variance of  $X, Y$  can be decomposed to its component parts:

$$\sigma_{XY}^2 = \sigma_X\sigma_Y\rho_{XY}$$

where  $\rho_{g_{XY}}$  is the genetic correlation, that we have set out to estimate.

This can now be estimated using the same maximum likelihood estimation we described above:

$$\begin{aligned} \mathcal{L}(\sigma_{g_X}^2, \sigma_{e_X}^2, \sigma_{g_Y}^2, \sigma_{e_Y}^2, \rho_{e_{XY}} | X, Y) = & -n\ln(2\eta) - \frac{1}{2}\ln|\Omega_B| \\ & - \frac{1}{2}([\frac{X}{Y}] - \mu_{[\frac{X}{Y}]})' \Omega^{-1} ([\frac{X}{Y}] - \mu_{[\frac{X}{Y}]}) \end{aligned}$$

As before, the three parts of this function are: a distribution parameter (now for bivariate normal), the genetic and environmental components (now including a mean), and the mean.

Similar to the test above, the p-values for s can be computed by estimating the same model with  $\rho = 0$ .

$$\chi_1^2[\rho_g] = -2\mathcal{L}_{\rho_g=0} + 2\mathcal{L}$$

#### *Computing: Center for High-Throughput Computing (CHTC) and the Open Science Grid*

This research was performed using resources and the computing assistance of the UW-Madison Center For High Throughput Computing (CHTC) in the Department of Computer Sciences. The CHTC is supported by UW-Madison and the Wisconsin Alumni Research Foundation, and is an active member of the Open Science Grid, which is supported by the National Science Foundation and the U.S. Department of Energy's Office of Science. All jobs were submitted using HTCondor.

#### *Data availability*

Voxelwise maps representing the AT-relatedness and heritability of brain volume and brain metabolism, can be found in supplementary Dataset 1.

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## Supplementary Legends

**Legend Figure S1:** Histograms displaying the distribution of t-values reflecting the relationship between brain metabolism and each component of AT, i.e. freezing, cooing, and cortisol can be seen in **(a)**. Grey arrows represent the threshold for reaching significance at a Šidák corrected  $p < .05$ . Although the components of AT are not highly related (see (1)), spatial correlations across voxels demonstrate a similar pattern of brain-phenotype relationships between components of AT **(b)**,  $r^2_{[Freezing,Cooing]} = .71$ ; **c**,  $r^2_{[Freezing,Cortisol]} = .37$ ; **d**,  $r^2_{[Cooing,Cortisol]} = .40$ ; all  $p$ 's  $< .0001$ .

**Legend Figure S2:** ROC curves using FDG-PET (red) and log jacobian determinant (green) to predict AT using elastic net regularized regressions. The dashed black line indicates chance predictions, and curves that near the upper left corner represent better predictors of AT.

**Legend Figure S3:** ROC curves using FDG-PET (red) and log jacobian determinant (green) to predict Freezing (left), Cooing (middle) and Cortisol (right) using elastic net regularized regressions. The dashed black line indicates chance predictions, and curves that near the upper left corner represent better predictors of AT.

**Legend Figure S4:** Regions where brain metabolism showed a significant genetic correlation with AT include include orbital preisocortex/anterior insula (OPro/AI; shown in [a]), bed nucleus of the stria terminalis (BST; shown in [b]), and periaqueductal gray (PAG; shown in [e]), with no significant results in central nucleus of the amygdala (Ce; shown in [c]), anterior hippocampus (aHip; shown in [d]) or other brainstem regions (shown in [e]).

**Legend Table S1:** Clusters that are significantly related to AT ( $p < .05$ , Sidak corrected), as well as each local maxima in each cluster that fell in a cytoarchitectonically distinct region and was at least 2mm from the nearest peak.

