TITLE: Novel transcriptome-based polygenic risk score for depression is associated with neural processing of social stimuli

AUTHORS AND AFFILIATIONS:

K. Marečková[1], C. Hawco [1], A. Bakht [1], N. Calarco, [1] A.N. Voineskos, [1,2] E. Sibille [1,2,3], A.R. Hariri [4], Y.S. Nikolova [1,2]

[1] Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health (CAMH), Toronto, ON, Canada
[2] Department of Psychiatry, University of Toronto
[3] Department of Pharmacology and Toxicology, University of Toronto
[4] Laboratory of NeuroGenetics, Department of Psychology & Neuroscience, Duke University, Durham, NC, 27708, USA

CORRESPONDING AUTHOR: Yuliya S. Nikolova, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health (CAMH), 250 College Street, Toronto, ON, M5T 1L8, Canada; **Email**: yuliya.nikolova@camh.ca; **Phone**: 416-535-8501 ext. 30934.

SHORT RUNNING TITLE: Novel polygenic risk score for depression

KEYWORDS: transcriptome, polygenic risk, depression, anhedonia, functional magnetic resonance imaging (fMRI), amygdala, partial least square (PLS) regression

NUMBER OF WORDS IN THE ABSTRACT: 251
NUMBER OF WORDS IN THE MAIN TEXT: 4060
NUMBER OF FIGURES: 6
SUPPLEMENTARY INFORMATION: 1

ABSTRACT

Background: Convergent data from imaging and postmortem brain transcriptome studies implicate corticolimbic circuit (CLC) dysregulation in the pathophysiology of depression. To more directly bridge these lines of work, we generated a novel transcriptome-based polygenic risk score (T-PRS), capturing subtle shifts towards depression-like gene expression patterns. We then mapped this T-PRS onto CLC function and related depressive symptoms in a non-clinical sample of young adults.

Methods: Genetic, self-report, and neuroimaging data were available in 482 Duke Neurogenetics Study participants (226 men; age 19.78+/-1.23). T-PRS was generated based on common functional SNPs shifting gene expression in the brain towards a depression-like state. We used multivariate partial least squares regression to map T-PRS onto whole-brain activity patterns during perceptual processing of social stimuli (i.e., human faces). Posthoc univariate analyses followed up on the link between T-PRS and amygdala reactivity to neutral and threatening faces. For comparison, we generated a PRS summarizing depression risk variants identified by the Psychiatric Genomics Consortium (PGC-PRS). Sex was modeled as moderating factor.

Results: T-PRS was associated with male-specific reductions in neural response to neutral faces in a widespread network of cortical and subcortical regions (multivariate $p=0.03$) including the amygdala (beta=-0.14, $p=0.04$). These results mirrored patterns associated with PGC-PRS independently of sex ($ps<0.01$). Reduced reactivity to neutral faces was further associated with increased self-reported anhedonia.

Conclusions: We demonstrate for the first time that in men functional SNPs mimicking the postmortem transcriptomic signature of depression are associated with blunted neural activity to social stimuli, which may be expressed as increased anhedonia.

INTRODUCTION

Major depressive disorder (MDD or depression) is the leading cause of disability worldwide (1), with lifetime prevalence up to 17% (2). Studies on the biological basis of depression using in vivo neuroimaging or postmortem gene expression often focus on the same neural circuits, but have largely run in parallel, precluding an integrative understanding of this complex and heterogeneous disease. In vivo neuroimaging (3-10) and postmortem transcriptome studies (11) have nonetheless independently converged to associate depression with dysregulation in a distributed corticolimbic circuit (CLC), which lies at the intersection of affective, cognitive, and perceptual processing.

Functional neuroimaging studies of the CLC in depression have typically focused on the amygdala as a primary region of interest, because this structure serves as a processing hub through which associative learning of threat and related physiological and behavioral responses are coordinated (3, 5, 6). These studies associate depression with increased amygdala reactivity to threat (3), which may pre-date the development of depressive symptoms (4) and persist after remission (3, 5, 6). Consistent with the heterogeneity of the disease, however, other studies have reported blunted amygdala reactivity in individuals with, or at risk for, depression (7-10, 12). Although the behavioral implications of these divergent neural phenotypes are incompletely understood, amygdala hyper-reactivity has generally been interpreted as reflecting a depression risk pathway associated with high anxiety and stress sensitivity (4), while hypo-activity may reflect reduced behavioral engagement, consistent with co-occurring trait-like psychomotor slowing (7, 10, 12) and motivational deficits (10). Functional connectivity studies using both hypothesis- and data-driven approaches have additionally reported reduced coupling between amygdala and prefrontal regulatory regions (13, 14), as well as broader dysconnectivity within and beyond the CLC (15-20).

In parallel to this functional neuroimaging work, postmortem studies in depression have demonstrated changes in gene expression patterns in key CLC nodes, which may partially explain the observed macroscale CLC dysregulation noted above. A recent meta-analysis of 8 transcriptome datasets (11) identified 566 genes consistently up- or down-regulated in individuals with depression across the amygdala, subgenual anterior cingulate (sgACC), and dorsolateral prefrontal cortex (dlPFC) (11). The altered molecular pathways suggested by these transcriptomic findings include reduced neurotrophic support, neuronal signaling, and GABA function. These pathways have also been implicated in normal lifelong aging of the brain (21, 22), lending support to the notion that depression may be associated with accelerated, or anticipated, molecular brain aging (21, 23). Finally, functional neuroimaging studies suggest normal aging is associated with blunted CLC processing of emotional faces (24), mirroring neural activity patterns observed in specific depression subtypes (7-10, 12).

Despite this promising convergence, postmortem findings have limited clinical utility, as they provide little insight into the temporal primacy of gene expression changes and any clinically relevant pre-mortem symptoms. However, the persistent neuroimaging findings of CLC dysregulation associated with risk for depression and its pathophysiology (3-6) raise the possibility that some of the depression-associated transcriptome changes in the postmortem CLC may be present before overt disorder develops. Thus, these changes may represent a vulnerability factor or pathway targetable in the context of early prevention efforts.

To test this novel hypothesis we evaluated associations between common functional variant-driven gene expression changes, partially mimicking the postmortem depression transcriptome, and in vivo CLC activity as well as related symptoms of depression in a non-clinical sample of young adults. Specifically, we leveraged a list of genes associated with depression on the whole-transcriptome level (21), and the PrediXcan tool for "imputing" brain-level gene expression based on peripherally assessed common single nucleotide polymorphisms (SNPs) (25) to develop a novel CLC transcriptome-based polygenic risk score (T-PRS) for molecular vulnerability to depression. We then used a data-driven multivariate approach (partial least squares regression) to map T-PRS onto brain activation to social stimuli (i.e., faces) measured using BOLD fMRI in young adults from the Duke Neurogenetics Study (DNS) (26). For continuity with prior work, we also conducted univariate analyses focused on amygdala reactivity. We further compared this novel T-PRS to a non-overlapping

PRS based on the latest genome-wide association study from the Psychiatric Genetics Consortium MDD working group (PGC-PRS; (27)). Given well-documented sex differences in depression risk (28-32), and CLC function (33-36), sex was included as a moderating factor in all analyses.

METHODS

Participants

Our sample included 482 young adult university students (226 men, 256 women; mean age 19.78 +-1.23) who successfully completed the DNS. All participants provided informed consent in accordance with Duke University guidelines prior to participation and were in good general health (see (37) for full exclusionary criteria). Participants were screened for DSM-IV Axis I and select Axis II disorders (Antisocial and Borderline Personality Disorder) using the eMINI (38), but a current or lifetime diagnosis of a disorder was not exclusionary (**Supplementary Table 1).** The Duke University Institutional Review Board approved all study procedures. We limited our analyses to Non-Hispanic Caucasians to match the ethnic background of the postmortem cohorts used to develop our T-PRS.

Calculation of polygenic risk scores

DNA extraction and genotyping was performed as previously described ((10); **Supplementary Methods**). A transcriptome-based polygenic risk score (T-PRS) characterizing depression-related gene expression changes in the CLC was developed based on a list of 566 genes generated by a prior meta-analysis of case-control postmortem brain transcriptome datasets (n=101 postmortem subjects; 50 MDD, 51 controls; 21). Relying on the GTEx "cortex" tissue as a reference transcriptome, the PrediXcan tool (25) successfully "imputed" relative cortical expression levels on the individual participant level for 102 out of these 566 genes based on peripheral SNPs assessed in the DNS (see **Figure 1**). A PRS was created as a sum of these imputed expression values (normalized to a consistent scale across genes), each weighted by previously reported effect size and direction of association with depression, such that higher T-PRS reflects a more depression-like CLC transcriptome. To allow easy computation of our novel T-PRS scores in other studies, we provide the list of the 102 genes in **Supplementary Table 2**. Thirty-two out of the 44 top hits from the latest PGC depression GWAS (27) were present in DNS and used to construct PGC-PRS, via the allelic scoring option in PLINK 1.9 (39).