**Legend Table S2:** Clusters that are: 1) significantly related to AT ( $p < .05$ , Sidak corrected), 2) significantly heritable (FDR  $q < .05$  within AT-related regions), and 3) significantly co-heritable with AT (FDR  $q < .05$  within AT-related heritable regions), as well as each local-maxima within each cluster that fell in a cytoarchitectonically distinct region and was at least 2mm from the nearest peak according to the Paxinos atlas. Importantly, because of the complexity of this analysis readers should not interpret point-estimates of rho-g or non-significant findings.

**Supplementary Table S1: AT-related regions**

Cluster Region	Direction of correlation	Cluster Hemisphere	Cluster volume (mm <sup>3</sup> )	Peak							Heritability of peak			
				Region	Peak Hemisphere	Maximum t-value	p-value	x	y	z	h <sup>2</sup>	p		
Anterior temporal lobe & Orbitofrontal Cortex	+	Right	1090 TPO	Right	8.0727	2.00E-15	21.25	2.5	-9.375		0	1		
				Ce	Right	7.6635	3.84E-14	12.5	2.5	-9.375	0.261337	0.000360489		
				TEa	Right	7.1609	1.22E-12	19.375	0	-12.5	0.174347	0.000328481		
				aHip	Right	7.0817	2.08E-12	15	-6.875	-10.625	0.257345	0.000152826		
				Opro	Right	6.4932	9.06E-11	16.25	9.375	-1.25	0.268771	0.000128925		
				TPPro	Right	6.3251	2.54E-10	21.25	6.875	-6.875	0.201427	0.00237447		
				Pir	Right	5.9655	2.13E-09	13.125	3.75	-4.375	0.188983	0.0121205		
				TLR(Area 36R	Right	5.6065	1.60E-08	13.75	3.75	-17.5	0.166364	0.00698572		
				TE1	Right	5.5715	1.94E-08	19.375	-2.5	-13.125	0.19479	0.000189722		
				ER	Right	5.4874	3.05E-08	11.875	1.875	-15	0.151739	0.00802457		
Brainstem, Thalamus, Hypothalamus	+	Bilateral	2167 PAG	Right	8.9211	3.03E-18	0.625	-15	-3.75	0.188205	0.0171766			
				PTg (region)	Left	8.2515	5.31E-16	-2.5	-13.75	-8.75	0.359567	3.81E-06		
				3N (region)	Right	7.5539	8.30E-14	0.625	-10.625	-6.875	0.513636	0		
				pHip	Left	7.2293	7.72E-13	-10	-16.875	1.25	0.188604	0.00589794		
				IPul	Left	6.8106	1.22E-11	-11.25	-14.375	-1.875	0.346932	1.25E-06		
				MVe (region)	Left	6.6964	2.53E-11	-1.875	-21.25	-15	0.369479	2.93E-05		
				SC	Right	6.6095	4.39E-11	5.625	-13.75	-13.75	0.263692	0.00019896		
				PH	Left	6.5273	7.33E-11	-1.25	-5.625	-4.375	0.324197	6.38E-06		
				PH	Right	6.385	1.76E-10	2.5	-5.625	-4.375	0.344087	2.09E-06		
				RLi (region)	Left	6.184	5.91E-10	-1.25	-8.75	-3.125	0.460485	1.79E-07		
				IPul	Right	5.979	1.97E-09	8.75	-13.75	0.625	0.490416	0		
				PR (region)	Left	5.9405	2.45E-09	-3.75	-8.125	-1.875	0.382055	5.36E-06		
				APul (region)	Left	5.7907	5.76E-09	-7.5	-13.125	6.875	0.36935	1.79E-07		
				MD	Right	5.6464	1.29E-08	1.25	-5.625	5	0.499351	5.96E-08		
				Anterior Temporal	Left	434 TPO	Left	7.1858	1.04E-12	-20	2.5	-12.5	0.153073	0.00567132
				TEa	Left	6.4102	1.51E-10	-16.25	2.5	-14.375	0.242928	9.40E-05		
				TEM	Left	6.2393	4.25E-10	-22.5	-0.625	-12.5	0.188555	0.00140309		
				Pir	Left	5.8871	3.33E-09	-11.25	5	-10	0.155765	0.0173544		
				TPPro	Left	5.7371	7.77E-09	-19.375	7.5	-7.5	0.169864	0.014115		
				AA	Left	5.6471	1.28E-08	-11.875	3.125	-6.25	0	1		
				ST1	Left	5.4891	3.03E-08	-21.875	5.625	-6.875	0.250196	0.0013544		
Hippocampus	+	Left	172 aHip	Left	7.0474	2.61E-12	-11.25	-3.125	-9.375	0.294571	4.89E-05			
Extended Amygdala, Subgenual Cingulate	+	Right	155 BST	Right	6.742	1.90E-11	5.625	3.125	-1.875	0.269537	1.63E-05			
				Area 25	Right	6.3999	1.61E-10	1.25	8.75	1.875	0.40679	1.19E-07		
Temporal Cortex	+	Right	41 TEM	Right	6.0422	1.36E-09	28.125	-8.125	-4.375	0.229413	0.00238067			
Parietal Cortex	+	Right	31 PGa	Right	6.9724	4.27E-12	20	-9.375	-1.875	0	1			
Temporal Cortex	+	Left	35 TEM	Left	6.459	1.12E-10	-26.25	-10.625	-3.125	0.184614	0.00328374			
Orbitofrontal Cortex	+	Left	26 Area 47	Left	5.8177	4.94E-09	-16.25	14.375	4.375	0.12429	0.0409642			
				Area 13	Left	5.7168	8.70E-09	-10.625	14.375	5.625	0.164557	0.00539714		
Septum	+	Right	30 LS	Right	6.0748	1.13E-09	1.875	0	6.25	0	1			