Self-report measures

The short form of the Mood and Anxiety Symptom Questionnaire (MASQ) (40) was used to provide information on general distress symptoms shared between depression and anxiety (general distress depression and anxiety scales; GDD and GDA, respectively) or symptoms unique to anxiety (anxious arousal, AA subscale) or depression (anhedonia, AD subscale) (40). Early and recent life stress were assessed using the Childhood Trauma Questionnaire (CTQ; (41) and the Life Events Scale for Students (LESS; (42)), respectively.

Acquisition of fMRI data

BOLD fMRI data were acquired during a face-matching task (43) at the Duke-UNC Brain Imaging and Analysis Center using one of two identical research-dedicated General Electric MR750 3T scanners. Briefly, participants were presented with blocks of neutral and emotional faces, interleaved with blocks of simple geometric shapes as a control condition, and were asked to indicate which one of two faces/shapes shown at the bottom of the screen was identical with a target face/shape shown at the top. Detailed information regarding the face-matching task as well as the acquisition protocol are provided in the **Supplementary Methods**.

Multivariate whole-brain analysis

Partial Least Square (PLS) regression (44, 45) was used to identify the functional impact of the T-PRS at the whole-brain level. PLS regression combines features of principal component analysis and multiple regression and is a multivariate approach that allows the simultaneous mapping of a multidimensional set of independent predictors (i.e. task design, polygenic risk, sex) onto a multidimensional set of intercorrelated outcome variables (i.e., voxel time series across the whole

brain). This is achieved through the identification of a smaller set of latent variables (LVs), which best capture covariance between the independent variables and distributed spatial patterns of neural activity, without the need for a priori selected contrasts across experimental conditions or regions of interest (46). Following LV identification, a bootstrapping procedure with 1000 iterations was used to test which specific voxels are reliably related to each significant LV. A bootstrap ratio for each voxel was calculated as the voxel salience divided by its bootstrap standard error. A bootstrap ratio of 2.5 (corresponding to >95% reliability) was used to threshold for all voxel pattern maps in PLS, as previously described (46). Individual-level "brainscores", reflecting subject-specific fit to multivariate brain activation patterns associated with each task condition, were extracted and correlated with self-reported symptoms in an exploratory analysis. A parallel analysis using the same parameters was conducted for the PGC-PRS to allow a side-by-side comparison between the two PRS. Further details regarding the PLS regression analysis are provided in the **Supplementary Methods**.

Posthoc univariate analyses focused on the amygdala

After preprocessing (see **Supplementary Methods**), a first-level, single-subject general linear model (GLM) was used to estimate BOLD responses during (1) Emotional/Threat (Angry and Fearful) vs. Shapes, (2) Emotional/Threat vs. Neutral faces, (3) Neutral faces vs. Shapes contrasts. We focused on these three contrasts, because taken together they allow for dissociating aspects of amygdala reactivity specific to threat from those associated with general social novelty (47). These individual contrast images (i.e., weighted sum of the beta images) were then used in second-level random-effects models to determine mean condition-specific neural reactivity using one-sample *t*-tests with a voxel-level statistical threshold of $p < .05$, family-wise error (FWE) corrected for multiple comparisons across the anatomically defined (Automated Anatomical Labeling [AAL] Atlas-based) amygdala. Hemisphere-specific estimates for the three contrasts of interest were extracted from functional clusters exhibiting a main effect of task using Marsbar (version 0.41) and exported to JMP statistical software version 10.0.0 (SAS Institute Inc., Cary, NC) to perform statistical analyses.

First, a single full-factorial repeated-measures GLM assessed the relationships between either PRS (modeled as a continuous between-subject factor), and sex (x2), hemisphere (x2), and contrast (x3) (modeled as within-subject factors) on amygdala reactivity. Post hoc linear regressions then followed any potential interactions. Next, multiple regression assessed the relationships between self-reported mood and anxiety symptoms and amygdala reactivity. Finally, a moderated mediation used a bootstrapping approach to assess the possible mediation of the relationship between T-PRS and depressive symptoms by amygdala reactivity. All GLM were conducted with and without age, early and recent life stress, and MDD diagnosis as covariates.

RESULTS

Polygenic risk and variability in whole-brain activation

Our multivariate PLS analysis identified one significant latent variable (LV1; p=0.03), which accounted for 38.8% of crossblock covariance (i.e., shared variance between T-PRS, task condition, and voxel-wise brain activity). The spatial pattern of brain activity in this LV mapped onto 50 clusters greater than 20 voxels, spanning a distributed network including the amygdala, hippocampus, caudate, and cerebellum as well as the ventromedial prefrontal and orbitofrontal cortices (see **Supplementary Table 3**). Activity in all clusters was negatively correlated with the Neutral faces condition in men, indicating that, across this network, higher T-PRS was associated with male-specific blunting of activity driven by the neutral faces condition. In women, higher T-PRS was not significantly related to activity in response to neutral faces but was instead coupled with increased activity in these regions in response to the baseline shapes condition (**Figure 2A**).

For comparison, we used the same data-driven multivariate framework to examine the whole-brain activity patterns associated with PGC-PRS, which was uncorrelated with T-PRS (r=0.05, p=0.31). This analysis identified one significant LV (p<0.001) which accounted for 51% of crossblock covariance and mapped onto 36 clusters greater than 20 voxels spanning larger regions of the dlPFC and parietal cortices, as well as regions overlapping with the T-PRS-associated network (see

Supplementary Table 4). Similarly to our T-PRS results, this LV was negatively correlated with activity in response to neutral faces in men (**Figure 2B**). However, unlike our T-PRS results, this multivariate effect emerged for women as well. Additionally, LV1 was negatively correlated with activity in response to emotional faces in women but positively correlated with activity in response to shapes in men. Notably, both scores were associated with activity in key CLC nodes such as the amygdala, anterior cingulate and midfrontal cortices, as well other regions spanning the dorsal striatum, insula, and cerebellum. However, while the T-PRS was more strongly and specifically associated with reduced activity in CLC regions, including the amygdala and hippocampus, caudate, and vmPFC (**Figure 3A-C**), the PGC-PRS effects were more widespread and more robustly associated with reduced activity in dorsal regions, including the dlPFC and parietal cortex (**Figure 3D-E**). Finally, PLS-derived "brainscores" reflecting T-PRS-associated blunting of neural response to neutral faces was further linked with higher anhedonia, but had no effect on other MASQ facets. No additional associations with self-report symptoms emerged for other brainscores associated with either PRS (**Supplementary Figure 1**).

Polygenic risk and amygdala reactivity

The GLM focusing on amygdala reactivity yielded a three-way interaction between T-PRS, sex, and task contrast ($F_{(2,2390)}$=9.95, p<0.0001), and no interactions with hemisphere (p>0.59). Posthoc analyses revealed that T-PRS interacted with sex to predict left amygdala reactivity for the contrast of neutral faces > shapes (beta=-0.10, p=0.02; **Figure 4A**). Broadly consistent with our multivariate results, higher T-PRS was associated with left amygdala reactivity in men (beta=-0.14, p=0.04, R^2=0.02), but not women (beta=0.06, p=0.36) and this relationship was robust to the inclusion of covariates (all p<0.05). The T-PRS-by-sex interaction for the right amygdala was directionally consistent but emerged only as a trend (beta=-0.09, p=0.06; effect in men: beta=-0.11, p=0.06; effect in women: beta=0.06, p=0.33). Consistent with the PLS results, no relationships were present for any contrast involving threat-related emotional expressions (see **Supplementary Table 5**).

The same GLM yielded a two-way interaction between PGC-PRS and contrast ($F_{(2,2390)}$=7.45, p=0.0006) and no interactions with sex (p>0.07) or hemisphere (p>0.27). Posthoc analyses revealed that, in agreement with our T-PRS results, higher PGC-PRS was associated with lower left amygdala reactivity for the contrast of neutral faces > shapes (beta=-0.12, p=0.01, R^2=0.01; corrected for age, stress and MDD diagnosis: beta=-0.11, p=0.01; **Figure 4B**), but not during the contrasts involving threat-related emotional expressions (see **Supplementary Table 6**). However, unlike our T-PRS results, this effect of PGC-PRS was independent of sex (see **Supplementary Table 6**). When included in the same model, both PRSs retained their independent effects (PGC-PRS: beta=-0.12, p=0.007; T-PRS*Sex: beta=-0.11, p=0.02), and collectively accounted for more variance in amygdala reactivity ($F_{(4,477)}$=3.52, p=0.008, R^2=0.03) than each individually.

Amygdala reactivity and depressive symptoms

Consistent with multivariate results, blunted amygdala reactivity to neutral faces was uniquely associated with the anhedonia subscale, even when controlling for other MASQ domains (left amygdala: beta=-0.11, p=0.049, R^2=0.01, **Figure 5A**; right amygdala: beta=-0.15, p=0.009, R^2=0.02; **Figure 5B;** adjusted for covariates: beta=-0.12, p=0.04 and beta=-0.16, p=0.007, respectively). As suggested by the above pairwise associations, there was evidence of statistical mediation wherein T-PRS mapped onto anhedonia indirectly through amygdala reactivity to neutral faces in men (*ab* = 0.01, *SE* = 0.01, 95% CI [0.0007, 0.0364]; **Figure 6**), but not women (ab=-0.005, SE=0.006, 95% CI [-0.02, 0.003]).

DISCUSSION

Here, we provide novel evidence that a polygenic score comprised of functional variants mimicking the postmortem transcriptomic signature of depression (T-PRS) is associated with male-specific widespread reductions in activity across cortex, subcortex, and cerebellum as well as blunted amygdala reactivity to neutral faces, and indirectly, with increased anhedonia, in a non-clinical sample of young adults . Similar blunting of neural reactivity to faces, albeit across sexes, was associated with

an independent polygenic score derived from non-overlapping genome-wide supported risk variants for depression (PGC-PRS). Collectively, these results suggest that some of the postmortem gene expression changes observed in individuals with MDD may be present before disorder develops and may bias neural circuit function to increase depression susceptibility.

Our multivariate PLS analyses associated both T-PRS and PGC-PRS with reduced activity to neutral faces across an extensive network of regions, including, but not limited to the corticolimbic circuit (CLC). Specifically, T-PRS was associated with male-specific reduction in activity to neutral faces in the amygdala and hippocampus, as well as clusters in the caudate, cerebellum, and orbitofrontal cortex. The PGC-PRS was similarly associated with lower activity to neutral faces, but this effect was present across both men and women, and was accompanied by a simultaneous reduction in activity to emotional faces in women. While the networks associated with each of the PRS partially overlapped, the PGC-PRS-associated network was more dorsal, including extensive portions of the dlPFC and lateral parietal cortices, while the strongest effects in the T-PRS-associated network were confined to clusters comprising classic CLC regions, including large parts of the medial temporal lobe (amygdala and hippocampus). This relative spatial specificity is consistent with the provenance of the T-PRS, which is specifically derived from MDD-associated transcriptomic changes in corticolimbic circuit regions.

Within its respective network, each PRS was also associated with relatively higher activity during the "shapes" blocks, which serve as the baseline or control condition for the faces blocks. This elevated baseline effect emerged in women for the T-PRS and in men for the PGC-PRS. Across the two parallel analyses, the shapes and neutral faces blocks generally contributed in opposite ways to the only significant multivariate LVs. This pattern is broadly consistent with the proposition that genetic effects are at least partially driven by the contrast between neutral faces and shapes, even when the two conditions are modeled independently within a multivariate framework. As the shapes blocks are by design less perceptually and cognitively demanding than any of the faces blocks, it is also possible that some of our results point to genetically driven increases in baseline brain activity across the identified networks, consistent with prior work associating depression with a similar neural phenotype (48). Within this framework, we speculate our results may suggest that while T-PRS effects are driven by reduced activity to neutral faces in men, in women these effects are driven by an elevated baseline – an effect which may appear identical in analyses directly contrasting the two conditions. Future work specifically modeling a non-task baseline (i.e., periods of rest) is required to elucidate the validity of this conjecture and the implications of its sex-specificity.

For continuity with prior work, we supplemented our multivariate analyses with traditional contrast-based measures focused on the amygdala. Consistent with our multivariate analyses, we found that T-PRS was associated with reduced amygdala reactivity specifically to neutral faces in men. While the majority of prior work on CLC function in depression has focused on threat-related amygdala reactivity, our results are broadly consistent with recent studies which have found hypoactivity to neutral, but not emotional faces, in youth with severe mood impairment (49), as well as trait-like reductions in amygdala reactivity to both negative and neutral faces, relative to non-face stimuli, in young adults with seasonal depression (7). Similarly, reduced CLC reactivity to faces, often accompanied by slower psychomotor speed (24), is also commonly observed in healthy older adults (40) or young adults at genetic risk for accelerated brain aging (10, 50). This phenotypic convergence is consistent with the overlap in molecular pathways implicated in depression and age-related processes, which is well-documented in postmortem gene expression studies (21) and captured by our T-PRS (11). Our T-PRS neural reactivity results may thus reflect a genetically driven risk pathway shared between specific depression subtypes, including but not limited to seasonal depression, and both pathological and accelerated aging.

Both univariate and multivariate analyses further linked this T-PRS-associated blunting of reactivity to neutral faces with anhedonia, the only depression-specific facet of the MASQ. Furthermore, this association was independent of the remaining three MASQ domains, which capture symptoms unique to anxiety (anxious arousal) or shared between depression and anxiety (general distress scales), and all

of these results were independent of early or recent life stress. While prior studies have associated depression with elevated threat-related amygdala reactivity (3-6), which may partially reflect enhanced neural sensitivity to environmental stress (4) and may be part of a pathophysiological mechanism shared with comorbid anxiety disorders (51), the putative novel risk pathway we identify here likely reflects reduced behavioral engagement, which may be depression-specific and relatively independent of experiential factors.

Despite the markedly distinct provenance of the PGC-PRS and T-PRS, we found that the two had convergent effects in both our multivariate and univariate analyses. Although in contrast to our T-PRS results, the PGC-PRS effect was independent of sex, both scores were associated with reduced neural reactivity to faces, in the amygdala and across a broader network of regions. This convergence is especially intriguing in light of the fact that the two scores likely reflect distinct biological processes. Specifically, the T-PRS is mainly defined by genes associated with cell signaling and age-related processes such as trophic factor function and cell death (11), while the independently derived and uncorrelated PGC-PRS is comprised of variants near genes associated with largely non-overlapping biological pathways pertaining to HPA axis hyperactivation, neuroinflammation, and obesity (27). Collectively, these results suggest that multiple distinct pathways of genetic risk may converge onto a common neural phenotype characterized by blunted neural response to faces. The results are also consistent with the possibility that this neural phenotype may be sensitive to overall genetic risk for depression, independently of any specific underlying molecular mechanism. In line with this proposition, a recent twin imaging study associated cumulative genetic risk for depression with a dampening of amygdala reactivity, in contrast to environmentally mediated risk, which was associated with the opposite phenotype (9).

Notably, our main results associating T-PRS with reduced activity to social stimuli emerged in men only. Given that the genes included in our T-PRS were associated with altered postmortem gene expression similarly in men and women with MDD (11), this specificity is more likely due to the moderating influence of sex on individual cis-eQTL SNP effects, cumulatively captured by the PRS. Indeed, sex can significantly moderate SNP effects on gene expression (52) as well as downstream phenotypic effects (53). The postmortem gene expression data available in the GTEx reference dataset used by the PrediXcan tool were primarily from men (66% of sample). Thus, it is possible the SNP-based gene expression prediction model we used to generate T-PRS may more closely approximate functional SNP effects in men. In addition, recent studies have shown distinct and even opposite signatures of MDD in postmortem gene expression in men and women (54, 55), which are highly suggestive of non-overlapping sex-specific molecular subtypes or pathophysiological mechanisms. It is thus also possible our results reflect a male-specific molecular pathway of depression risk. Finally, the cis-eQTL approach may be better suited to model genetic risk in men due to the stronger modulatory effect of steroid hormones on transcription in women (e.g., via estrogen response elements), which may obscure SNP effects on gene expression.