Hippocampus	+	Right	16 Hip	Right	5.7616	6.78E-09	16.875	-10.625	-8.75	0.334407	5.90E-06
Visual Cortex,											
Parietal Cortex	-	Bilateral	7255 V1	Left	-9.1295	5.72E-19	-7.5	-20.625	13.75	0.187854	0.00480813
			PGM	Right	-8.9205	3.05E-18	9.375	-21.25	16.875	0.17998	0.00264174
			V4	Left	-8.7649	1.04E-17	-8.125	-18.75	13.125	0.158826	0.0188324
			PGM	Left	-8.7309	1.36E-17	-11.875	-26.875	0.625	0.335256	3.40E-06
			V1	Right	-8.5666	4.85E-17	6.25	-30.625	0	0.344527	6.56E-07
			V2	Left	-8.4085	1.63E-16	-5	-26.25	17.5	0.251739	9.38E-05
			LIP	Right	-8.2127	7.10E-16	10.625	-29.375	1.25	0.33002	4.05E-06
			V2	Right	-8.1754	9.37E-16	5	-34.375	-0.625	0.337413	4.17E-07
			PEa	Left	-8.0415	2.52E-15	-5	-36.25	1.25	0.30136	1.51E-05
			PEC	Right	-7.9715	4.21E-15	6.875	-33.75	8.75	0.348955	4.05E-06
			PEa	Right	-7.5088	1.14E-13	10.625	-35	1.875	0.329565	1.13E-06
			V3	Right	-7.3254	4.02E-13	2.5	-32.5	15.625	0.24428	2.72E-05
Superior Temporal											
Cortex	-	Right	78 MSTD	Right	-7.0493	2.57E-12	15	-21.875	6.875	0	1
Temporal Cortex	-	Left	83 TPOC	Left	-7.2556	6.47E-13	-12.5	-22.5	9.375	0.138651	0.0373646
Superior Temporal											
Cortex	-	Left	83 MSTD	Left	-6.1216	8.55E-10	-16.25	-24.375	10.625	0.231535	0.00296545
Motor Cortex	-	Left	497 Area 4	Left	-7.9745	4.11E-15	-12.5	-3.75	20	0.211055	6.09E-05
			Area 3	Left	-7.4147	2.18E-13	-6.875	-9.375	23.75	0.33474	0
Motor Cortex	-	Right	445 PE	Right	-7.8161	1.30E-14	11.25	-10.625	18.75	0.194055	0.00117958
			Area 3	Right	-7.2242	8.00E-13	7.5	-6.875	23.125	0.314775	0
			Area 4	Right	-7.1861	1.03E-12	10.625	-1.25	19.375	0.238066	1.31E-06