Our results should be interpreted in the context of several limitations. First and foremost, while our PLS analyses accounted for 30-50% of variance shared between PRS and brain activity, effect sizes in our amygdala-focused univariate analyses were relatively small, on the order of 1-3% variance explained. Although these effect sizes are consistent with those seen in other PRS studies using similar or smaller sample sizes (56, 57), our results suggest multivariate methods leveraging whole-brain voxel-wise co-activation patterns may be better powered to capture complex polygenic effects than analyses focusing on a single region or contrast of interest. Relatedly, task-elicited functional activity in *a priori* regions of interest, including the amygdala, have generally poor reliability thereby limiting the utility of such measures in individual differences research such as that reported herein (58-61). Our multivariate analyses may further mitigate this limitation. Another limitation of this work is that our results are restricted to Non-Hispanic Caucasians. We focused our analysis on this group in order to match the demographic characteristics of the GTEx reference dataset as well as the Ding et al. postmortem dataset, both of which included primarily individuals of European ancestry (85% in GTEx; 97% in Ding et al.; (11)). While this decision likely increased our power to detect significant effects, it limits the generalizability of our results to other ethnic groups. Future research using larger

multi-ethnic samples for both postmortem and neuroimaging analyses is required to elucidate the functional impact of depression-related transcriptomic changes on brain function across ethnicities.

These limitations notwithstanding, our results introduce a novel tool for modeling genetic risk for depression and identify a pathway of depression risk associated with reduced neural activity to social stimuli, which may be partially shared with age-related processes. Pending further refinement, this analytic framework bridging postmortem gene expression work with neuroimaging data may offer novel insight into molecular mechanisms underlying MDD or risk thereof and serve to inform future individualized treatment or prevention efforts.

ACKNOWLEDGEMENTS

YSN is supported by a NARSAD Young Investigator Award from the Brain & Behavior Research Foundation, a Koerner New Scientist Award, and a Paul Garfinkel Catalyst Award administered by the CAMH Foundation. ARH received support from NIH grants R01DA033369 and R01AG049789. ES is supported by NIH Grant R01MH077159 and a NARSAD Distinguished Scientist Award. CH is supported by a NARSAD Young Investigator Award. ANV is currently supported by the Canadian Institutes of Health Research, Canada Foundation for Innovation, the Natioanl Institute of Mental Health, and the CAMH and University of Toronto Foundations.

DISCLOSURES

All authors have no conflicts of interest or any financial disclosures.

LIST OF FIGURES

Figure 1. Study design. A list of 566 genes associated with depression on the transcriptome-wide level was selected based on a prior meta-analysis of 8 postmortem brain transcriptome datasets (Ding et al., 2015). Building on reference data from the combined genomic-transcriptomic GTEx database, the PrediXcan tool (25), was then used to "impute" cortical expression of 102 of these genes based on 482 young adults participating in the Duke Neurogenetics Study (DNS). The imputed values for each of these genes were weighted by their original association with depression and summed into a polygenic risk score (T-PRS), such that higher values indicated a more depression-like transcriptome. This novel T-PRS was then mapped onto brain function during face processing.

Figure 2. Higher levels of T-PRS were associated with lower activity in the LV1 clusters during the Neutral faces condition in men and higher activity in the LV1 clusters during the Shapes condition in women (**2A**). Higher levels of PGC-PRS were associated with lower activity in the LV1 clusters during the Neutral faces condition in both men and women, lower activity in the LV1 clusters during the Emotional faces condition in women, and higher activity in the LV1 clusters during the Shapes condition in men (**2B**).

Figure 3. Brain regions negatively correlated with the novel T-PRS and the PGC-PRS that survived the 2.5 bootstrap ratio (corresponding to 95% reliability) and were greater than 20 voxels. The T-PRS was more strongly and specifically associated with activity in CLC regions including the amygdala and hippocampus (**3A**), caudate (**3B**), and vmPFC (**3C**). The PGC-PRS-based response was more widespread and more strongly associated with activity in dlPFC (**3D**) and parietal inferior cortex (**3E**).

Figure 4. The T-PRS interacted with sex to predict BOLD response in left amygdala during the Neutral faces vs. Shapes contrast (**4A**; beta = -0.10, p=0.02). Higher T-PRS was associated with lower response in the left amygdala in men (beta=-0.14, p=0.04, R^2=0.02) but not women (beta=0.06, p=0.36). In contrast, the PGC-PRS predicted BOLD response in left amygdala during the Neutral faces vs. Shapes contrast independently of sex (**4B**; beta=-0.12, p=0.01, R^2=0.01).

Figure 5. Lower response in amygdala to neutral faces was associated with greater self-reported anhedonia (**5A** – left amygdala: beta=-0.11, p=0.049, R^2=0.01; **5B** – right amygdala: beta=-0.15, p=0.009, R^2=0.02) independently of general distress symptoms shared with anxiety, or anxiety-specific arousal symptoms. These effects remained significant when correcting for sex, early life stress and MDD diagnosis.

Figure 6. The relationship between the T-PRS and anhedonia was mediated by BOLD response in left amygdala during the Neutral faces vs. Shapes contrast and moderated by sex (*ab* = 0.01, *SE* = 0.01, 95% CI [0.0007, 0.0364]).

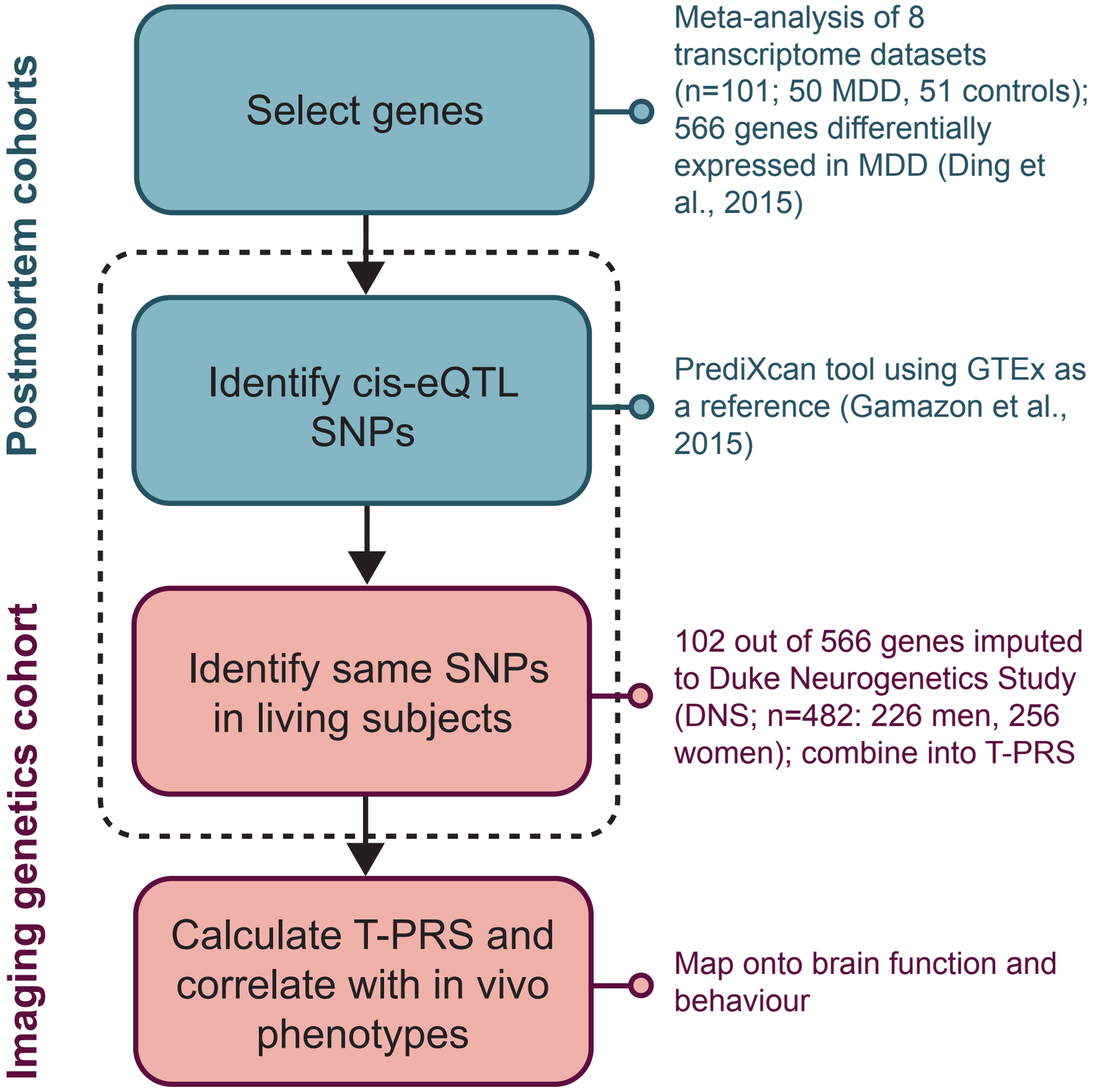
REFERENCES

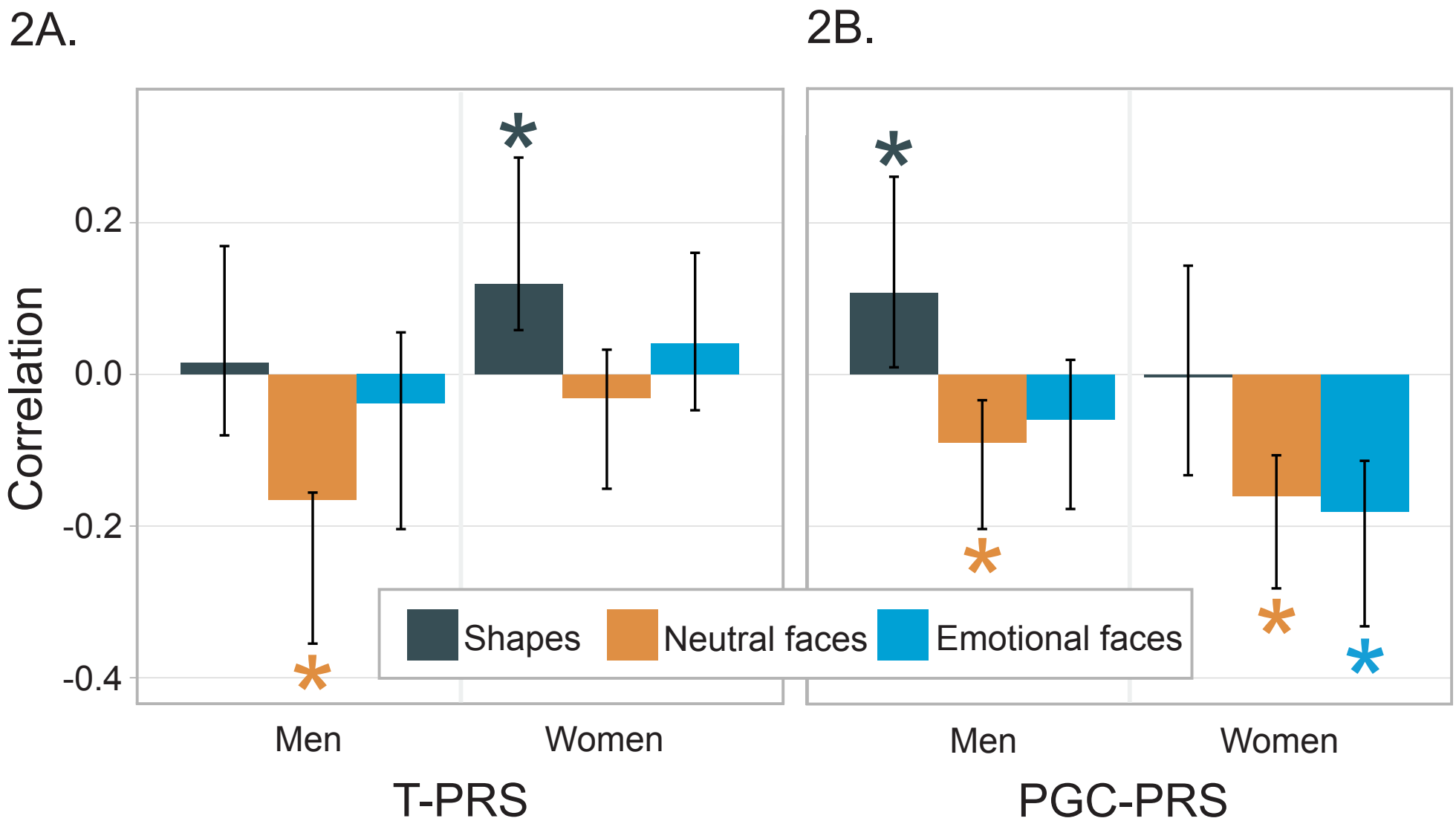
1. Ustun TB, Ayuso-Mateos JL, Chatterji S, Mathers C, Murray CJ (2004): Global burden of depressive disorders in the year 2000. *Br J Psychiatry*. 184:386-392.
2. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005): Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*. 62:593-602.
3. Victor TA, Furey ML, Fromm SJ, Ohman A, Drevets WC (2010): Relationship between amygdala responses to masked faces and mood state and treatment in major depressive disorder. *Arch Gen Psychiatry*. 67:1128-1138.
4. Swartz JR, Williamson DE, Hariri AR (2015): Developmental change in amygdala reactivity during adolescence: effects of family history of depression and stressful life events. *Am J Psychiatry*. 172:276-283.
5. Arnone D, McKie S, Elliott R, Thomas EJ, Downey D, Juhasz G, et al. (2012): Increased amygdala responses to sad but not fearful faces in major depression: relation to mood state and pharmacological treatment. *Am J Psychiatry*. 169:841-850.
6. Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME (1992): A functional anatomical study of unipolar depression. *J Neurosci*. 12:3628-3641.
7. Borgsted C, Ozenne B, Mc Mahon B, Madsen MK, Hjordt LV, Hageman I, et al. (2018): Amygdala response to emotional faces in seasonal affective disorder. *J Affect Disord*. 229:288-295.
8. Williams LM, Korgaonkar MS, Song YC, Paton R, Eagles S, Goldstein-Piekarski A, et al. (2015): Amygdala Reactivity to Emotional Faces in the Prediction of General and Medication-Specific Responses to Antidepressant Treatment in the Randomized iSPOT-D Trial. *Neuropsychopharmacology*. 40:2398-2408.
9. Wolfensberger SP, Veltman DJ, Hoogendijk WJ, Boomsma DI, de Geus EJ (2008): Amygdala responses to emotional faces in twins discordant or concordant for the risk for anxiety and depression. *Neuroimage*. 41:544-552.
10. Nikolova YS, Iruku SP, Lin CW, Conley ED, Puralewski R, French B, et al. (2015): FRAS1-related extracellular matrix 3 (FREM3) single-nucleotide polymorphism effects on gene expression, amygdala reactivity and perceptual processing speed: An accelerated aging pathway of depression risk. *Front Psychol*. 6:1377.
11. Ding Y, Chang LC, Wang X, Guilloux JP, Parrish J, Oh H, et al. (2015): Molecular and Genetic Characterization of Depression: Overlap with other Psychiatric Disorders and Aging. *Mol Neuropsychiatry*. 1:1-12.
12. Hjordt LV, Stenbaek DS, Ozenne B, Mc Mahon B, Hageman I, Hasselbalch SG, et al. (2017): Season-independent cognitive deficits in seasonal affective disorder and their relation to depressive symptoms. *Psychiatry Res*. 257:219-226.
13. Dannlowski U, Ohrmann P, Konrad C, Domschke K, Bauer J, Kugel H, et al. (2009): Reduced amygdala-prefrontal coupling in major depression: association with MAOA genotype and illness severity. *Int J Neuropsychopharmacol*. 12:11-22.
14. Satterthwaite TD, Cook PA, Bruce SE, Conway C, Mikkelsen E, Satchell E, et al. (2016): Dimensional depression severity in women with major depression and post-traumatic stress disorder correlates with fronto-amygdalar hypoconnectivty. *Mol Psychiatry*. 21:894-902.
15. Yang J, Yin Y, Svob C, Long J, He X, Zhang Y, et al. (2017): Amygdala Atrophy and Its Functional Disconnection with the Cortico-Striatal-Pallidal-Thalamic Circuit in Major Depressive Disorder in Females. *PLoS One*. 12:e0168239.
16. Jacobs RH, Barba A, Gowins JR, Klumpp H, Jenkins LM, Mickey BJ, et al. (2016): Decoupling of the amygdala to other salience network regions in adolescent-onset recurrent major depressive disorder. *Psychol Med*. 46:1055-1067.
17. Yue Y, Yuan Y, Hou Z, Jiang W, Bai F, Zhang Z (2013): Abnormal functional connectivity of amygdala in late-onset depression was associated with cognitive deficits. *PLoS One*. 8:e75058.
18. Lui S, Wu Q, Qiu L, Yang X, Kuang W, Chan RC, et al. (2011): Resting-state functional connectivity in treatment-resistant depression. *Am J Psychiatry*. 168:642-648.
19. Luking KR, Repovs G, Belden AC, Gaffrey MS, Botteron KN, Luby JL, et al. (2011): Functional connectivity of the amygdala in early-childhood-onset depression. *J Am Acad Child Adolesc Psychiatry*. 50:1027-1041.e1023.