**Supplementary Table S1:** Clusters that are significantly related to AT ( $p<.05$ , Sidak corrected), as well as each local maxima in each cluster that fell in a cytoarchitectonically distinct region and was at least 2mm from the nearest peak.

**Supplementary Table S2**  
**Regions showing a significant genetic correlation with AT.**

Region	Peak									
	Direction of correlation	Cluster Hemisphere	Cluster volume (mm <sup>3</sup> )	Region	Peak Hemisphere	Maximum rho-g	p-value	x	y	z
Brainstem	+	Left	5.3711	Left	Edinger Westphal/ Oculomotor Nucleus (3N)	0.64	0.0080	0	-10	-3.125
BST / Nucleus Accumbens	+	Left	7.5684	Left	BST	0.75	0.0005	-2.5	3.125	-1.25
BST / Subventricular extended amygdala / Nucleus Accumbens	+	Right	47.1191	Right	BST/Nacc	1.00	0.0006	1.25	4.375	0
Orbital/Insular Cortex	+	Right	32.7148	Right	13L	0.82	0.0005	16.875	11.25	1.875
	+			Right	OPro	1.00	0.0011	16.875	9.375	1.25
	+	Left	6.5918	Left	Opro	0.74	0.0023	-16.875	9.375	-2.5
	+			Right	47O	0.93	0.0024	20	11.25	-0.625
	+			Right	AI	0.80	0.0025	15.625	10	2.5
PAG	+	Left	15.1367	Left	PAG	0.86	0.0042	-0.625	-15.625	-3.75
White-matter	+	Right	8.7891	Right	White-matter near Area 47L and Area 45A	0.97	0.0024	18.75	15	6.25
Parietal Cortex	-	Left	5.3711	Left	MSTD	-0.94	0.0018	-11.875	-23.75	11.875
Visual and Parietal Cortex	-	Bilateral	1746.0938	Left	PEa (MIP)	-1.00	0.0000	-8.125	-23.75	16.875
Visual and Parietal Cortex	-			Right	PEa (MIP)	-1.00	0.0001	5.625	-21.875	13.125
	-			Right	V6	-0.73	0.0002	6.875	-30.625	3.75
	-			Right	V2	-0.72	0.0002	11.875	-31.25	0
	-			Right	PO (V6)	-1.00	0.0006	3.125	-33.75	7.5
	-			Left	PEa	-0.88	0.0009	-7.5	-16.25	13.125
	-			Left	V1	-0.69	0.0029	-11.25	-36.25	-1.25
	-			Right	V2	-0.63	0.0042	5	-36.25	5.625
Visual Cortex	-	Left	8.7891	Left	V2	-0.92	0.0006	-8.125	-20.625	-4.375
Visual cortex	-	Right	31.7383	Right	V1	-0.82	0.0038	5.625	-43.125	-5.625
Visual Cortex	-	Left	2.4414	Left	V3	-0.71	0.0071	-11.25	-22.5	-4.375

**Supplementary Table S2:** Clusters that are: 1) significantly related to AT ( $p<.05$ , Sidak corrected), 2) significantly heritable (FDR  $q<.05$  within AT-related regions), and 3) significantly co-heritable with AT (FDR  $q<.05$  within AT-related heritable regions), as well as each local-maxima within each cluster that fell in a cytoarchitectonically distinct region and was at least 2mm from the nearest peak according to the Paxinos atlas. Importantly, because of the complexity of this analysis readers should not interpret point-estimates of rho-g or non-significant findings.