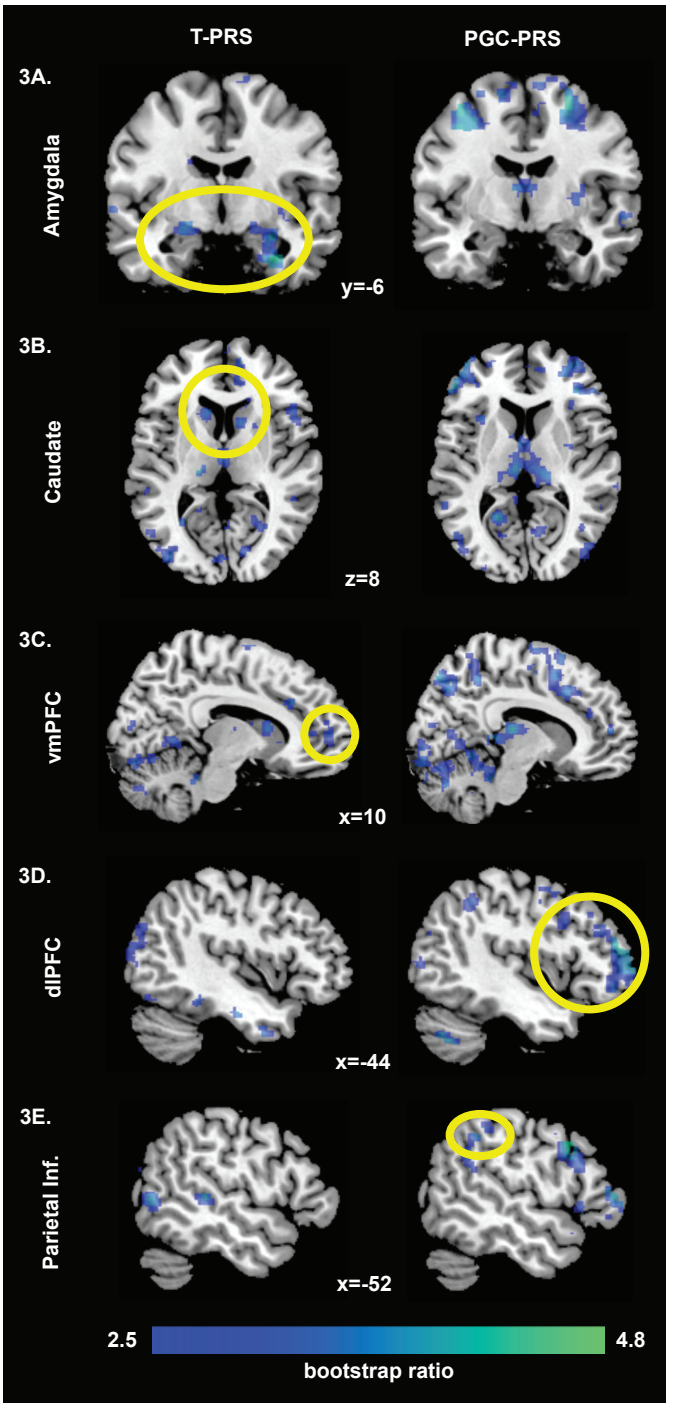
20. Anand A, Li Y, Wang Y, Wu J, Gao S, Bukhari L, et al. (2005): Activity and connectivity of brain mood regulating circuit in depression: a functional magnetic resonance study. *Biol Psychiatry*. 57:1079-1088.
21. Sibille E (2013): Molecular aging of the brain, neuroplasticity, and vulnerability to depression and other brain-related disorders. *Dialogues Clin Neurosci*. 15:53-65.
22. Rozycka A, Liguz-Lecznar M (2017): The space where aging acts: focus on the GABAergic synapse. *Aging Cell*. 16:634-643.
23. Douillard-Guilloux G, Guilloux JP, Lewis DA, Sibille E (2013): Anticipated brain molecular aging in major depression. *Am J Geriatr Psychiatry*. 21:450-460.
24. Tessitore A, Hariri AR, Fera F, Smith WG, Das S, Weinberger DR, et al. (2005): Functional changes in the activity of brain regions underlying emotion processing in the elderly. *Psychiatry Res*. 139:9-18.
25. Gamazon ER, Wheeler HE, Shah KP, Mozaffari SV, Aquino-Michaels K, Carroll RJ, et al. (2015): A gene-based association method for mapping traits using reference transcriptome data. *Nat Genet*. 47:1091-1098.
26. Koenigs M, Grafman J (2009): The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. *Behav Brain Res*. 201:239-243.
27. Wray NR, Ripke S, Mattheisen M, Trzaskowski M, Byrne EM, Abdellaoui A, et al. (2018): Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nat Genet*. 50:668-681.
28. Organization WH (2016): Global Health Estimates 2015: Disease burden by Cause, Age, Sex, by Country and by Region, 2000-2015.
29. Albert PR (2015): Why is depression more prevalent in women? *J Psychiatry Neurosci*. Canada, pp 219-221.
30. Seney ML, Sibille E (2014): Sex differences in mood disorders: perspectives from humans and rodent models. *Biol Sex Differ*. 5:17.
31. Kessler RC (2003): Epidemiology of women and depression. *J Affect Disord*. 74:5-13.
32. Cyranowski JM, Frank E, Young E, Shear MK (2000): Adolescent onset of the gender difference in lifetime rates of major depression: a theoretical model. *Arch Gen Psychiatry*. 57:21-27.
33. Hardee JE, Cope LM, Munier EC, Welsh RC, Zucker RA, Heitzeg MM (2017): Sex differences in the development of emotion circuitry in adolescents at risk for substance abuse: a longitudinal fMRI study. *Soc Cogn Affect Neurosci*. 12:965-975.
34. Mareckova K, Holsen LM, Admon R, Makris N, Seidman L, Buka S, et al. (2016): Brain activity and connectivity in response to negative affective stimuli: Impact of dysphoric mood and sex across diagnoses. *Hum Brain Mapp*. 37:3733-3744.
35. Andreano JM, Dickerson BC, Barrett LF (2014): Sex differences in the persistence of the amygdala response to negative material. *Soc Cogn Affect Neurosci*. 9:1388-1394.
36. Hamann S (2005): Sex differences in the responses of the human amygdala. *Neuroscientist*. 11:288-293.
37. Nikolova YS, Knodt AR, Radtke SR, Hariri AR (2016): Divergent responses of the amygdala and ventral striatum predict stress-related problem drinking in young adults: possible differential markers of affective and impulsive pathways of risk for alcohol use disorder. *Mol Psychiatry*. 21:348-356.
38. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. (1998): The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 59 Suppl 20:22-33;quiz 34-57.
39. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. (2007): PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 81:559-575.
40. Watson D, Weber K, Assenheimer JS, Clark LA, Strauss ME, McCormick RA (1995): Testing a tripartite model: I. Evaluating the convergent and discriminant validity of anxiety and depression symptom scales. *J Abnorm Psychol*. 104:3-14.

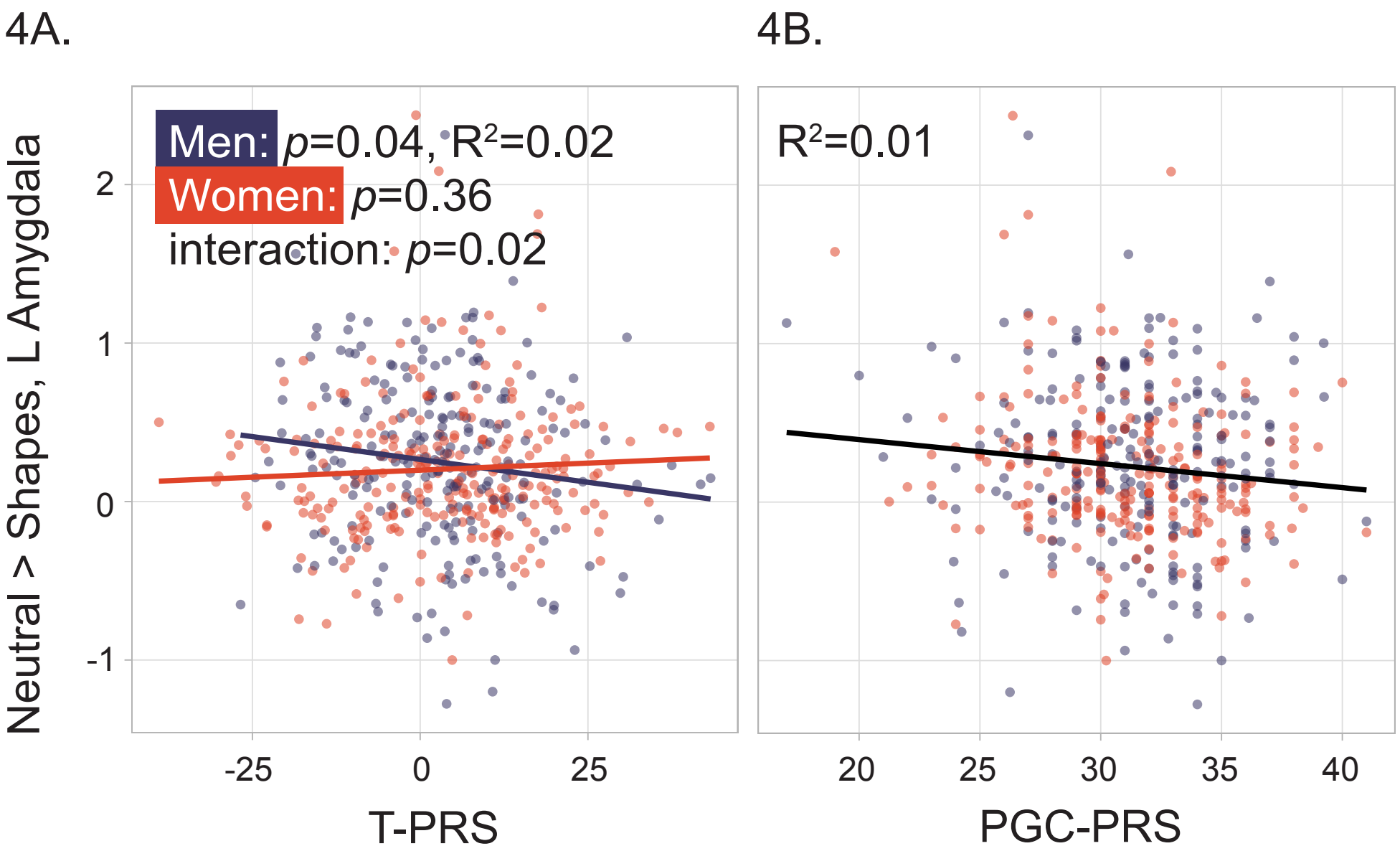
41. Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T, et al. (2003): Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse Negl*. 27:169-190.
42. W L (1984): Development and initial validation of a life event scale for students. *Canadian Counsellor*. 18.
43. Carre JM, Hyde LW, Neumann CS, Viding E, Hariri AR (2013): The neural signatures of distinct psychopathic traits. *Soc Neurosci*. 8:122-135.
44. McIntosh AR, Lobaugh NJ (2004): Partial least squares analysis of neuroimaging data: applications and advances. *Neuroimage*. 23 Suppl 1:S250-263.
45. Krishnan A, Williams LJ, McIntosh AR, Abdi H (2011): Partial Least Squares (PLS) methods for neuroimaging: a tutorial and review. *Neuroimage*. 56:455-475.
46. Hawco C, Kovacevic N, Malhotra AK, Buchanan RW, Viviano JD, Iacoboni M, et al. (2017): Neural Activity while Imitating Emotional Faces is Related to Both Lower and Higher-Level Social Cognitive Performance. *Sci Rep*. 7:1244.
47. Arloth J, Bogdan R, Weber P, Frishman G, Menke A, Wagner KV, et al. (2015): Genetic Differences in the Immediate Transcriptome Response to Stress Predict Risk-Related Brain Function and Psychiatric Disorders. *Neuron*. 86:1189-1202.
48. Northoff G, Sibille E (2014): Why are cortical GABA neurons relevant to internal focus in depression? A cross-level model linking cellular, biochemical and neural network findings. *Mol Psychiatry*. 19:966-977.
49. Brotman MA, Rich BA, Guyer AE, Lunsford JR, Horsey SE, Reising MM, et al. (2010): Amygdala activation during emotion processing of neutral faces in children with severe mood dysregulation versus ADHD or bipolar disorder. *Am J Psychiatry*. 167:61-69.
50. Avinun R, Nevo A, Knodt AR, Elliott ML, Hariri AR (2017): Replication in Imaging Genetics: The Case of Threat-Related Amygdala Reactivity. *Biol Psychiatry*.
51. Etkin A, Wager TD (2007): Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry*. 164:1476-1488.
52. McRae AF, Matigian NA, Vadlamudi L, Mulley JC, Mowry B, Martin NG, et al. (2007): Replicated effects of sex and genotype on gene expression in human lymphoblastoid cell lines. *Hum Mol Genet*. 16:364-373.
53. Rawlik K, Canela-Xandri O, Tenesa A (2016): Evidence for sex-specific genetic architectures across a spectrum of human complex traits. *Genome Biol*. 17:166.
54. Labonte B, Engmann O, Purushothaman I, Menard C, Wang J, Tan C, et al. (2017): Sex-specific transcriptional signatures in human depression. *Nat Med*. 23:1102-1111.
55. Seney ML, Huo Z, Cahill K, French L, Puralewski R, Zhang J, et al. (2018): Opposite Molecular Signatures of Depression in Men and Women. *Biol Psychiatry*. 84:18-27.
56. Yuksel D, Dietsche B, Forstner AJ, Witt SH, Maier R, Rietschel M, et al. (2017): Polygenic risk for depression and the neural correlates of working memory in healthy subjects. *Prog Neuropsychopharmacol Biol Psychiatry*. 79:67-76.
57. Walton E, Geisler D, Lee PH, Hass J, Turner JA, Liu J, et al. (2014): Prefrontal inefficiency is associated with polygenic risk for schizophrenia. *Schizophr Bull*. 40:1263-1271.
58. Lois G, Kirsch P, Sandner M, Plichta MM, Wessa M (2018): Experimental and methodological factors affecting test-retest reliability of amygdala BOLD responses. *Psychophysiology*. 55:e13220.
59. Nord CL, Gray A, Charpentier CJ, Robinson OJ, Roiser JP (2017): Unreliability of putative fMRI biomarkers during emotional face processing. *Neuroimage*. 156:119-127.
60. Lipp I, Murphy K, Wise RG, Caseras X (2014): Understanding the contribution of neural and physiological signal variation to the low repeatability of emotion-induced BOLD responses. *Neuroimage*. 86:335-342.
61. Plichta MM, Schwarz AJ, Grimm O, Morgen K, Mier D, Haddad L, et al. (2012): Test-retest reliability of evoked BOLD signals from a cognitive-emotive fMRI test battery. *Neuroimage*. 60:1746-1758.

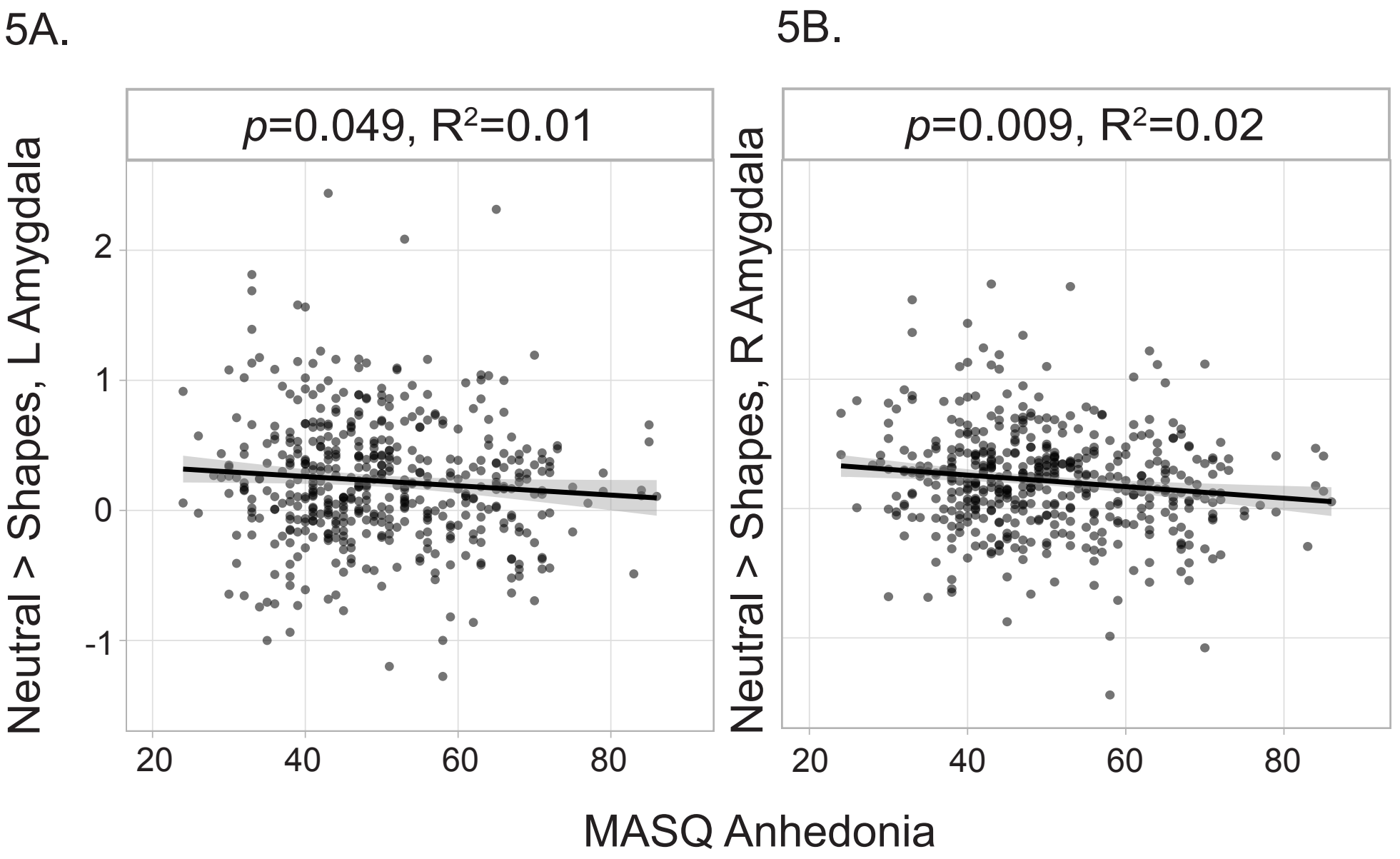
1.


Postmortem cohorts
Imaging genetics cohort
Select genes
Meta-analysis of 8 transcriptome datasets (n=101; 50 MDD, 51 controls); 566 genes differentially expressed in MDD (Ding et al., 2015)
Identify cis-eQTL SNPs
PrediXcan tool using GTEx as a reference (Gamazon et al., 2015)
Identify same SNPs in living subjects
102 out of 566 genes imputed to Duke Neurogenetics Study (DNS; n=482: 226 men, 256 women); combine into T-PRS
Calculate T-PRS and correlate with in vivo phenotypes
Map onto brain function and behaviour

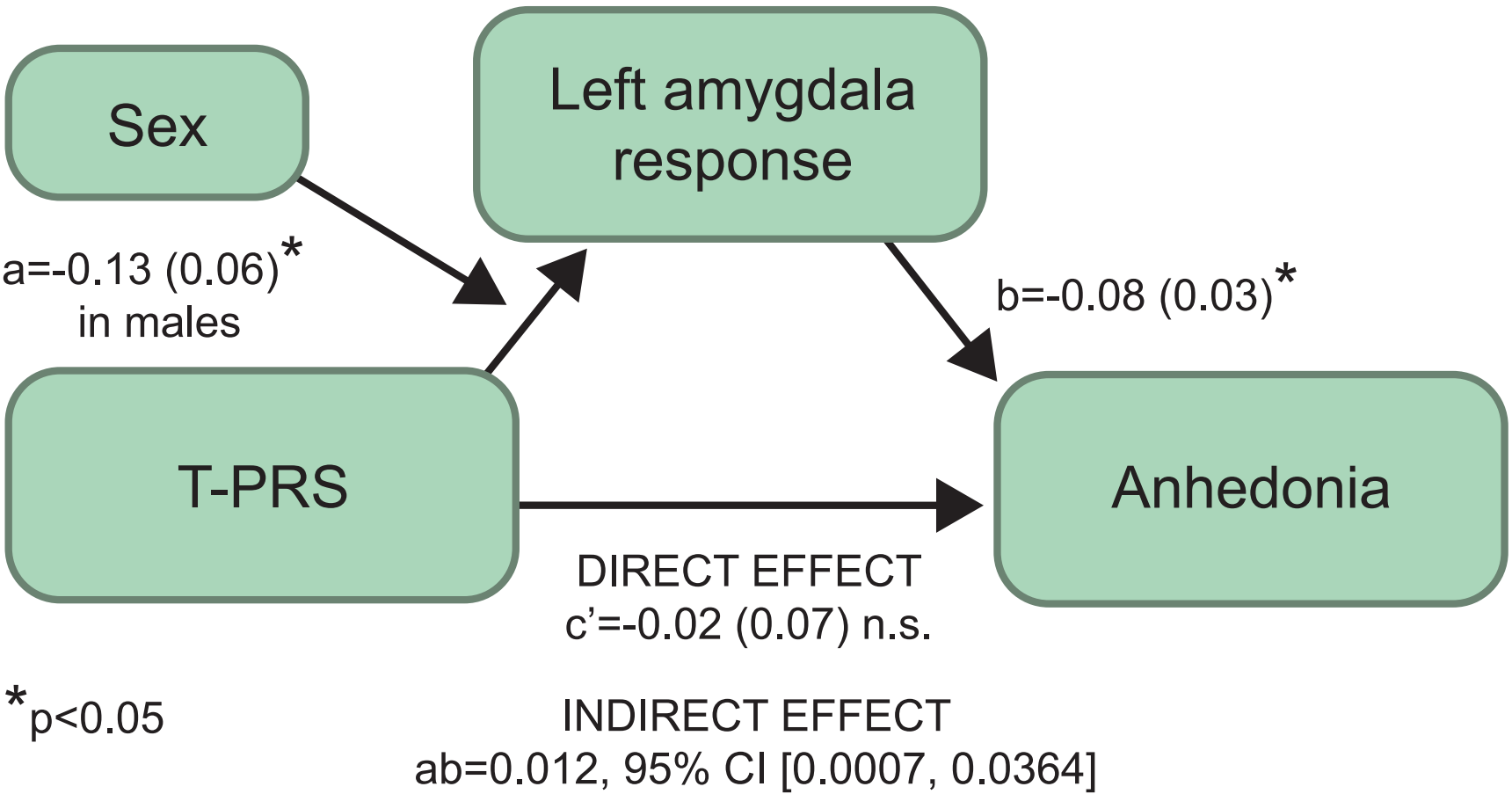

2A.
2B.
Correlation
0.2
0.0
-0.2
-0.4
Shapes
Neutral faces
Emotional faces
Men
Women
Men
Women
T-PRS
PGC-PRS


T-PRS
PGC-PRS
3A.
Amygdala
y=-6
3B.
Caudate
z=8
3C.
vmPFC
x=10
3D.
dlPFC
x=-44
3E.
Parietal Inf.
x=-52
2.5
4.8
bootstrap ratio


4A.
4B.
Men: p=0.04, R²=0.02
Women: p=0.36
interaction: p=0.02
R²=0.01
Neutral > Shapes, L Amygdala
2
1
0
-1
-25
0
25
T-PRS
20
25
30
35
40
PGC-PRS


5A.
5B.
p=0.049, R²=0.01
p=0.009, R²=0.02
Neutral > Shapes, L Amygdala
Neutral > Shapes, R Amygdala
2
1
0
-1
20
40
60
80
MASQ Anhedonia

6.


Sex
Left amygdala response
a=-0.13 (0.06)*
in males
b=-0.08 (0.03)*
T-PRS
Anhedonia
DIRECT EFFECT
c'=-0.02 (0.07) n.s.
*p<0.05
INDIRECT EFFECT
ab=0.012, 95% CI [0.0007, 0.0364]